Taint and Odour Phenomena in Carton-board Packaging Systems

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The candidate confirms that the work submitted is his own and that appropriate credit has been given where reference has been made to the work of others.

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ABSTRACT

Unsaturated lipids exist in carton-board and can be the precursors of odorous compounds. Two unsaturated lipids that can be present are oleic acid (*cis* 9-octadecenoic acid) and linoleic acid (*cis, cis* 9,12-octadecadienoic acid). These lipids can be present in the free acid form, as alkyl ester derivatives and in the triglyceride form. These lipids are able to undergo oxidation. Such oxidation leads to the formation of a number of odorous compounds. Oxidation proceeds via hydroperoxide intermediates to produce aldehydes, ketones, alcohols, furans, lower fatty acids, alkenes and alkanes, the majority of which are odorous. The aldehydes, in particular, have very low odour and taste detection thresholds, needing to be present only in very small amounts in a packaging material to cause a taint problem. Typically, taints associated with these compounds are described as 'rancid', 'pungent', 'soapy' and 'green',

Static headspace GC/MS analyses have identified a number of odorous compounds formed from oxidation reactions, within standard samples of wood pulp obtained from carton-board manufacturers. These oxidation reactions are affected to various extents, by a number of factors such as the temperature, the nature of the fatty acids, the presence of oxidation catalysts such as transition metal ions, the availability of oxygen, the humidity and the presence of photosensitising agents. Breakdown pathways and reaction schemes are presented in context as are kinetic details of odorous product formation.

UV curable inks are commonly used in the printing of carton-board packaging materials. These inks contain photoinitiators which have the potential to affect the oxidation rate and extent of formation of unsaturated lipids, resulting in the formation of odorous compounds within the carton-board matrix. Static headspace analysis, dynamic headspace analysis and vacuum extraction procedures have identified a number of volatile components, that are odorous, in the inks and varnishes. These compounds, which include by-products arising from photoinitiators, amine photoactivators and residual solvents, have the potential to cause 'ink', 'varnishy', 'plasticy' taints in a packaged food system if these components are able to migrate from the carton.

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CONTENTS

1 GENERAL INTRODUCTION

In an initial purchase of food, it is probable that many factors not related to the senses operate. These include exposure through advertising, positioning on the supermarket shelves, packaging and pricing. For that product type to be purchased again, however, it is important that the customer enjoys its organoleptic quality, i.e. it's taste and smell. The perception of quality usually depends upon our expectations of the product, which may be affected by factors such as strong brand image, attractive packaging and printing, and premium pricing. If a product does not live up to our expectations other products will be chosen. Moreover, if the expectations are violated, complaint to the retailer or manufacturer may arise. Although foods have different sensory characteristics, flavour is normally the most significant sensory determinant of food choice.

Problems associated with the organoleptic quality of a food product can be brought about by, amongst other things, the packaging the food is contained in. The main function of a food package is to transport, effectively, the food product from the producer to the consumer. In addition to this key role, modern packaging may also display other important attributes such as preserving the quality of the food product and protecting the food product from external contamination. Food packaging systems are carefully designed to enhance, and not degrade, the desired sensory attributes of food. If the design of the food packaging combination is deficient, or if the food is stored under inappropriate conditions, degradation can occur. If transparent films are used in the packaging then colour fading can occur in some foods, on exposure to daylight that has an ultra-violet component. The texture of foods can deteriorate if the foods are packaged in a film of the inappropriate permeability: snack foods will lose their crispness if packed in media of high water permeability.

Two major mechanisms exist for the deterioration of food flavours. These are,

(I) physicochemical changes taking place within the food, and

(II) ingress of foreign flavours into the food from external sources.

Flavour deterioration may also occur by the additional mechanism of 'flavour scalping' which is the reverse migration of flavour volatiles from the food product to the packaging material. This can reduce the overall characteristic flavour intensity of the food. In addition changes can be less easy to detect. Moreover, this phenomenon may result in the selective absorption of volatiles which may change the flavour balance of the food.

Packaging is very important in the effective marketing of a food product. Today's consumer demands ever higher food quality coupled with requirements for increased storage times and shelf life. The significance of choice of appropriate food packaging, therefore, cannot be overstated.

Taste and flavour are important properties of foods therefore 'taints' by foreign substances are usually not acceptable. Similarly, odours arising from foreign substances that are not associated with the food product will also be found unsatisfactory by the customer.

English language dictionaries define 'taint' in such terms as a blemish, contamination, corruption, infection, pollution and defect (Kilcast, 1993). The ISO (International Standards Organisation) definition of taint is a taste or odour foreign to the product (Kilcast, 1993). This ISO standard also distinguishes an off-flavour as an atypical flavour usually associated with deterioration. The important distinction between these definitions and those found in dictionaries is that food taints are perceived by the human senses. The term 'taint' within the context of food packaging materials, can be defined as the unpleasant odours or flavours imparted to food through external sources.

Every year the food industry receives complaints concerning off-flavours in packaged foods from consumers. Usually, many of these are isolated cases but, on occasions, major problems are encountered which may involve, for example, the destruction of one, or more, batches of packaged food. Off-odours and tainting of food products can result in considerable economic losses to the manufacturer, both in terms of sales and production reputation. In

the last 10-15 years, consumers have become more aware of the occurrence of such off-flavours and are now more vocal in their criticism of those suppliers marketing inferior quality products. The food industries and food packaging industries, in turn, have become more sensitive to off-flavour complaints and now seek rapid identification of the cause of their problems. Industry odour and taint tests on food packaging normally only indicate that a potential problem exists. Only rarely is the cause identified. Large amounts of time, effort and money may be saved if the source and the cause of the contamination can be determined, in order for normal production quality to be restored. Such information may also be used to prevent a recurrence of the problem.

In order to gain a better idea of how taints can arise from the migration of substances from packaging materials, an insight into the human senses, and into the way organoleptic methods can be used for detection purposes, is needed.

The primary human senses involved in organoleptic assessment of food are sight, hearing, touch, taste and smell, although physiological responses are thought to be of some lesser importance. The importance of each of these individual senses will depend on factors such as the food type, the consumption situation and the psychological state of the subject. It is also important to realise that different senses can have antagonistic effects on one another. They may not operate in isolation. For example, dark foods will be judged to have a stronger flavour than lighter foods even when it is only the visual colour intensity that differs.

Generally, the sensory properties of food are placed into three categories: appearance, flavour and texture. The following definitions have been proposed by the International Standards Organisation (Kilcast, 1993):

Appearance: This is defined as *all the visible attributes of a substance or object.*

In addition to colour, the appearance includes other characteristics such as opacity and surface gloss. Even the form and the shape can be of great importance. Considering that the sense of vision is probably the first

awareness that we have with a food, appearance is a characteristic that can determine subsequent perception and reaction.

Flavour: This is a *complex combination of the olfactory, gustatory and trigeminal sensations perceived during tasting.*
This definition recognises the relationship between the sense of smell, as perceived through the nose; the sense of taste, as perceived on the tongue; together with the pain response that gives irritant effects and sensations such as cool and hot.

Texture: Here, attention is given to *all the mechanical, geometrical and surface attributes of a product perceptible by means of mechanical, tactile and, where appropriate, visual and auditory receptors.*
Significantly, this definition highlights the importance of other sensory modalities.

Taint problems arising from packaging materials can be particularly serious for long shelf-life foods that are stored for considerable periods of time, in intimate contact with the packaging material. This problem is significant for liquid food products and other systems that maximise the risk of migration. Even if contamination does not occur, release of volatiles on opening a food pack can generate consumer complaints. Such release should be regarded as a taint problem.

A printed carton-board package is a complex system consisting of many different components within which are several chemical species. A large number of these species can be classed as being volatile. They have the potential to change from a solid phase or liquid phase to the vapour phase. This can occur at ambient temperatures or at temperatures that are not much higher than room temperature. When a compound enters the liquid phase and/or the vapour phase, it becomes mobile. Depending on whether or not the compound has an odorous nature, it can cause taint problems for packaged foods in two ways:

(I) By transfer from package to the food product, via the air space between the two. This is more significant in situations involving secondary packaging, where the food and package are not in direct contact.

(II) By the transfer from package to the food product through direct contact. This is significant in primary packaging, where the food and the package are in direct contact.

Odour molecules dissolve into, or are absorbed by the surface layer of the food and start to diffuse into its interior. The equilibrium between molecules in the solid state and the molecules in the vapour state is now upset. Any equilibrium tends to readjust itself by further evaporation of molecules into the vapour state. This results in an increase in the amount of the odorous substance in the foodstuff. In this way, some foods which easily dissolve or absorb odour, for example, chocolate, can develop a strong taint even though only a slight smell can be detected in the packaging material.

Not all taints are necessarily unpleasant. For example, octenol, sometimes present in board, emits an aroma of mushrooms. In some foods this may be acceptable. However, the consumer is unlikely to want his, or her, chocolate to taste of mushrooms.

Smell and taste are the basis for the customer's judgement and, therefore, should never be neglected. The customer judgement may remain subjective, ambiguous and have poor reproducibility. Throughout the food and packaging industries, taint and odour tests are routinely carried out on the packaging materials before use. This testing usually occurs along the production chain from the material suppliers, through to the package printing, to the food manufacture and the retailers. These tests usually determine whether the particular batch of packaging material under test is organoleptically acceptable to the consumer. For a chemical to be perceived as a taint, it does not need to be positively recognised, but must be identified as a deterioration in flavour quality. When sensory test methods are used, it is important to consider human sensitivity to chemical stimuli and how tainting species are perceived.

Whether, or not, a chemical species can be perceived in a food depends on the chemical structure, the concentration, the type of food and the sensitivity of the human subject. Characteristically, food taints are often detectable at sub-parts per million (ppm) levels and even down to parts per trillion levels (ppt). However, the detection thresholds of chemical species vary widely between individuals. It is important, therefore, if untrained panellists are to be used in a sensory test, that as many panellists as possible are used to ensure the greatest amount of sensitivity within the test.

One type of taint and odour test that is used extensively in the food and packaging industries is the Robinson test (Robinson, 1964). The test method is intended for sensory evaluation of any transfer of off-flavour from packaging material, particularly paper and carton-board, to food. The method is an accelerated storage test, which corresponds to the storage of food in its packaging, under controlled conditions, for a period of several months. For secondary packaging materials, the test is performed with the test food not in direct contact with the packaging material test specimen. For primary packaging materials, the test is performed with the test food in direct contact with the packaging material specimen.

The packaging material to be tested is stored with a test food, for example, chocolate, for 48 hours at room temperature (20±2°C) and nominal humidity, in a closed stainless steel container. A control test food is stored in the same way, but without packaging material. Any off-flavour transferred to the test food from the packaging material under test is subjected to sensory analysis using the triangle test (BS 5929: Part 3, 1984). The evaluation is made by a highly experienced panel of eight to twelve people, of proven unimpaired sensory perception.

Chocolate is the standard test food used in this test due to its high ability to acquire off-flavours from substances which can migrate from packaging materials. Sometimes it may be more appropriate to use other test foods that are more like the foodstuff for which the packaging material is intended. Such products are 'water biscuits', icing sugar, and sponge cake.

Paper-board used for carton packaging is made from pulp, which is turn is derived from lignocellulosic materials (wood). Such carton-board commonly contains various additives such as fillers, sizing agents, retention aids, pigment brighteners, and co-polymer binders. Board used for packaging applications normally consists of more than one layer. It is known as composite board and may have adhesives to bond the various layers. The main component of the carton-board, wood, contains traces of many hundreds of organic compounds and inorganic compounds produced as a result of biosynthesis of a living system. On processing of the wood, many of these compounds are removed. However, some compounds remain and are often chemically modified as a result of oxidative decomposition that occurs during pulping processes. These compounds may be odorous, or be precursors of odorous compounds formed, through oxidative reactions, over a period of time under certain conditions. Trace monomers associated with the styrene/butadiene or the styrene/acrylate copolymers used as binders in coatings for board materials are also potential taint sources.

Usually, the external surface, and sometimes the internal surface of the carton-board package, will be printed with inks and over-coated with a varnish to give added gloss and abrasion resistance to the package. Risks of taints lie in solvents used to dissolve pigments and resins, and in the active components of 'solvent-free' systems, such as ultraviolet (UV)-cured systems. UV-cured systems can produce taint from trace residues of acrylate monomers and from benzophenone, and other photoinitiators.

Food packaging cartons are normally held in their final shape by adhesives. These can be water based formulations made up of dextrins, starch, and poly(vinyl acetate). As with the printing processes, solvent residues and other volatile substances from the formulations can remain in the packaging.

Permeation of components of the inks, varnishes, adhesives, binders, and other compounds may occur unless there is a functional barrier in the packaging material between the printed and adhesive layers and the food product. Normally such migration does not occur significantly enough to cause a taint problem.

The food industries and food packaging industries are well aware that food packaging should be free both of unacceptable odours and of the potential to taint the food product.

The 1958 U.S. Food Additives Amendment to the Federal Food, Drug and Cosmetic Act (Katan *et al.*, 1996) defines a food additive as:

'...any substance, the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component ... of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, transporting, or holding food...)'.

Therefore, even substances that may migrate into food in very small quantities from packaging materials, are regarded as indirect food additives. Because of this they have been the subject of regulations and pre-market approval. With advances in analytical methods of detection over the years, lower and lower levels of migrating substances have become technically subject to food regulation.

The UK Statutory Instrument No. 1523 *The materials and Articles in Contact with Food Regulations, (1987)* (Tice, 1993) state:

'Materials and articles... shall be manufactured in accordance with good manufacturing practice, i.e. in such a way that under normal, or foreseeable, conditions of use they do not transfer their constituents to foods with which they are, or likely to be, in contact in quantities which could (i) endanger human health or (ii) bring about a deterioration in the organoleptic characteristics of such food...'.

Statutory Instrument No. 1523 originated from EC Directive 76/893/EEC, which was the first Directive on the materials and articles intended to come into contact with foodstuffs (EC, 1976) (Tice , 1993).

In 1988, a new EC framework Directive 89/109/EEC was passed by the Council of European Communities (Katan *et al.*, 1996). However, the requirements, with respect to safeguarding human health and preserving the organoleptic properties of the food, remain the same (EC, 1989). The other EC Member states also incorporated the regulations of EC Directive 76/893/EEC into their national laws. An organoleptic characteristic, with respect to humans, can be defined as that characteristic capable of being perceived by one or

more of the sense organs. Therefore, the regulation covers any deterioration in the taste of food due to tainting by contaminating substances originating from the packaging, and any deterioration in the aroma of food due to odours originating from the packaging.

It is very important, therefore, that manufacturers and regulators in the food and packaging industries can identify migrating species originating from packaging materials and, as far as taint and odour are concerned, particularly if these migrating species are odorous. Bad odours and, to some extent bad tastes, in foods characteristically arise from low molecular weight compounds which exhibit significant vapour pressure at room temperature, or from higher molecular weight compounds which are volatilised during processing at elevated temperatures.

The objectives of this research work were:

- To use and develop sensory testing procedures to gain organoleptic characterisation of the carton-package system and all its various components.
- To use appropriate analytical techniques to characterise odorous volatile compounds present in the carton-package that are capable of causing taint within a packaged food. This characterisation would include the determination of the source of odorous compounds and the pathways to the formation of these compounds.
- To correlate the sensory testing data with the volatile compounds detected within the packaging system and determine the potential of different compounds to cause taint and odour problems.
- To determine the different factors and conditions that can affect the rate and extent of the formation of odorous compounds.

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2 SENSORY TESTING FOR TAINT

2.1 INTRODUCTION

Packaging contents and storage factors often are reflected in a packaged food product's shelf-life, with respect to taint occurring from external sources. These external sources need to be identified. The picture that emerges means that the use of chemical analytical methods can lead to several sources of complexity. The task can be simplified by the use of appropriate sensory methods.

Characteristic food taints can have detection thresholds at the parts per trillion (ppt) level. The concentrations at which chemical species can be detected varies considerably between individuals.

The term threshold, commonly defined as the concentration in a specified medium that is detected by 50% of a specified population, is widely used in describing sensory perception of stimuli. Unfortunately the term is frequently misused and misunderstood (Kilcast, 1993). A threshold value indicates the level of stimulus that is sufficient to trigger perception. A number of thresholds can be defined, none of which is invariant. ISO 5492 (ISO, 1992) gives the following definitions for thresholds:

Detection threshold:	the minimum value of a sensory stimulus needed to give rise to a sensation.
Recognition threshold:	minimum value of a sensory stimulus permitting identification of the sensation perceived.
Difference threshold:	value of the smallest perceptible difference in the physical intensity of a stimulus.
Terminal threshold:	minimum value of an intense sensory stimulus above which no difference in intensity can be perceived.

Detection thresholds are usually of concern when dealing with taints.

Published chemical threshold values differ significantly among research groups. This variation is illustrated in the table below (Table 2.1).

Table 2.1 Examples of odour and taste thresholds reported for hexanal (Fazzalari, 1978 and Pangborn, 1981).

Threshold	Medium	Value/range (ppb)
Odour detection	Air	4.5
	Water	0.19-30.0
Odour recognition	Water	4.5-400
Taste detection	Water	0.2-10
	Paraffin	150-300

These ranges reflect variations in number of test subjects, the nature of instructions given to test subjects, the degree of experience of the test subjects, the nature of instructions to test subjects, the test procedure and whether the procedure can be replicated, and details of any statistical analysis. However, taking these factors aside, variations in measured thresholds must be expected as a result of the enormous range of human sensitivities. Measurements using inexperienced panellists should, therefore, use as many human subjects as possible, and should also include provision for repeat testing since subject performance improves with practice. It can be seen, from Table 2.1, that the medium in which the stimulus is present has a substantial effect on the measured thresholds.

A variety of single sample, paired, and triangle presentations has been used to determine detection, difference, and recognition thresholds. These tend to be variations of one of the following types of procedure (Pangborn, 1981):

(i) The Methods of Limits (Method of Least Noticeable Difference, Method of Minimal Changes, Method of exploration). The detection threshold is determined by approaching and receding from the standard stimulus by

short concentration steps. The threshold is that step where the response shifts from one perceived category to another.

(ii) The Method of Average Error (Method of Adjustment, Ad-Libitum Mixing.) This difference threshold technique allows the subject to adjust the concentration of the comparison stimulus to apparent equality with a standard. This comparison sample is then analysed by an appropriate physical or chemical technique. This type of technique is rarely used for food taints, however.

(iii) The Frequency Method. This matching procedure is used to establish the frequency distribution of perception of a specific additive. Each comparison stimulus is tested against the standard stimulus an equal number of times, and the relative frequency plotted against concentration.

Threshold data can be misused in a number of ways (Pangborn, 1981). These include:

(i) Attempting to express relative sensory intensity. Thresholds are of restricted value, because they are only single points on a dynamic concentration continuum. Perceived intensity of taste and odour are not linked linearly to concentration. For example, in a test, subjects detecting one compound at a concentration 150 times lower than they detect another compound, it is incorrect to conclude that the first compound is 150 times more potent than the second, because the ratio is valid only at the threshold concentration.

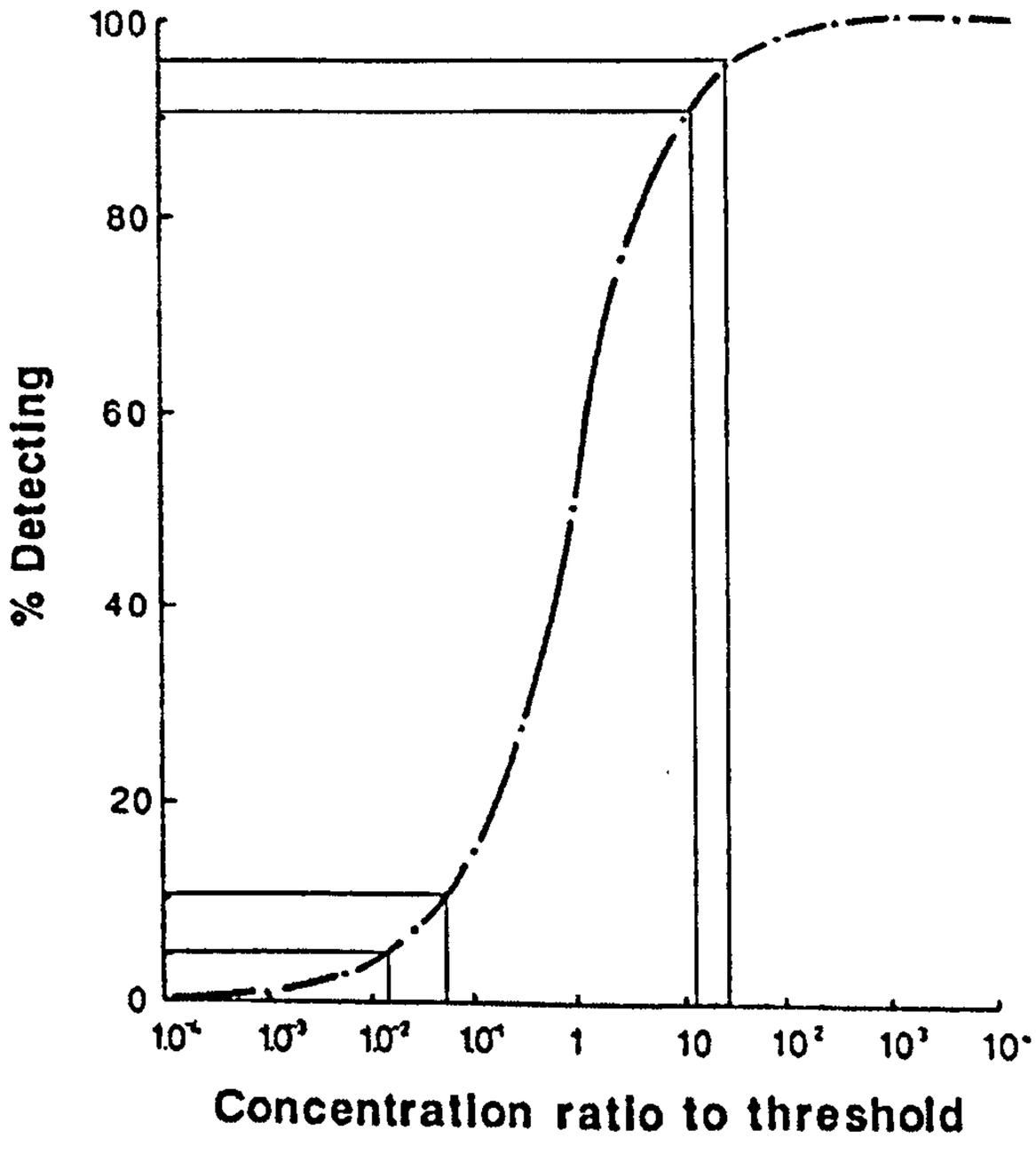
(ii) Attempting to determine the influence of procedural or experiential effects on sensitivity. Threshold values for individuals may vary depending on factors such as whether the panellist smokes and the extent of training received. Thresholds differ. However, perceived intensities at higher concentrations tend not to differ.

(iii) Attempts at selection of sensitive people as subjects for sensory panels. No correlation exists that relate a subject's sensitivity to dilution of odours or

tastes and their performance in judging the sensory attributes of complex food systems. It is necessary, therefore, to introduce new subjects to a variety of odorous compounds to determine their correct use of sensory descriptors.

The wide range of human thresholds to chemical stimuli is a major reason for the difficulties in preventing food taints and in positively identifying the causes of taints. Figure 2.1 (taken from studies of Zoeteman and Piet (1973)) shows threshold data on taints in drinking water (Kilcast, 1993). The figure shows the normalised cumulative distributions of the concentrations of 1,3,5-trimethylbenzene, geosmin, dimethylsulphide and 2-chlorophenol. These compounds differ widely in their chemical structure and in their mean threshold value.

Figure 2.1 Normalised cumulative distributions of the concentrations of 1,3,5-trimethylbenzene, geosmin, dimethylsulphide and 2-chlorophenol (Kilcast, 1993).



The above graph shows that the difference in the concentration ratio to threshold values between the 10% most sensitive human subjects and the 10% least sensitive human subjects is approximately 200-fold, and between the 5%

most sensitive and 5% least sensitive approximately 5000-fold. Considering the 1% most sensitive and 1% least sensitive, this difference rises to approximately 10^5.

The food and food packaging industries must ensure that any tainting species present in the food and packaging are not only below the mean threshold levels, but also below the levels that can be detected by the small, though highly important, proportion of consumers with high sensitivity to food taints. For example, in a case where 1 in every 100 consumers complained to a retailer that a food product had a particular taint, it would be considered unacceptable, if this 1% represented the most sensitive proportion of consumers. Ideally, in light of this, the industry tends to aim for zero levels of tainting species.

It is important, when carrying out sensory testing for taint identification and prevention, to use human subjects who are known to represent, most effectively, consumers with the highest sensitivities. Human subjects may be selected for this on the grounds of their performance in a series of threshold measurement tests.

The wide range of individual sensitivities to a stimulus should be considered in the design of a sensory test procedure. The stimulus should be presented in a range that is sufficiently wide to represent this. It is also important to present the stimulus in a medium that is similar to that which will be encountered in the real, packaged food product system. If little information on the likely threshold concentration is available, a wide concentration range can be spanned using a logarithmic presentation sequence, in which the stimulus concentration increases in a constant ratio. An arithmetic presentation sequence, in which the stimulus concentration increases by a constant added increment, covers a smaller concentration range but offers the possibility of more precise threshold data. In practice, logarithmic sequences are usually more appropriate when a wide range of sensitivities is anticipated.

Depending on the nature of the test, subjects are given coded stimuli which they can detect or recognise. The risk of 'error of anticipation' exists for tests in which stimuli are presented in a series of samples of increasing

concentrations. This can result in subjects incorrectly anticipating the presence of a stimulus. At the other extreme, the 'error of habituation' may exist when a series of samples, containing decreasing concentrations of stimuli, is used. This may result in the tendency to continue to report false positive recognition and detection. Normally, these errors can be averaged out using a randomised or balanced order of sample presentation.

Many taint stimuli, however, are characterised by extremely persistent flavour carry-over. A high level of such a stimulus can desensitise the palate and result in unreliable judgements thereafter. Consequently, presentation orders of increasing concentration are favoured within tests, normally incorporating modifications to minimise the 'error of anticipation'. A simple way of achieving this is to use random blank samples in the presentation sequence. A more elaborate method is to use the '*choose 1 of n*' procedure (Kilcast, 1993), where *n* is normally 2 or 3 samples at each level. For two stimuli at each level, one sample contains the stimulus and the other a blank. The sequences are shown below (Table 2.2).

Table 2.2 Sample presentation sequences.

Straight sequential	0,1,2,3,4,5,6,7, ...
Modified sequential	0,1,0,2,3,0,0,4,0,5, ...
Choose 1 of *n*: *n*=2	0;0,1;2,0;0,3;0,4; ...
***n*=3**	0;0,0,1;2,0,0;0,3,0; ...

0 = blank (no stimulus)

1,2,3, ... = increasing stimulus concentration

Regardless of the test method used, it is considered to be essential to reduce the risk of subject fatigue and carry-over effects of stimuli. This can be achieved by limiting the number of stimuli presented in any given session and by allowing enough time for palate recovery between sample tastings.

The significance of these factors will depend on the nature of both the stimulus and subject response. As a general rule, no more than 4-8 stimuli should be presented in a single session, allowing approximately 1-2 minutes between stimuli. Palate cleansers may be used where stimuli are presented in food media, to reduce the risk of carry-over and fatigue effects.

2.1.1 SENSORY DESCRIPTIONS OF TAINT

Subjects may have difficulty in verbalising their perceived sensory experience. This is a major factor and should be addressed. During sensory testing it is usually quite easy to obtain reliable information on a subject's level of liking of a stimulus. However, it is more difficult to obtain a breakdown of the sensory characteristics of the stimulus that contribute to these levels. Therefore, selection of individuals who are capable of verbalising sensory perceptions, especially in the presence of other sensory stimuli is of great importance.

It is widely accepted that subjects achieve better description of sensory terms when they are familiar with those stimuli as a result of past experience. This is most easily seen in the case of chlorophenols, since exposure to oral mouthwashes (e.g. TCP) results in a ready production of terms such as TCP, medicinal and antiseptic.

To overcome the problems associated with verbalisation of unfamiliar stimuli, a pre-selected set of odour descriptor terms has been developed (Dravnieks *et al.*, 1978). During direct comparison of two odours, the odours are rated for their similarity or dissimilarity, using some scale. In an indirect comparison, each odour is described separately in terms of applicability of various character aspects, and the resulting profiles compared. Profiling permits the accumulation of data on very many odours, tested at different times, while the direct comparison would require comparing a new added sample with all previously compared samples, which can be time consuming.

This type of profiling involves a descriptor set consisting of 136 terms arranged in a sequence that facilitates an independent consideration of each descriptor. The odour to be tested is scored for each term on a 0 to 5 scale. It has been reported that the use of so many terms does not create practical problems, one sample being evaluated in 5-10 minutes. One advantage of the use of a number of very similar terms lies in the possibility of a panellist, though being unfamiliar with one term, being able to find a representative term more common to him, or her. Thus, the scale of terms can be seen as a compromise between a small, economical, non-redundant scale and a free choice of descriptors. It may serve as a prompting list containing a sufficient choice for matching each subject's own semantic structures (Dravnieks *et al.*, 1978). In the study by Dravnieks *et al.* descriptor terms were left undefined and panellists

were instructed to interpret the meanings in accordance with their own perceptions. A requirement of the test procedure was that a panel consisted of at least 9 subjects, experienced or inexperienced, with no limitations being imposed on the selection of panellists. Changes in panel composition were difficult to avoid. The effect of such changes was disregarded.

Results of profiling were displayed as composite values of so-called 'percent applicability' of each descriptor. This parameter was derived from profiling score data as follows:

(i) descriptors used for a particular odour only by one subject in an entire panel were disregarded;

(ii) for descriptors used by two, or more, subjects in the panel, the percentage of subjects who used it was calculated; also, the sum of scores for the panel was calculated, and expressed in terms of percent of the maximum possible score, which is 5 x (number of panellists);

(iii) the geometric mean of the above two percentages was calculated, and termed the percent applicability.

This adjustment was used to help equalise cases where only a few subjects used the descriptor, but gave the descriptor a high score and cases where many subjects used the descriptor, but gave the descriptor a low score level. It can also help equalise the inputs of panels of different sizes.

The odour descriptor terms used were further developed by The Finnish Pulp and Paper Research Institute to make the terms more suitable for fibre-based products (Söderhjelm and Pärssinen, 1985). The descriptors set consists of approximately 140 terms arranged in a sequence which facilitates independent consideration of each descriptor (Table 2.3). As before, the intensity of the odour associated with each descriptor was scored on a scale of 0 to 5.

When analysing the results, the descriptors are rearranged into 24 groups so that similar terms are close to each other. This eliminates the confusion caused by different panellists using different terms for a particular odour. The groups are further arranged to form a hedonic sequence with very pleasant smells such as apple and pear to the far left and unpleasant smells such as sewage and cadaver on the far right.

Table 2.3 List of descriptor terms used to define odours associated with fibre-based materials (Söderhjelm and Pärssinen, 1985).

Index	Descriptor
1	Fragrant
2	Sweaty
3	Almond-like
4	Burnt, smoky
5	Herbal, green, cut grass
6	Etherish, anaesthetic
7	Sour, acid, vinegar
8	Like blood, raw meat
9	Dry, powdery
10	Like ammonia
11	Disinfectant, carbolic
12	Aromatic
13	Meaty (cooked, good)
14	Sickening
15	Musty, moldy
16	Sharp, pungent, acid
17	Camphor-like
18	Black liquor
19	Kraft pulp
20	Wood (material)
21	Printing ink (fresh newspaper)
22	Metallic
23	Glue (PVAc)
24	
25	Cinnamon
26	Popcorn
27	Pipe tobacco
28	Vinegar
29	Tar-like
30	Eucalyptus
31	Oily, fatty
32	Like mothballs
33	Like gasoline, solvent
34	Cooked vegetables
35	Sweet
36	Fishy
37	Spicy
38	Paint-like
39	Rancid
40	Minty, peppermint
41	Sulphidic, rotten egg
42	Earthy
43	Fresh sawdust
44	Putrid, foul, decayed
45	Pitch (resin)
46	DDT, insecticide
47	Ethyl acetate (solvent)
48	Onion
49	Animal
50	Vanilla-like
51	Fecal
52	Floral
53	Yeasty
54	Wood bug
55	Honey-like
56	Aniseed (Liquorice)
57	Turpentine
58	Cabbage, cooked
59	Medicinal
60	Fruity (citrus)
61	Buttery (fresh)
62	Like burnt paper
63	Plastic (PE)
64	Caraway
65	Bark-like, bitch bark
66	Burnt plastic
67	Celery
68	Burnt candle
69	Mushroom-like
70	Wet wool, wet dog
71	Chalky
72	Leather-like
73	Peat (fruit)
74	Stale tobacco smoke
75	Raw cucumber-like
76	Raw potato-like
77	Conifer
78	Casein glue
79	Bean-like
80	Banana-like
81	Burnt rubber-like
82	Geranium leaves
83	Urine-like
84	Beery (beer-like)
85	Pine wood-like
86	Coconut-like
87	Root cellar
88	Washing-up liquid
89	Tall soap
90	Cardboard-like
91	
92	Dirty linen-like
93	Smoked fish
94	Caramel
95	Sauerkraut-like
96	Crushed-grass
97	Chocolate
98	Molasses
99	Alcohol-like
100	Dill-like
101	Chemical
102	Creosote
103	Allspice
104	Household gas
105	Hexanal
106	Dish-cloth
107	Tea-leaves-like
108	
109	Stale
110	Sulfite pulp
111	Sour milk
112	Cat-urine-like
113	Pineapple (fruit)
114	Fresh tobacco smoke
115	Nutty (walnut, etc.)
116	Fried chicken
117	Wet paper-like
118	Coffee-like
119	Peach (fruit)
120	Laurel leaves
121	Burnt milk
122	Sewer odour
123	Sooty
124	Cooked milk
125	Rubbery (new rubber)
126	Bakery (fresh bread)
127	Clabbered milk
128	
129	Latex
130	Eggy (fresh eggs)
131	Bitter
132	Cadaverous, like dead mouse
133	Bitter almond
134	Seasoning (for meat)
135	Apple (fruit)
136	Bouillon
137	Grainy (as grain)
138	
139	Tallow
140	Dried fruit
141	Kerosene
142	Acetone
143	Fermented (rotten) fruit
144	
145	Varnish
146	Groundwood

The applicability value, E_i, reflects both the frequency of use of each descriptor, i, and the level of the scores given. It is calculated from the formula:

$E_i = 100\ (n/N \times \Sigma p/\Sigma P)^{1/2}$. Here,

n = number of panellists who used the descriptor

N = total number of panellists

Σp = sum of scores given to i

ΣP = sum of maximum possible scores.

The higher the value of E_i, the greater the significance of the particular odour characteristic represented by i. An odour 'finger-print' can be obtained from these data and used to evaluate the similarity or dissimilarity between two odours. This can be achieved when the hedonic profiles from two odours are compared, using the following measure of the degree of likeness:

$$Z = \left({}_{j=1}^{q}\Sigma\ (X_{aj}-X_{bj})^2 \right)^{1/2}$$

Here, in the case of hedonic profiles, the symbols denote:

Z = the measure of similarity (degree of likeness)

X_{aj} = mean value of E within a group of descriptors, j: sample a

X_{bj} = mean value of E within a group of descriptors, j: sample b

q = number of groups, in this case 24.

Z increases when the profiles are dissimilar and decreases when they are similar.

Handling approximately 140 descriptors proved to be quite tedious. Also some of the descriptors were very seldom used by panellists. Therefore, if this method of odour characterisation is adopted, then a shorter list of odour descriptors may be needed.

It should be noted that even minor differences within classes of chemical compounds can give rise to markedly different sensory perceptions. The odour descriptions of chloroanisoles, together with the detection thresholds

measured in water for a selection of these materials, is shown in Table 2.4 (Griffiths, 1974).

Table 2.4 Odour description (% of terms used) and odour detection thresholds for chloroanisoles (Griffiths, 1974).

Stimulus	Musty and related	Medicinal and related	Solvent/ alcoholic	Sweet/ fruity	Detection threshold in water (ppm)
Penta CA	86	3	-	3	$4x10^{-3}$
2,3,4,5-TeCA	39	9	30	9	
2,3,4,6-TeCA	83	7	-	3	$4x10^{-6}$
2,3,5,6-TeCA	44	12	32	4	
2,3,4-TrCA	56	-	24	20	
2,3,5-TrCA	19	37	-	41	
2,3,6-TrCA	80	-	-	12	$3x10^{-10}$
2,4,5-TrCA	41	28	-	21	
2,4,6-TrCA	96	-	-	4	$3x10^{-8}$
3,4,5-TrCA	27	12	12	48	
2,3-DCA	30	20	15	20	
2,4-DCA	56	-	-	44	$4x10^{-4}$
2,5-DCA	25	20	20	30	
2,6-DCA	62	23	15	-	$4x10^{-5}$
3,4-DCA	16	-	3	72	
3,5-DCA	25	16	-	50	
2-CA	-	35	43	22	
3-CA	-	22	30	48	
4-CA	4	37	41	18	
Anisole	-	30	27	47	$5x10^{-2}$

- Penta CA = 2,3,4,5,6 pentchloroanisole
- TeCA = tetrachloroanisoles
- TrCA = trichloroanisoles
- DCA = dichloroanisoles
- CA = chloroanisole (C_7H_7OCl)

Relatively small structural differences can result in major changes, not only in odour thresholds but also in the sensory descriptions.

Searching for reliable descriptor sets for chemical stimuli can be difficult. In a study of 101 petrochemicals, odour thresholds, odour qualities and hedonic characteristics were recorded (Hellman and Small, 1974). The reported descriptors were limited to only one or two per compound, though the hedonic rating, (pleasant/neutral/unpleasant), was potentially useful. However, with few exceptions, the compounds studied were not commonly associated with food taints.

In addition, many sensory descriptors are not unique to one compound but can be associated with a large number of different compounds. Most of the possible compounds associated with three common sensory descriptors are shown below (Table 2.5).

Table 2.5 Possible chemical compounds related to specific sensory descriptors (Kilcast, 1993).

Descriptor	Chemical compound
Musty	2,6-Dimethyl-3-methoxypyrazine
	2-Methoxy-3-isopropylpyrazine
	2,4-Dichloroanisole
	2,6-Dichloroanisole
	2,3,6-Trichloroanisole
	2,4,6-Trichloroanisole
	2,3,4,6-Tetrachloroanisole
	Pentachloroanisole
	2,4,6-Tribromoanisole
	Geosmin
	2-Methylisoborneol
	1-Octen-3-ol
	Octa-1,3-diene
	α-Terpineol
	4,4,6-Trimethyl-1,3-dioxan
Painty	Heptan-2-one
	Trans,trans-hepta-2,4-dienal
	Trans 1,3-pentadiene
	2-(2-pentenyl) furan
Plasticy	Benzothiazole
	Methyl acrylate
	Methyl methacrylate
	Trans 2-nonenal
	1-Octen-3-one
	Trans-1,3-pentadiene
	Styrene

Each of the above descriptors may be associated with very different classes of chemical structures. This increases the difficulties encountered by the sensory analyst when attempting a positive identification. A phenomenon that further increases the task for the analyst is the fact that the sensory descriptor, associated with a particular compound, may change with compound concentration and with the medium in which it is present. During work carried out on coffee flavours (Parliment *et al.*, 1973), *trans* 2-nonenal was bubbled through soluble coffee to a level of 1 ppb. The effect on the organoleptic

properties of the coffee was noted using a profile evaluation panel. The effect was that of adding a desirable woody, fresh brewed flavour to the coffee brew. At a lower level (0.2 ppb), a more subtle flavour was detected. At a higher level (8.0 ppb) a more pronounced woody flavour was experienced and at 40 ppb, a burnt, fatty, rancid flavour was perceived. The range of different organoleptic perceptions from the effect of 2-nonenal, at different concentrations, is illustrated in Table 2.6. The sensory descriptors obtained from the same levels of *trans* 2-nonenal in water are also shown.

Table 2.6 Organoleptic evaluation of the effect of 2-nonenal at various levels (Parliment *et al.*, 1973).

Concentration (ppb)	Evaluation in	
	Soluble coffee[a]	Water[b]
0.2	Detectable subtle flavour	Slightly plastic-like
0.4	Subtle woody	Woody
1	Woody, smoother	Woody
2	Woody, groundsy	Woody
8	High woody impact	Fatty
16	Tallowy	Fatty
30	Burnt, fatty	Unpleasant oily, fatty
40	Burnt, fatty, rancid	Unpleasant fatty
1000	Strong cucumber	Strong cucumber

[a] Evaluated at 140-160°F

[b] Evaluated at room temperature

The medium in which the compound of interest exists has a particular influence on its organoleptic quality, though this effect is not too severe in these cases.

2.1.2 SENSORY EVALUATION

A number of different methods exists for the sensory analysis of foods and food packaging materials. However, it is important to select the appropriate type of assessment for each case to be evaluated. It should also be recognised that

many of these methods need to be adapted, both in use and in interpretation, for various different applications.

Sensory testing methods can be grouped into two broad classes:

(i) analytical tests

(ii) affective (or hedonic) tests (IFT, 1981)

The test procedure may be the same for each class. However, the purposes of the test, in each case, are quite different. In analytical tests, which can be subdivided into discriminative and descriptive categories, the perceptions of the human subjects are used to provide information on the organoleptic characteristics of the food or the packaging system. When used in this way, the responses from human subjects can be compared with those from analytical instruments. Procedures are normally adopted to reduce the various forms of bias that can effect their performance and to reduce the influence of natural biological variation. Hedonic tests, in contrast, are used to assess the effect of the food, or packaging material, on human response, usually in terms of preference or acceptability. Natural human reactions are therefore needed, that reflect biases normally encountered and also biological variations that can result from both physiological and psychological sources. The major difference between tests, within each class of testing, is that analytical tests tend to utilise small numbers of carefully selected and trained assessors, whereas hedonic tests involve relatively large numbers of untrained assessors, used to indicate the likely response of a larger population. Normally, hedonic information is not gathered using a panel of trained assessors nor is analytical information gathered using untrained assessors. However, the diverse nature of problems associated with taint, sometimes require developments of the above principles.

2.1.2.1 Types of analytical tests

Discriminative, or difference, tests. This type of test is used to determine whether, or not, a sensory difference exists between two samples. Such tests can also be used as a means of measuring thresholds and sensitivity. Three types of test are most frequently encountered:

(i) paired comparison tests

(ii) triangular tests

(iii) duo-trio tests

In addition, three other tests, two out of five, 'A' or 'not A', and R-index are used less commonly.

Paired comparison test. In this test, two coded samples are offered in either a sequential or simultaneous sequence (i.e. AB and BA). Two variations of the test exist. In the simple difference variant, the panellists are asked if there is a difference between the two samples, having been informed previously that there may, or may not, be a difference. In the directional difference variant, the panellists are asked to choose the sample with the greater or lesser amount of a specified characteristic. Panellists may be allowed to choose a 'no-difference' response, or may be asked to make a 'forced-choice' judgement. The forced-choice procedure is more statistically correct, though, no-difference responses can provide a useful source of information. The probability of a judgement being made purely by chance is 0.5, and responses are analysed in terms of statistical significance levels calculated by using a binomial distribution. Statistical tables are normally used to calculate these fixed levels of significance, (e.g. 1% and 5%). It is recommended, however, that exact significance levels should be used where ever possible (Kilcast, 1993).

Triangular test. In this approach, three coded samples are presented to panellists, two of which are identical, using all possible sample permutations, i.e.:

ABB AAB

BAB ABA

BBA BAA

Panellists are asked to select the odd sample in either a no-difference or a fixed-choice procedure. The possibility of choosing the odd sample purely by chance is 0.333. Thus, the test is more statistically powerful than the paired comparison and, therefore, fewer panellists are required. The increased

number of samples can, however, result in problems with flavour carry-over when using strongly flavoured samples, making identification of the odd sample more difficult. Difficulties can also be encountered in trying to achieve identical presentation of samples of some foods and packaging materials. Statistical significance values can again be calculated from a binomial distribution. No-difference responses can either be ignored and the number of judgements reduced relatively or, alternatively, one third of the no-difference responses can be added to the correct responses.

Duo-trio test. In this test, the panellists are given a sample that is identified as the standard, followed by two coded samples, one of which is the same as the standard and the other different. They are then asked to decide which sample is the same as the standard. The sample presented as the standard may be the same for all panellists, (fixed reference standard), or may be split between the two types. This type of test has the same statistical power as the comparison test and can be analysed using the same procedures. The need for expert or selected assessors is not so important for this test method due to the use of the identified reference standard. In addition, the use of a fixed reference standard can reduce problems associated with flavour carry-over.

Difference from control. This test can be used when a control sample is available. Panellists are presented with an identified control and a range of test samples. They are asked to rate the samples on suitable scales anchored by the points 'different from control' to 'very different from control'. This kind of test is more commonly known as similarity/dissimilarity scaling and is analysed using multidimensional scaling methods (Kilcast, 1993).

R-index test. This approach is quite a recently developed method (Kilcast, 1993), and can be classed as a short-cut signal detection method. The test samples are compared against a previously presented standard, and rated in one of four categories. The categories for difference testing are (i) standard; (ii) perhaps standard; (iii) perhaps not standard; and (iv) not standard. The test can also be carried out as a recognition test, in which the categories are: (i) standard recognised; (ii) perhaps standard recognised; (iii) perhaps standard not recognised; and (iv) standard not recognised. The results are expressed in

terms of R-indices, which represent probability values of correct discrimination or correct identification.

2.1.2.2 Descriptive tests

Classification. Descriptive testing is used to sort items into a pre-defined number of categories, normally using a small panel of expert or selected assessors.

Ranking. Ranking tests are used to sort several samples into an order of intensity of a specific sensory characteristic. The ranking totals are calculated from the input of all panellists, and differences are interpreted by statistical tests, e.g. the Friedman test. This type of test may be carried out as a screening test, or pre-test, prior to more precise assessments. No quantitative information can acquired from this type of test and no comparison of data between sets of results is possible.

Rating. These tests involve classification into categories in the form of an ordered scale. It is important that the panellists have a good understanding of the various categories. In contrast to ranking tests, data concerning the magnitude of attributes are produced. These magnitude scales can take various forms, for example graphic or descriptive and uni-polar or bi-polar. With this type of test, it should be pointed out that differences in magnitude, across the various categories, is not uniform.

Scoring. Scoring is a form of rating using a numerical scale, in which the scores hold significant mathematical weight. Coded samples are given to panellists in a simultaneous or sequential order. These scales can be category scales, unstructured line scales (or graphic scales) with verbal anchors, or in a form of scaling known as magnitude estimation ratio scales.

The scored data from samples may be analysed by looking at the variance and carrying out comparison tests. The data from this type of test should be normally distributed.

Simple descriptive tests. These are purely qualitative in nature and require each panellist to assess one or more samples independently to identify and describe the sensory attributes. Following the independent assessments, the panel leader collates the data and generates a list of sensory attributes based on frequency of use within the test. In some tests, the panel leader and the panel will discuss their perceptions and draw up a list of descriptive terms that are acceptable to all panel members. In order to reach this agreement, several discussions may be necessary. It is important that panellists can also agree a definition of each descriptive term.

Sensory profile tests. This type of test is used to quantify the sensory attributes of foods and packaging materials that have been established using descriptive tests. Two major classes of test are used. Consensus profiling, based on a procedure termed quantitative descriptive analysis (QDA), uses the list of attributes agreed in a prior simple descriptive test. Suitable scoring scales (usually unstructured line scales) are constructed with appropriate anchors, and panellists are trained in scoring reproducibly the intensity of the chosen attributes using a training set of samples. This training may be time consuming for systems in which large number of different sensory attributes exist. On achieving satisfactory reproducibility, replicated tests are used to score test samples using appropriate statistical methods. Data generated may be analysed using variance and multiple difference testing.

The second profile method is known as free choice profiling. Panellists use an individual attribute list, produced from the first stage of a simple qualitative test. Each assessor builds an individual profile based on his/her own attributes, and a consensus profile is generated mathematically using a technique known as generalised Procustes analysis. This method reduces panel training times significantly, but data interpretation can be less reliable.

2.1.2.3 Hedonic tests

Hedonic tests are used to determine a measure of liking, usually in terms of acceptability or preference. This type of test uses some of the procedures associated with analytical tests. Hedonic tests demand the use of sufficient numbers of panellists who are, ideally, typical of a larger consumer population.

In addition, the test environment may need to reflect the appropriate consumption situation, i.e. where, and under what conditions, the food product is likely to be consumed. The most common tests used are: (i) paired comparison tests, (usually paired preference); (ii) ranking tests; and (iii) scoring tests using hedonic category scales.

It is important that analytical and hedonic tests should not be carried out with the same assessors. However, some adaptation may be needed depending on the nature of the taint and the assessors available.

The number of assessors for both analytical and hedonic sensory testing recommended by ISO 6658 (ISO, 1985) are shown in Table 2.7.

Table 2.7 Minimum recommended numbers of assessors (ISO, 1985).

Tests	Number of assessors		
	Experts*	Selected*	Assessors*
Analytical tests	-		
Paired comparison	7	20	30
Triangular	5	15	25
Duo-trio	2		20
Ranking	2	5	10
Classification	1	1	
Rating	1	5	20
Scoring	-	5	20
Simple descriptive	5	5	
Profile	5	5	
Hedonic tests			
Paired comparison	-	-	100
Ranking	-	-	100
Rating	-	-	50 (2 samples)
			100 (>2 samples)
Scoring	-	-	50 (2 samples)
			100 (>2 samples)

* as defined in ISO 6658

2.1.2.4 Sensory testing procedures

Practical operating restraints will normally determine the degree to which the rigid guidelines for test methods can be met. Although it is not possible to keep to all these criteria, some important guidelines must be maintained (Kilcast, 1993):

(i) The testing must be carried out in a suitable environment. In particular, analytical testing requires an environment that is free from distraction, noise and odours, so that panellists can give their tasks sufficient concentration. The provision of individual booths, with lighting control and temperature control may be necessary.

(ii) Sources of external bias must be minimised. This will involve, among other possible steps, minimising panellist interaction, (e.g. by using individual booths), coding samples with different random number codes for each test, balancing presentation order, careful questionnaire design, and using statistical tests that are appropriate to the data and the purposes of the test.

(iii) Panellists possessing the appropriate level of training must be used for the tests. For example, there is no value in operating profile tests using panellists that are untrained or unfamiliar with the products to be profiled.

(iv) Panel managers must be prepared to allow the conclusions from the panel test to override their own personal judgements and expectations. This is particularly important if the confidence in panel operations is to be maintained. The only exception to this is when the panel manager possesses special information or expertise that can complement panel findings.

2.1.3 EVALUATION OF TAINT USING SENSORY TESTING PROCEDURES

A major problem associated with the use of sensory testing procedures for the identification of taints lies in the possibility of a taint not being detected. This

may arise when a taint problem exists that is only detectable by the small proportion of highly sensitive consumers. This problem could have serious commercial implications. However, the small set of panellists used in the tests may not detect the problem.

Though no procedure exists to remove this problem, steps can be taken to minimise the risk of not identifying a taint stimulus. The most important of these are the following.

(i) When the identity of the tainting species is known, panellists of known sensitivity to that stimulus should be used. Threshold measurements may be used to determine panellist sensitivity to a stimulus, but it may be useful to measure sensitivity at higher stimulus concentrations to reflect levels present in real systems. Unfortunately, it cannot be assumed that a panellist sensitive to one taint stimulus will be sensitive to another taint stimulus. As a result, members of a testing panel should be selected on their sensitivities to several different taint stimuli. However, this is not always possible when testing for unknown tainting species. A compromise has to be reached in which panellists that are generally sensitive and reliable in their judgements are used.

(ii) If it is not possible to have a test panel consisting of highly sensitive members, a larger number of panellists should be used in the hope that someone present is sensitive to the taint. The recommended minimum numbers of panellists of various levels of expertise is given in Table 2.7. When testing for unknown taint stimuli, at least the minimum number of panellists stated should be used, and even increased, if possible. Sometimes, practical operating restraints will restrict the number of people available to perform sensory testing. Therefore, it is common for a small number of panellists to make replicate judgements; for example a triangular test can use a panel of five to carry out three tests. This is acceptable in normal difference testing, since statistical analysis is, strictly, based on the number of independent judgements and not on the number of panellists. This procedure is not of any use, however, for taint testing; if the five panellists used are insensitive to a taint, then no amount or replication will increase the likelihood of the detecting the taint.

(iii) An appropriate test procedure that has a high sensitivity to the situation should be used. For example, a difference test may be more suitable than a profile-type test, due to the former being more rapid and not requiring intensive training of panellists. A difference test, matched against an appropriate untainted control should provide a reasonably easy task even for a panellist of little experience.

In choosing a high sensitivity difference test, the potential for flavour carry-over between tainted samples must be addressed. This can be demonstrated using a triangular test in which two tainted samples and one control sample are presented. Identification of the different sample is less easily achieved than when only one tainted sample and two control samples are used (Kilcast, 1993). However, limiting the tests to permutations containing two control samples may introduce unacceptable bias and should only be used in panellist selection or threshold measurement procedures.

Paired comparison tests and duo-trio tests, although statistically less powerful, have an advantage in that only one taint sample is presented to each panellist. In general, the paired comparison test is not considered suitable for taint testing. However, the duo-trio test, in which a control reference sample is included, is seen as an effective means of determining taint. A drawback of this type of test lies in its lower statistical power. Thus, a higher number of panellists is needed than would be used in operating the triangular test.

(iv) The amount of information gathered from a test should be maximised. Some authors (Kilcast, 1993) suggest that panellists should only be asked to identify a difference between samples presented within a taint test. The reason for this is that panellists may need to use different mental processes to supply the additional information. This may invalidate the test. Normally, it is accepted that descriptive information concerning the nature of any identified difference between test samples should be recorded.

In one sensory testing situation (Kilcast, 1993), two other forms of information were gathered from taint tests. Firstly, since taints were, by definition, disliked, preference information was recorded. This can be seen as a rare exception to the rule that hedonic and analytical tests must not be mixed. This preference information is not interpreted as a likely measure of

customer response, but is used as a directional indicator in conjunction with descriptive information. Secondly, panellists are required to rate how confident they were in their choice of the odd sample in triangular test on a 3-point scale.

These confidence indicators can be used to resolve indeterminate results by giving an indication of the extent of guessing by panellists. It should be noted that a confidence score for a particular panellist must only be considered if he/she has given an incorrect identification of the odd sample.

(v) Appropriate statistical tests should used with respect to taint testing. These depend on how sure the analyst wishes to be that he/she has found a real difference result within a test. A probability value of 0.05 (5% significance) in a difference test can be interpreted as indicating that a difference appears to exist, but with a 5% (1 in 20) probability that the result could be due to chance. If more assurance is required that a difference has really been found, a lower significance level of 1% could be used, giving a 1 in 100 probability of a chance result. However, as a greater assurance of a real difference is sought after, the risk of not identifying a real difference is increased. As the significance levels are increased to 10%, 15% or even 20%, the risk of not identifying a real difference diminishes. The choice of a suitable significance level will depend largely on the level of risk that is required. The consequences of incorrectly identifying a difference are relatively minor, compared with the consequences of not identifying a difference resulting in a taint that reaches the customer. As a result, levels of up to 20% should be used to minimise this risk, considering the fact that, overall, 1 in 5 judgements may be incorrect.

(vi) Irrespective of the data produced by statistical tests, the judgements from the minority of panellists, especially from those of proven sensory perception, should be considered and, if necessary, appropriate retesting carried out.

2.1.3.1 Diagnosis of taint using sensory testing

The first indication of a taint problem that is associated with a food product, is usually through consumer complaints of the sensory quality. More often than not, the taint detection by the consumer will be low. Therefore, the complaints will come in at a low rate over an extended period of time, and recognition of the taint may be slow. Another problem exists in that the investigation of a product item that has been returned may be considered compromised if the product has been removed from its packaging. In such cases, examination may be restricted to odour evaluation and, if possible, chemical analysis.

It may be possible to examine products from the same batch as that containing the tainted item, both having gone through the same packaging and distribution channels. This would be in addition to using control samples of a similar age.

Consumer descriptions of the majority of taints are unreliable and should not be used as a basis for chemical analysis investigations. Sensory analysis of suspect batches should be carried out to generate reliable descriptive information which may be of benefit to chemical analysts.

On establishment of a taint problem, the major tasks are to isolate and to identify the source of the taint. Sensory testing can be used to investigate if the problem lies within a single transport container, production run, the ingredients batch or packaging batch. If the problem has occurred over a longer time span, it may be caused as a result of the food or packaging production process environment, or water/solvent-borne contamination.

2.1.3.2 Use of sensory testing to prevent taint

Preventative testing can be used to reduce taint problems that may arise from the introduction of new materials and from changes in environmental conditions. The tests involve exposing food to potential sources of taint under severe, but not unrealistic, conditions. Severity factors of up to ten times are usually used, but higher factors can be used for critical applications.

The design of the exposure system will differ markedly depending on the test application. For packaging materials, the system is designed depending on

whether direct contact or remote contact is sought between the packaging and the food substrate.

Important points to be considered when designing model systems for testing materials, such as the production environment, the storage environment and the type of packaging materials are:

- the type of food/packaging;
- the volume or surface area of food/packaging material compared to the volume of test vessel;
- the volume or surface area of packaging material compared to volume or surface area of food substrate;
- the stage of exposure (i.e. perform test at a time when the environment may be affected by external factors, such as installation of new materials at a production line);
- the length of exposure;
- the temperature and the humidity of exposure;
- the exposure method, (e.g. by direct contact or vapour phase transfer);
- the lighting level during exposure time (particularly relevant when oxidative rancidity may occur);
- the ventilated or unventilated exposure system;
- the period of storage of the food substrate between exposure and sensory testing;
- the type of sensory test method and the statistical interpretation of results.

The type of food substrate chosen for a particular test is important. When a specific ingredient or product is considered to be at risk, the test methods can be built around it. Simple foods or food simulants are often used when more general information is required from sensory testing. In this case, solvent or adsorptive properties are usually the more important factors to be considered in selecting appropriate general food simulants. Fats and oils tend to absorb water-insoluble tainting species, and food substrates such as butter are known to be sensitive to taint transfer. In addition, high surface area powders with hydrophilic characteristics, tend to be sensitive to taints via the migration of water-soluble species. The use of such materials should simulate the majority

of the solvent and adsorptive characteristics of real foods. Food simulants should have a relatively bland flavour so that tainting species can easily be recognised and should, also, be of reasonably high palatability. However, this last requirement renders some food simulants, recommended for packaging migration tests (EC, 1985), unsuitable for taint transfer tests. For example, the 3% acetic acid in water system may be inadequate.

Some test foods used for general purpose testing are given in Table 2.8.

Table 2.8 Test foods used for taint transfer testing (Kilcast, 1993).

Type	Food/simulant	Comments
Fat	Chocolate	Bland variety (e.g. milk) preferred.
	Unsalted butter	Mixed prior to sensory testing or outer surfaces only tested for severe taint.
Hydrophilic powder	Sugar	High surface area preferred (e.g. icing sugar). Test as 5% solution.
	Cornflower	Test as blancmange formulation (can achieve textural variation).
Combined	Biscuits	High-fat (e.g. shortbread).
	Milk	Full cream. For short-term exposure tests only, or rancidity problems can interfere.

2.1.3.3 Standard sensory testing methods

A number of different test procedures for taint transfer to foods from packaging materials has been published; these include BSI, 1964; OICC, 1964; DIN, 1983; ASTM, 1988. The British Standard and the American Standard covered packaging materials in general. The OICC standard, or 'Robinson test', concerned specifically the taint transfer to cocoa and chocolate products, although it is frequently used for testing other food products. The German DIN standard also concerned food packaging, but, in addition, provided information for the testing of other materials.

The British Standards Institution (BSI) published BS3755 in 1964. This document was entitled *Methods of Test for the Assessment of Odour from Packaging Materials used for Foodstuffs* (BSI, 1971). The test was designed to be used to evaluate whether, or not, packaging samples were odorous. As the odour characteristics of paper and board vary somewhat with moisture content and with the surrounding atmosphere, the standard was designed to accommodate the testing of the packaging material in the conditions of actual use. The moisture content could be modified by the addition of a quantity of water.

Consideration was made for the fact that the level of odorous substances that were acceptable in a particular packaged food depended, somewhat, on the period of storage and on the storage temperature. Therefore, the test conditions applied for a particular packaging system were allowed to be varied to be representative of that system.

The standard recommended the use of between 2 and 12 people in the sensory panel. The assessment room should be free from odours and any other distractions, and individual testing booths were recommended.

The test procedure for evaluating odour involves placing portions of packaging materials in jars, which are sealed and stored at room temperature for 24 hours. For tests carried out at higher humidities, a small quantity of water is also added to jars. Individual panellists are asked to open jars presented to them and make an assessment of any odours detected by placing them in one of three categories: 'A' - odourless; 'B' - slight odour; and 'C' - odour present at a level strong enough to render the sample unacceptable. Due to the fact that tainting can occur without odour, samples with results in categories A and B are then subject to a taint test. Samples which are placed in category C are usually rejected and are not subject to a taint test. This is due to the fact that a consumer would probably reject a food product if a strong odour was present, on opening the package, before they actually tasted it.

The basic taint test procedure laid out in the standard, involve placing the packaging material in a container, with the food suspended above the packaging, in the case of a secondary packaging system. For primary packaging systems, the test food is placed in direct contact with the packaging

material. A second container is also prepared with the packaging material and test food, with the addition of a small quantity of water. A third container is used for a reference sample, or control, in which a sample of the food under test is placed without the packaging material. The containers are usually stored for a period of 24 hours, at ambient temperature (~20°C).

The test foods which have been exposed to the packaging materials, under both wet and dry conditions, are removed from the containers and each divided in to the appropriate number of portions corresponding to the number of panellists. The reference food is also removed and split in to two lots of portions, each number of portions corresponding to the same number as with the test foods. Pairs are prepared from two sets of 12 portions of test and reference food for assessment by the panel. The pairs may consist of two 'exposed' portions, two 'reference' portions, or one of each. The panellists are then asked to decide whether they think pairs are the same or different, on sampling.

It is considered that exposure of the foodstuff to the packaging material will have made a difference for the purpose of interpretation of results. It is, therefore, assumed that a correct answer will have taken this difference into account. The number of correct answers is compared with the possibility of obtaining a result by chance. When a panel of 12 is used, a sample is considered to be tainted when there are 10-12 correct answers. With 6 correct answers, or less, the conclusion is 'no detectable taint'. If 7-9 correct answers are obtained, the difference produced is considered not statistically significant and the test should be repeated. Ideally, the same set of panellists should be used for both tests and the two sets of results combined with the following conclusions: greater than 17 correct answers, taint has occurred; 17 or less correct answers, no detectable taint.

The BSI standard was revised in 1971 with some minor amendments, and some users of the test procedure may now use the triangular test instead of the paired test.

The ASTM Standard E 462-84 (reapproved 1989) *Standard Test Method for Odor and Taste Transfer from Packaging Film* (Tice, 1993) uses large test samples ($0.9m^2$) with test conditions of at least 20 hours at room temperature. Odour testing is carried out from test jars containing the test sample directly, or

by evaluation of odours absorbed on to mineral oil, water or butter. Water, butter and chocolate are used for testing of any taints originating from the packaging material. Assessments can be made using any appropriate category scale. However, the standard recommends the use of scales with either four or seven levels.

The ASTM Standard E 619-84 (reapproved 1989) *Standard Practice for Evaluating Foreign Odors in Paper Packaging* (Tice, 1993) suggests the procedures for the evaluation of odours from paper packaging. The standard extends to paper packaging that has been printed and formed with adhesives, and to paper/plastic laminates.

The actual methods for odour assessment include direct examination without confinement of single sheets, sheet stacks and bundles, and examination with confinement at room temperature and at an elevated temperature of 52°C, either dry or at higher humidities. Procedures also exist for the evaluation of the transfer of odorous substances to mineral oils (as a simulant for fatty foods), tests foods and odour-sensitive commercial products. Taste tests using butter, cream and milk chocolate are considered for the assessment of taints resulting from the adsorption of odorous substances from paper packaging.

The most extensively used method for the evaluation of taint transfer from paper and board packaging, both printed and unprinted, is a version of the Robinson Test (Robinson, 1964). It has been considered for use as an EN (European Committee for Standardisation) standard.

The method is an accelerated storage test, which corresponds to the storage of food in its packaging, under ambient conditions, for a period of several months. There are several slight variations on the test procedure. The method described here is the Pira International version.

The packaging material to be tested is stored with a test food (chocolate), for 48 hours at room temperature (20±2°C) and nominal 75% relative humidity, in a closed stainless steel container. The control test food is stored in the same way, but without the packaging material.

Any off-flavour transferred to the test food from the packaging material under test is subjected to sensory evaluation using the triangular test. The evaluation

is made by a highly experienced panel of eight to twelve people of proven unimpaired sensory perception.

The sample supplied for testing should be representative of the batch/type of packaging material. This is particularly important when evaluating printed samples. All samples supplied for testing should come individually wrapped in aluminium foil or other suitable material to prevent the sample becoming contaminated during transit to the test laboratory, and to reduce to a minimum the loss of odorous compounds due to volatilisation. Both these factors could result in the sample becoming unrepresentative of the batch/type of packaging material.

It is vital that control samples and test samples are isolated from one another to prevent cross contamination. The time between receipt of samples and testing should be kept to a minimum.

A test organiser is responsible for preparing the test specimens, arranging the taste panel test, calculating the results and preparing the report. The test organiser must not be a member of the taste panel.

A relative humidity of 75% is achieved by placing a petri dish filled with a suitable saturated salt solution plus a little solid salt in the bottom of the stainless steel test vessel.

Assessment of secondary packaging is carried out by taking a sample of packaging material that is approximately the size of an A4 sheet. This is cut in strips of about 25mm in width, folded to form a concertina and placed around the petri dish with the saturated salt solution in the bottom of the test vessel.

A stainless steel gauze is placed over the top of the packaging material and petri dish, and 12 chocolate buttons/food portions (or other number corresponding to the number of panellists), laid on top. A second test vessel is prepared with the test food and saturated salt solution, but with 24 buttons/food portions instead of 12 buttons/food portions. These are used as the control samples.

Assessment of primary packaging is carried out by taking a sample of the packaging in the form of a disc that is approximately 180mm in diameter. If the packaging sample is printed, it is important to take a representative area of printed material. As before, with the testing of secondary packaging, a gauze

is placed over the petri dish containing saturated salt solution. This time, the packaging material is placed on top of the gauze with the food contact side upper most. The chocolate buttons/food portions are then laid evenly on the packaging material.

A vessel is set up for the control samples using twice the number of chocolate buttons/food portions, but without the packaging material, as before.

The chocolate buttons/food portions are subsequently evaluated, after storage, using the triangular test. Three coded food samples are presented to each panel member and the question asked:

Which of the three buttons has an off-flavour ?

Two of the chocolate buttons are control food and one is sample test food. A request is then made:

Please rank any off-flavour using the following scale

0 = no off-flavour, same as 'control test sample'
1 = off-flavour, just perceptible
2 = definite off-flavour
3 = strong off-flavour

Part points (+ or -, ½) can be used. Any off-flavour may also be described in words by the panel taster.

The statistical significance of the difference between the sample test food and the control food is read from the triangular test significance table (Table 2.9).

Considering the number of panellists and the number of correct answers, one can determine from the table, whether any flavour difference between the control food and the sample food is statistically highly significant (p = 0.1%), significant (p = 1%), almost significant (p = 5%), or non-significant (all correct answers less than that listed in the p = 5% column).

Table 2.9 Triangular Test Significance Table (Robinson, 1964).

Number of Answers	Minimum number of correct answers for a significance level of: (p value)		
	5%	1%	0.5%
8	6	7	8
9	6	7	8
10	7	8	9
11	7	8	10
12	8	9	10

If the test food was found to be tainted by the packaging material, then the result would be ranked, highly significant, significant, or almost significant. The test result indicates that the packaging sample has the (high, medium or low) tendency to taint foodstuffs represented by the test food used in the test.

Individual taint scores and, also, mean score values are only used when the test results show that the test food has a significant off-flavour. In addition, verbal description of the off-flavour should only be quoted when more than two panellists have recorded the same off-flavour description.

The German DIN 10 995 standard is applicable to container materials and containers for packaging products. The purpose of testing is to establish whether a container material or container possesses an inherent odour or contains substances which, under the specified test conditions, may be transmitted through the air space or through direct contact to a test substance and affect its taste. Either a test food product or an original food product may be used as the test substance.

The standard states that an unsuitable test arrangement may cause the test system to undergo changes resulting from its susceptibility to oxygen, light and heat, or from the action of micro-organisms for which the container can not to be blamed. The test conditions are, therefore, chosen to exclude such changes.

It should also be established whether, or not, the sensory objections associated with a packaging sample are due to secondary influences mentioned above, or from permeability or absorptivity of the containers for odorous substances which exclude application of this test standard.

The test food substance, in the case of taste testing, is the original food product intended as the goods to be packaged, or some other suitable test food product. In the case of odour testing, it is the air space above the packaging sample which is tested. The choice of test food is normally the original food product for which the container material or container is intended. However, some times other test foods that have properties suitable for the intended purpose of the test material are used. The standard suggests that the following may be used as test food products:

- for predominantly greasy food products, containing water: unsalted butter, without taint, in respect of taste and odour;
- for predominantly greasy food products, free of water: neutral cooking fat, neutral edible oil or grated milk chocolate;
- for dry food products: icing sugar, butter biscuits
- for watery and acid food products: tap water or mineral water low in carbon dioxide content, apple juice and 0.2% acetic acid, (content by mass).

When taking samples of packaging materials, the outer layers of a stack or roll should be discarded. When taking samples from stacked containers the outer units should be discarded, and when taking samples from continuous production the first 20 packages produced should be discarded. When taking samples from consumer packs, representative specimens should be taken from the interior of the stack or bulk package.

The test method itself involves taking packaging samples of 10dm^2, ensuring that, if printed, the ratio of area of printed area to non-printed area is the same for each sample to be tested, and representative of the finished package. If the test preparations make it necessary to reduce the size of the test pieces, the individual test pieces should not be made smaller than 1 dm^2.

In testing for odour transfer and for taste transfer through the air space, it is important that the test pieces are as accessable as possible to the surrounding air within the test vessel. In the case of multi-layered packaging samples with appreciable surface differences and separation of the layers by a virtually impermeable inner layer, it is essential, particularly when the samples are printed, that the test is performed on one side. This should be the side

intended to face the packaged food product. For this purpose, receptacles should be made from the test pieces, e.g. tetrahedrons or bags, in such a way that the inside constitutes the surface to be tested.

When testing for off-odour, test pieces of 10 dm^2 should be introduced loosely into a wide-necked, flat bottomed flask or domestic preserve jar. The test vessels should then be stored in the dark for 20±2 hours, 23°C.

When testing for taste transfer from the air space, (for secondary packaging materials), in the case of board materials, 4 dm^2 test pieces should be introduced loosely into the test vessel. A maximum of 30g of test food are then placed in the vessel on a petri dish. The vessel is then stored in the dark for 20±2 hours at 23°C, or at a temperature appropriate to the test substance. In some cases, a longer storage time may be employed. If original packages are available, or if receptacles have been shaped, the test substances can be placed in the empty receptacles, on petri dishes. Additional portions of test food are placed in test vessels, without packaging materials, and are used as control samples.

When testing for taste transfer through direct contact, (primary packaging materials), either one side or both sides of the test piece may be brought into contact with the test food. The ratio of test piece surface area to volume of test substance should correspond to practical conditions. If these are not known, the ratio should be 2-4 cm^2: 1cm^3. If both sides of the test piece are in contact with the test substance, the area of the test piece should be considered as doubled. For testing one side of the package only, flat bags may be used. The test substance is placed in these bags.

Pieces of the container material or container, measuring 1 dm^2 should be placed in petri dishes and covered with a layer of test substance to a depth of about 5 mm. For solid or semi-solid test substances the sandwich test should be applied. This involves placing approximately a 10 mm thick layer of the test food substance between test packaging pieces, each of which measures 1 dm^2, ensuring that the inner sides of the latter are in all-over contact with the food product. The test preparation may be covered on both sides with sheets of glass, wrapped in foil, placed on a petri dish, and stored as described above. Reference samples may be prepared in a similar way, but without the packaging material.

The number of samples presented to the individual panellist should match the level of difficulty but, as a rule, not more than 6 tests should be performed in succession. In odour tests, each assessor should be given a sample of his/her own. In taste tests, not less than 15 cm^3 of liquid test substance, or 5 grams of solid substance, per individual sample should be provided for each assessor.

When evaluating odour, the sample should be smelt immediately on opening the vessel. If repetition is needed, the vessel should be kept closed for a period of approximately 5 minutes. The test procedure should be a ranking test, a paired comparison test, or a triangular test. Each test should be performed versus the reference specimen. If no reference specimen is available, then the odour intensity should be assessed individually according to the following intensity scale:

0 = no perceptible off-odour
1 = off-odour just perceptible (but still difficult to define)
2 = slight off-odour
3 = distinct off-odour
4 = strong off-odour

Alternatively, odour intensities of the samples may be ranked in increasing intensity to suit the purpose of the test.

Normally odour intensities of below 2.5 are considered sufficient for the test. If values of 2.5 or above are found, then the packaging samples are tested for taste transfer.

Taste transfer assessment is carried out using the following intensity scale relative to a control sample:

0 = no perceptible off-taste
1 = off-taste just perceptible (but still difficult to define)
2 = slight off-taste
3 = distinct off-taste
4 = strong off-taste

However, little or no information is provided within these test methods for their appropriate use in testing for taint problems. It has been suggested that more

sensitive test procedures should be used based on ISO 6658 (Kilcast, 1993). Guidelines laid out in a code of practice (Goldenberg and Matheson, 1975), state the importance of testing by the packaging supplier before dispatch, and by the food manufacturer before use.

This code of practice relies on a close working relationship between retailer and suppliers, and their production managers and technologists. It has been reported that the resulting joint study and follow-up of significant 'off-flavour' customer complaints has often led to the identification of the cause of the complaint, with subsequent benefit to all concerned. In some cases, complaints associated with particular taints from food packaging have been completely eliminated. Table 2.10 summarises this code of practice with respect to food packaging.

Table 2.10 Code of practice with respect to food packaging materials (Goldenberg and Matheson, 1975).

Food quality packaging to avoid 'taints': Summary of recommendations

1 Packaging manufacturer to be informed when 'food quality' packaging is needed.
2 Printed material to be adequately aired before dispatch.
3 Where board and food both overwrapped: board to be white Scandinavian board; not chipboard, strawboard or 'manilla' board, whether lined or unlined.
4 Ink for printing: Care in selection of solvents. Toluene and xylene should not be used.
5 Recommended airing periods for different types of inks:

Oil-based	21 days
Moisture-set	5 days
Gravure	14 days (or sufficient to remove solvents).

6 Plastics: Specific danger points:

All plastics	Careful control of all additives
Poly(ethylene) and Poly(propylene)	Overheating
PVC	Organic sulphur derivatives not to be used as stabilisers
Poly(styrene)	% Monomer to be as low as possible: (a) for foods with water as continuous phase (b) for foods with fat as continuous phase.

7 Adhesives: To be free of offensive smells.
8 Lacquers: To be used as little as possible, and to be free of offensive smells.
9 Analysis:
(a) Testing by packaging manufacturer before dispatch
(b) Testing by food manufacturer before use
(c) 'Performance' tests by food manufacturer, if necessary.

Some points in this code of practice do not apply to ultra-violet radiation curable inks and varnishes used on packaging materials, particularly those points concerned with oil-based inks and lacquers, but the principles on which they are based do apply. The code relies on adequate communication and

understanding between the retailer, the manufacturer, and the packaging supplier.

2.1.3.4 Use of sensory taint testing in the quality assurance environment

The various testing methods described in previous sections are needed to provide high sensitivity screening for taint. However, in a quality assurance (QA) environment, the use of relatively large numbers of panellists is not always possible. In addition, a high workload normally rules out tests in which only one sample is compared to a control to keep a high degree of sensitivity. In such circumstances, it is often necessary to adopt short-cut screening procedures, as part of a more general QA system and to have in place an alerting system and a back-up system for examining suspect foods or materials.

The significance of having effective procedures for guarding against taint is reflected both in recommendations for good quality management in the food and packaging industries and in legislation.

2.1.3.5 Storage and shelf-life testing

It is inevitable that the sensory characteristics of some foods will change with time. These changes can be relatively rapid as, for example, in fresh food produce, or very slow as, for example, in canned foods. In general, changes in the sensory characteristics of foods on storage are undesirable, producing unpleasant changes in appearance, odour, flavour and texture. These changes are used to determine the shelf-life of a product. Shelf-life has been defined as 'the period between manufacture and retail purchase of a food product during which the food is of satisfactory quality' (IFT, 1974).

The three more common situations for a product reaching the end of its shelf-life at the point of purchase are (Labuza and Schmidl, 1988):

(i) The product is sufficiently changed from the initial product specification to make it entirely unacceptable to all consumers. It is basically inedible to 100% of the general population of consumers, and could be considered adulterated under section 402(a)(3) of the Food, Drug and Cosmetic Act.

(ii) The product is changed only to some degree. It is still acceptable to a certain percentage of consumers but not to most of them. Management must decide what percentage of consumers (P_5) they will allow to be dissatisfied. The lower the value of P_5, the lower the risk but the shorter the shelf-life.

(iii) The product is acceptable for the remaining distribution time because the rate of quality loss is so slow that all the product will be consumed before any significant changes occur to cause customer rejection.

An important point to consider when assessing shelf-life is that change in sensory quality, with time, can often be a result of the formation of off-flavours within the food. These off-flavours can be confused with taint from external sources. Sensory test methods designed to establish the nature of such changes must address some important issues:

(i) Sensory evaluation of the effects of storage on food products should be carried out using descriptive testing and the results correlated to simple hedonic information obtained from consumer testing.

(ii) The tests used should be relatively resistant to changes in the makeup of the panel that can occur over extended test periods. Profile testing suffers from the need to re-train replacement panellists. Difference tests, such as paired comparison tests, duo-trio tests, and triangle tests, are less demanding on panellists and may be the preferred option, in spite of their lower information content. Hedonic rating scales are of limited use in shelf-life testing, yet they are probably more demanded by some marketing groups, especially if they want to determine if the control is better than the competitor's product. Hedonics measure the degree of acceptability on some scale. The problem is that acceptability can go up or down due to changes which occur during storage and panellists respond differently to these factors, (e.g. rancidity, moisture loss, staling, crispiness, browning). Proper hedonic testing, as related to shelf-life testing, requires asking more specific questions (which sample is more crisp compared to the control?) but can produce more accurate information.

(iii) Storage changes may result in the development of off-flavours that were not present at the start of the storage trial. Difficulties can result in problems when using profiling methods, since panellists may not have experience in quantifying these new off-flavour attributes. Again, this may result in the use of difference testing which does not suffer from this problem.

(iv) It is important to have suitable material to represent the fresh food product under test, particularly when panellists are not highly trained. This can be achieved by using fresh product for each test for which deviation between food batches is negligible. Alternatively, one can use food samples, from the same batch, that have been stored under conditions for which change is insignificant compared to the samples under test.

(v) Some philosophical questions need to be asked when shelf life testing of foods using sensory analysis is carried out. One can ask is the information obtained from panel tests representative of consumers in the real world. The answer to this question is likely to be negative. In proper testing, in-house panels are pre-selected, trained and have acquired considerable experience. They are usually given an anchor point for comparison, which the consumer does not have. In addition, the panel judge is not influenced by factors such as product price, packaging, and brand name.

A second question that needs to be asked is based on whether the true end of the shelf-life of a product needs to be known. Many times this information is not needed and only an assurance that the product will still be acceptable for a set period in the distribution system is required. Long storage test periods often conflict with the need to introduce new product lines into retail outlets, with minimal delays. This problem is heightened by the requirement for 'sell by' or 'use by' labelling information on foods. A commonly used procedure to combat this problem is accelerated shelf-life testing (ASLT) (Kilcast, 1993). The product/packaging system is stored under some specified abuse system, usually at raised temperature and humidity. Difficulties may arise from these procedures when attempting to extrapolate, from changes under abuse conditions, back to storage under normal (ambient) conditions. This can be carried out using the Q_{10} approach (Kilcast, 1993), where Q_{10} is defined as the ratio of the reaction

rate constants at temperatures differing by 10°C. Equivalently, the time for an unwanted change to reach unacceptable levels, when the food is stored at a temperature higher than 10°C, may be used. More commonly, however, many companies have developed a rule-of-thumb approach based on extensive experience with a product range. For example, the snack food industry often relates storage for one week at 37°C, to storage for four to six weeks at an 'ambient' temperature, taken to be approximately 20-22°C.

Possibilities of errors arising when using this rule-of-thumb approach arise due to the lack of consideration of variations within the physical characteristics of the test system at elevated temperatures. These include changes to the ratio of solid/liquid fat content and the resulting shifts in solvent and migration characteristics.

2.1.3.6 Ethics surrounding sensory testing for taint

It is important to consider the potential hazards exposed to human subjects during sensory evaluation testing. Procedures need to be in place to protect panellists from consuming unsafe foods. Consuming, or testing food, that may be contaminated with tainting species carries a specific toxic risk, and additional procedures may be required to protect panellists against such risks. Suitable procedures will depend heavily on company safety policies, but a number of more general requirements exist:

(i) It may be necessary to develop a risk classification system for the food types and ingredients to be presented to panellists. A simple three category system may classify materials as: (a) standard foods/ingredients/processes; (b) non-standard foods/ingredients/processes for which evidence of safety is available; and (c) non-standard foods/ingredients/processes for which there is little or no safety information. When testing for an unknown taint, no safety information is available, and tests should be carried out at the highest level of risk. However, if evidence suggests that contamination is at a low level, then a lower risk contamination can be given.

(ii) It may be necessary to have in place an appropriate advisory body with expertise in the specific areas of interest and also with medical knowledge.

(iii) It may be necessary to develop information and consent procedures designed to give panellists as much knowledge as possible regarding the risks associated with the tests (whilst not introducing unacceptable bias), and to request their written consent.

2.1.4 HUMAN PERCEPTION OF TASTE AND SMELL

The human senses of odour and taste perception are less well adapted than the senses of sight and hearing. In modern society, humans are continuously exposed to optical and acoustical stimuli. In contrast to this frequent use of our physical senses, the chemical senses may only be called into use periodically at special fixed times, for example, at meal times. The senses of smell and taste are known as chemical senses. The term 'chemical senses' means that the primary sensory process, initiating a taste or smell sensation, should be a chemical binding of two substances, with taste or smell properties, to the surfaces of the membranes of the respective receptor cells. Like the senses of audition and vision which can act over long distances, smell makes use of molecular odorants, which may approach the subject carried by the wind over quite long distances. In contrast, taste is a close range or contact sense, operating only when direct contact is achieved with the source of the taste.

The efficiency of the human senses of smell and test are characterised not only by high sensitivity, but also, by a very good ability to discriminate between many different stimuli. Normal adults can distinguish between nearly 2000 odour impressions. However, this may not be so apparent due to their low claim to recognition and to their definition of known odour effects. On training, this recognition and defining process can be greatly improved, as discussed earlier, and a trained sensory panellist may have the ability to discriminate between up to 10 000 odour impressions (Rothe, 1988).

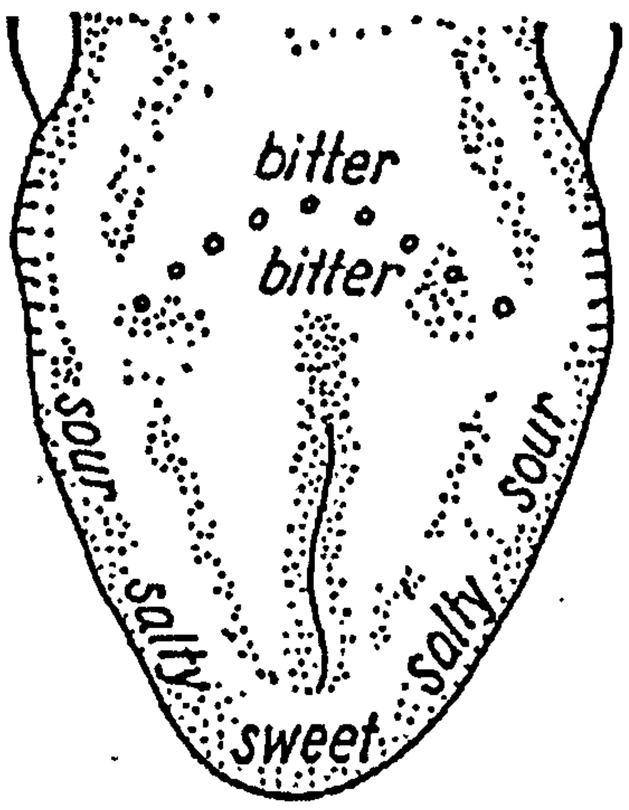
Flavour can be defined as the complex combination of olfactory, gustatory, and trigeminal sensations perceived during tasting. This definition recognises the interaction of the sense of smell, perceived through the nose; the sense of taste, perceived on the tongue; and the pain response that gives irritating effects and sensations such as hot and cold. Frequently there is some difficulty

in differentiating between odour and taste as two separate physiological senses. Of these two senses, odour perception is much more complicated. In contrast to the ability of adults to discriminate between approximately 2000 different odour impressions, there are only four basic taste qualities.

The main physiological function of the mouth is to prepare food for chemical digestion. A number of things happen during this process that are important for chemosensory analysis. Firstly, food is chewed into smaller pieces and moved around the mouth in a process called mastication. During this process, chewing movements cause tastants and pungent compounds in the salvia to stimulate the gustatory and trigeminal receptors throughout the mouth.

It is widely accepted that all of gustation is initiated by adsorption and chemical binding of the tastant molecules to receptors on the surface of the tongue, possessing quality-specific cellular acceptor molecules. These receptors register the four basic taste qualities sweet, sour, salty and bitter. The perceptions of each of these taste qualities are located in special areas of this sense organ, as shown in Figure 2.2.

Figure 2.2 Sensitivity of tongue areas for the basic tastes (Rothe, 1988).



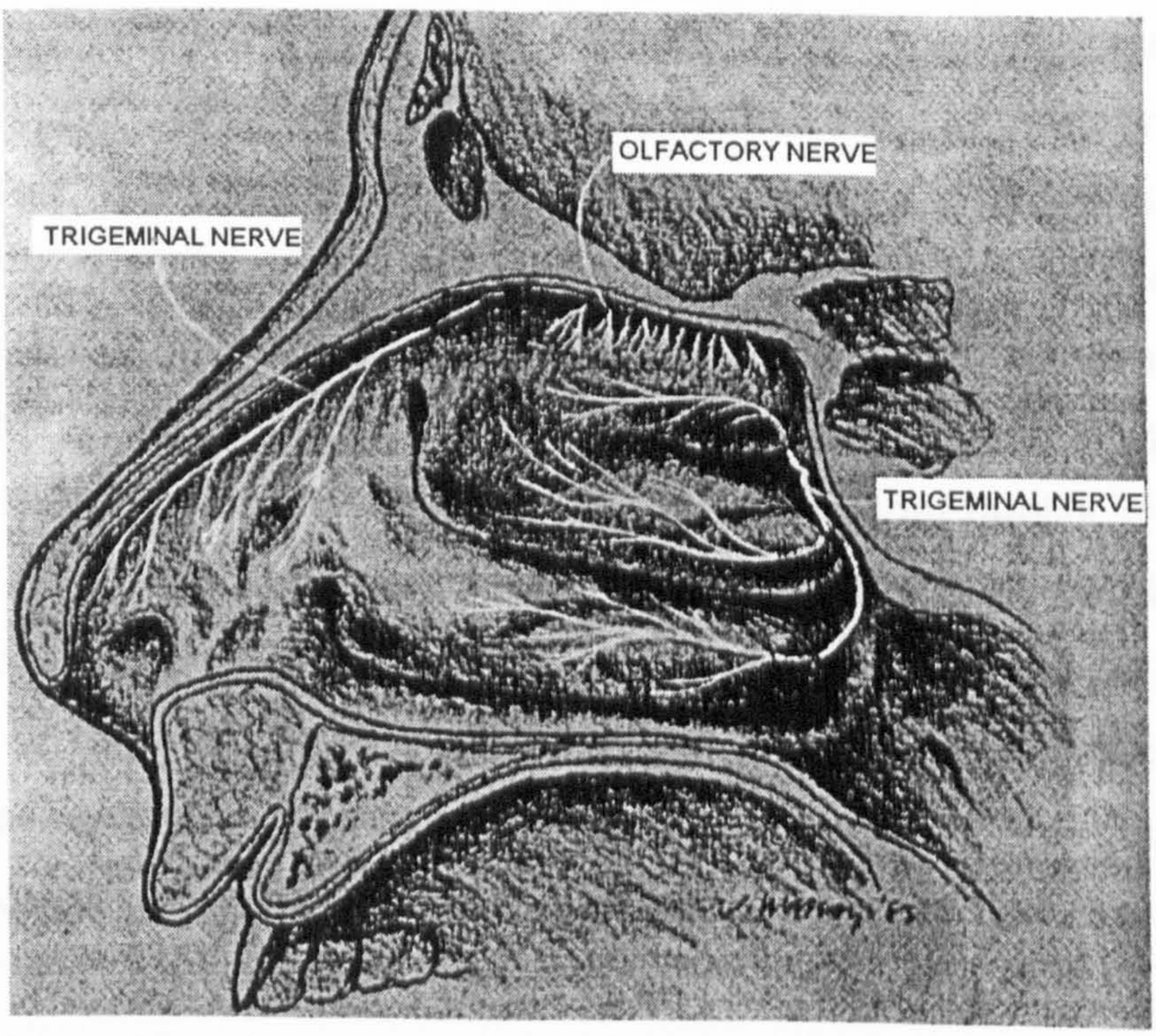
The receptors on the surface of the tongue consist of papillae. The tip and edges of the tongue possess mushroom-shaped papillae which bear 8-10 taste buds. The base of the tongue possesses circumvallate papillae which bear 100-300 taste buds. Each taste bud lies beneath a pore into which microvilli

extend. Interaction between taste stimulus and the receptors is thought to occur at the microvilli (Rothe, 1988).

During mastication, food volatiles pass to the back of the mouth and into the respiratory airstreams of the nasopharynx. During expirations, these volatiles are carried up into the nose and stimulate the olfactory receptor cells and nasal trigeminal receptors via the so-called 'retronasal' route. The sense of smell in humans results from the stimulation of chemoreceptors of the olfactory and trigeminal nerves. Olfaction, however, contributes by far the greater component of this sensation.

Olfaction, or odour perception, takes place in the upper part of the nasal cavity, at the regio-olfactoria, (olfactory sensory epithelium), which contains some 10 million receptors. Figure 2.3 shows the location of the olfactory and trigeminal nerves in the nasal cavity of man.

Figure 2.3 Location of the olfactory and trigeminal nerves in the nasal cavity of man (Maruniak and Mackay-Sim, 1984).



The trigeminal nerves respond to many different odorants, (Maruniak and Mackay-Sim, 1984) and are involved in a host of physiological reflexes which can affect the olfactory system. The trigeminal system is involved in pain

perception and in the perception of noxious stimuli within the nasal cavity and may also be involved in the discrimination of odorants.

Responses of both oral and nasal chemoreceptors contribute to a food's flavour. The olfactory receptor neurons are stimulated by odours travelling via the retronasal route. Whenever an odour stimulus in the mouth reaches the olfactory receptor neurons, via the retronasal route, it is perceived as an oral and not a nasal sensation, i.e. taste not smell.

Whether an odour reaches the olfactory receptor neurons via the nose or the retronasal route, it appears the odour sensations are brought about by the same basic mechanisms. However, there would appear to be some differences. Certain foods such as Limburger cheese have a pleasant flavour, but do not smell good (Maruniak and Mackay-Sim, 1984).

2.1.4.1 The Olfactory Code

The structure of any molecular species represents, under specific physical conditions, the only source of information carried by molecules (Maruniak and Mackay-Sim, 1984). However, the relationship between molecular structure and odour quality, known as the olfactory code, is far from clear. There are many theories relating odour quality to various molecular properties. The most popular of these theories is the 'stereochemical theory of odour quality' (Maruniak and Mackay-Sim, 1984). It suggests that the three-dimensional shape of the molecule, or part of a molecule, is the most important molecular parameter that the olfactory system uses to assign odour quality.

A strong correlation has been made between the psychophysical assessment of odour quality for a number of odours and the size and shape of the odour molecule. It has also been suggested that only part of a molecule may be responsible for the quality of an odour. It is known that molecules with very different overall structures, but similar partial structures can have similar odour qualities.

An 'earthy' perception has been linked to a decalin molecule due to the presence of a 5- or 6-membered carbon ring, with α-methyl or methylene groups on both sides of a hydroxy group, within the molecule structure, (Maruniak and Mackay-Sim, 1984).

It is thought that the odour quality of smaller molecules depends mainly on the nature of the functional groups present. The electronic properties of the functional groups and the presence of hydrogen bonding are critical factors in the interaction of olfactory receptors with these small molecules.

For large molecules, it is thought that both stereochemistry and functional groups are involved in determining odour quality. For a group of molecules with a flowery odour, with up to 250 atomic mass units (a.m.u.), possessing hydroxyl and carbonyl functional groups in the hydrophobic region, it has been found that, if the two functional groups were separated by less than 300 pm, the molecules had an odour. If this separation was more than 300 pm, then the molecules were odourless. It was thought that the functional groups interacted with a complementary system in the receptor site through intramolecular hydrogen bonding, (Maruniak and Mackay-Sim, 1984).

Further evidence for the importance of functional groups, with respect to odour quality, comes from work in which a panel of 73 organic chemists were reasonably successful in identifying the functional groups of 36 unknown and unfamiliar odorants, (Maruniak and Mackay-Sim, 1984). Their correct identification of functional groups ranged from 86% to 50% for odours containing amines, sulphur, esters, phenols, and carboxylic acids, respectively. Aldehydic and ketonic groups were recognised on 42% of the occasions; hydrocarbons, 36%; alcohols, 25%; ethers 20%; and halides, 16%. In a follow-up study, when the best panel subjects were tested for their ability to identify odorants that contained sterically hindered functional groups, success was significantly lower than that seen with the unhindered groups.

From work carried out to determine the odour and taste threshold values of some aliphatic aldehydes dissolved in paraffin oil (Meijboom, 1963), no distinct relationship could be defined between threshold value and the chain length of the saturated aldehydes, from propanal up to dodecanal. It would also appear that the boiling point of the saturated aldehyde, which increases with chain length, gave no indication of the threshold value. In light of these findings, it was thought that the number of molecules required to stimulate the olfactory receptors decreased with molecular weight, at least in the range of C_9-C_{12}. In

all cases, the threshold values for taste were found to be lower than those for odour.

In the series of 2 *trans*-alkenals, from C_5-C_{10}, it was found that the presence of a *trans* double bond in the α-ß position results in a rise in threshold value, compared to the saturated aldehydes with the same number of carbon atoms. The exception is the C_9- homologue. The taste threshold values were reported to increase with chain length of these alkenals. This trend was found to alternate. The aldehydes in this series with an uneven number of carbon atoms appeared to have lower threshold values than the corresponding aldehydes with even numbers of carbon atoms.

In the series of 2 *trans*, 4 *trans*-alkadienals, it was found that lower threshold values existed for the aldehydes with an even number of carbon atoms than for those with an uneven number of carbon atoms, in contrast to the previous series. This trend was also seen for the homologous series of alkadienals with the *trans*-double bond in the α-ß position and a *cis*-double bond between carbon atoms 3 and 4 (terminal position).

It was also apparent that in the case of the 2 *trans*-alkenals, introduction of a *trans*-double bond in the C_4 position (conjugated diene system) decreases the threshold value with respect to the alkenal. This may result from the presence of a second double bond in the terminal 3-4 position.

It can also be concluded from this work that one aldehyde can have an antagonistic effect on another. This is seen when 3 *cis*-hexenal is mixed with decadienal. 3 *cis*-hexenal was only weakly observed, at a concentration of 13 ppm, in the presence of decadienal, at a concentration of 21 ppm, even though 3 *cis*-hexenal was found to have a odour threshold value of 0.1 ppm. The same effect was seen when decadienal was replaced with 2 *trans*-nonenal, to the extent where the 2 *trans*-nonenal was present below its threshold value.

2.1.5 ELECTRONIC NOSES

A number of artificial smelling devices have been developed that are designed to discriminate between different odours. The advantages of a non-human system are:

- The electronic nose is available twenty four hours a day.
- Odours can be compared to those existing in tests carried out in previous months, whereas a human panel may drift over time.
- The electronic nose does not suffer from colds, the effects of eating spicy foods or anything else which may effect the performance of the human nose.
- Results are repeatable, reliable, and non-subjective.

One such electronic nose, developed by Aromascan (Aromascan, 1997), employs a conducting polymer sensor. The headspace vapours associated with the particular sample under analysis are passed across an array of 32 conducting polymers. These polymers rapidly adsorb and desorb volatiles at their surfaces. This results in a temporary change in the electrical resistance which is measured. The combined responses from each sensor are used to generate a pattern which is characteristic of that headspace. These patterns can be represented visually on a 2D map or a 3D map, or presented to an Artificial Neural Network for characterisation. An unknown odour can be evaluated by reference to a database of previously encountered patterns, the degree of correlation being displayed as a percentage confidence level.

The polymer sensors are based on the polymers of pyrrole, aniline, and thiophene to which different functional groups have been added. The sensor array of 32 different polymers detects a spectrum of compounds in a similar manner to that of the 30 receptor families, within the olfactory epithelium of the human nose.

If two, or more, aromas are to be compared, then Aroma Mapping can be used. These are based on the calculation of the Euclidean Distance (E.D.) between two aroma patterns. E.D. is a statistical measurement of the difference between two patterns and can be calculated using the sums of the squares of the differences between each sensor in the normalised aroma patterns, i.e.:

$$ED = (\Sigma^{32}_{1} (x-x^{1})^{2})^{1/2}$$

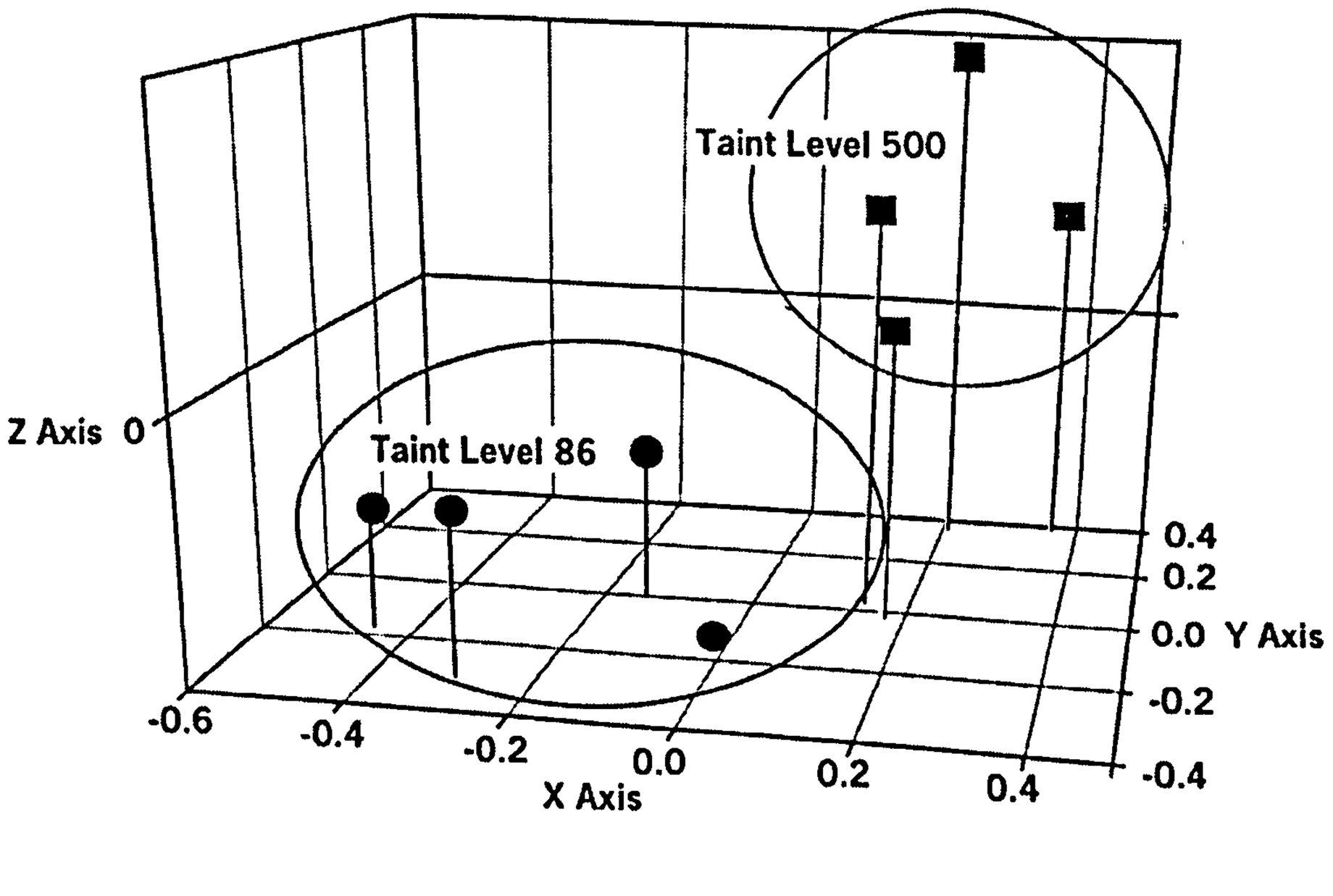
x = response of sensor element n to sample A

x^1= response of sensor element n to sample B

The technique used to represent these E.D.'s is a non-linear mapping technique, referred to as Sammon mapping. Sammon maps can give a representation of how similar or dissimilar a set of aroma patterns are. The closer together the points are on the map, the more alike are the compared smells.

This aroma mapping technique is illustrated on the 3D map below shown in Figure 2.4, representing samples of carton-board with a low level of taint and a high level of taint, respectively.

Figure 2.4 Carton-board taint at 86 mg/m^3 compared with 500 mg/m^3 evaluated using AromaScan system (AromaScan, 1997).



For these samples, a clear difference in distribution on the map exists, depending on the degree of taint.

A second type of electronic nose, developed by Neotronics, also uses conducting polymer sensors or Metal Oxide Sensors (M.O.S.). The system

uses a 12 sensor array and analyses the headspace vapours associated with the sample, as with the AromaScan system.

Metal oxide gas sensors are based upon ceramic elements such as tin oxide or zinc oxide. A metal oxide sensing element is placed between two electrodes, and the sensing element is maintained at elevated temperature (~350°C). Electrical current is passed between the electrodes, through the metal oxide. Interactions between gaseous species and the metal oxide sensor surface give rise to a change in resistance, measured across the sensor.

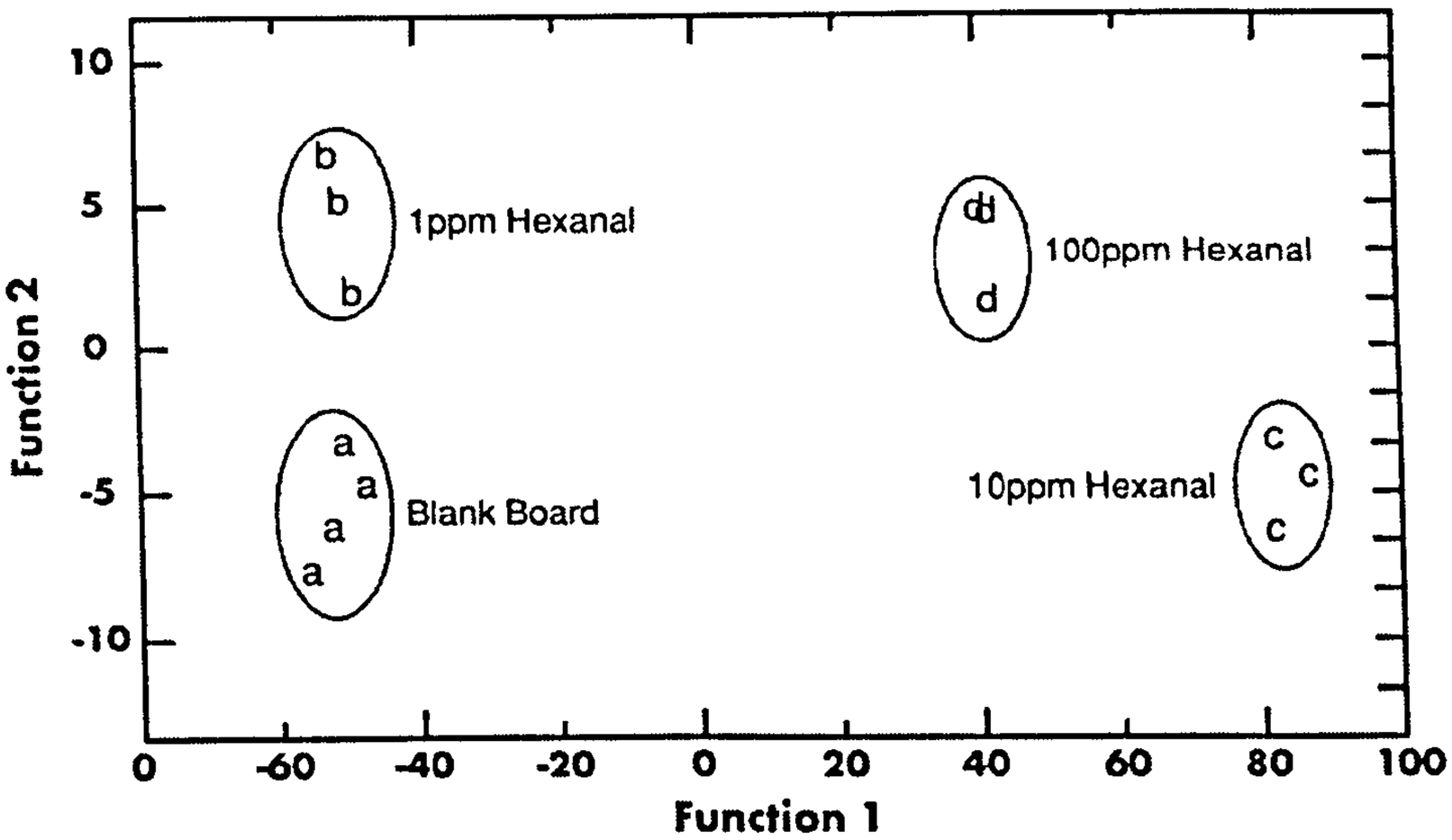
The system can use a number of statistical methods, as can the AromaScan system, to translate data into information presentable to the human analyst. One such method is Multivariate Data Analysis (MDA), which can transform multidimensional, (multi-sensor data), into just 2 or 3 dimensions. The routines available can be divided into 2 types: untrained (unsupervised) and trained (supervised) techniques.

Untrained techniques are often used for exploratory data analysis to investigate hidden relationships between samples. An example of this technique is that of Principle Component Analysis (PCA). The purpose is to develop a number of functions, (principle components), each of which is uncorrelated and derived from the sensor responses. Two or three of these responses may then be plotted against one another, and the relationships between samples in the data set observed graphically.

Trained techniques require training data, and should be used when trying to correlate instrument response to perceived odours. An example of this technique is Multiple Discriminant Analysis (MDA). The purpose is to develop a number of functions that emphasise differences between various sample classes that correlate to odour, and de-emphasise differences between samples due to system or method related parameters.

Figure 2.5 shows how the Neotronics system differentiated between samples of carton-board that had been dosed with different concentrations of hexanal, a compound associated with carton-board taint. The chart represents Multiple Discriminate Analysis translated into two dimensional functions.

Figure 2.5 Classification of hexanal levels in carton-board using the Neotronics system (Neotronics, 1997).



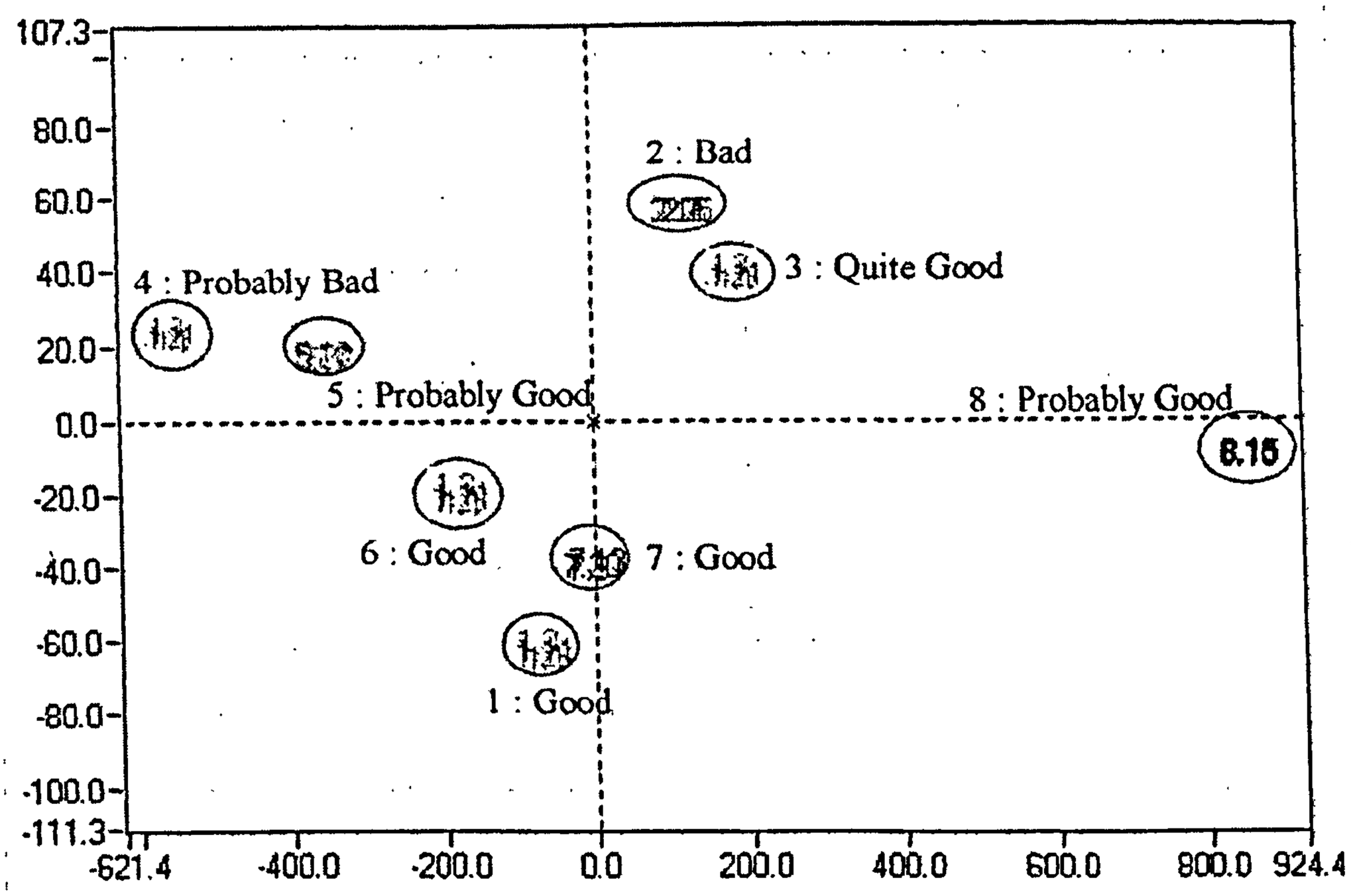
Another type of electronic nose is the Alpha M.O.S. system (Alpha M.O.S., 1996), which has a basic detection system made up of 6 small semi-conducting odour sensors and can be upgraded with several more arrays of 6 elements (for a total of 6,12 or 18 sensors). These can include M.O.S., conducting polymers, or Surface Acoustic Wave (S.A.W.) arrays.

The M.O.S. have different partial selectivities and respond to a wide range of odours. M.O.S's can be selected from a range of more than 40 different types (SnO_2, ZnO, WO_3...) to suit the application.

Figure 2.6 shows the plot using Factorial Discriminant Analysis (F.D.A.), representing 8 samples of unprinted and printed carton-board, analysed using an Alpha M.O.S. Fox 4000 system with 18 M.O.S. sensors. F.D.A. allows the plotting, on two axes, of the information given by the 18 sensors. The majority of information content is represented on the X axis and only a small proportion on the Y axis.

The F.D.A. plot allows the definition of a territory for each sample. 'Good samples' 1,6 and 7 are displayed in the bottom of the chart. 'Probably good' samples 5 and 8, and 'quite good' sample 3 are displayed above the 'good sample' group. 'Bad samples' 2 and 4 are displayed outside the other two groups.

Figure 2.6 A two dimensional plot, using Factorial Discriminant Analysis, representing samples of carton-board analysed using an Alpha M.O.S. Fox 4000 system (Alpha M.O.S).



2.2 EXPERIMENTAL

2.2.1 TAINT AND ODOUR TESTS - SERIES I

Initially a taint/odour test that was based on the 'Stora - Newton Kyme Ltd. Laboratory Test Method 22 *Evaluation of the Presence of Odour and Taint in Cartonboard and Pulp Samples*' was developed. This is a non-standard derived accelerated test for evaluating the presence of odour and/or taint in food contact carton-board. The reason for choosing and developing this method was that a short packaging sample/test substrate exposure time was employed, allowing a high turnover of tests in a relatively short period of time. This accelerated the development of the test procedure and the panel training. This developed method, 'Method 1 - taint test', was an accelerated test carried out over four hours at 40°C. The test substrate was water. The nature of

information sought from this test was purely descriptive and did not rely on panellists' liking or disliking of a perceived taint. Data obtained were used for the purpose of sensory characterisation of the packaging system and not for determining whether or not a sample was tainted or untainted.

2.2.1.1 Method 1 - Test procedure for taint (taste) test

740 cm^2 of carton-board to be tested was cut into small pieces. These pieces were then placed in a 3 dm^3 Kilner jar and sprayed with 2 cm^3 of high purity water (HPLC grade). A 25 cm^3 Pyrex beaker, containing 20 cm^3 of high purity water (HPLC grade), was then placed in the jar. The jar was then sealed and placed in an oven at 40°C, for 4 hours. In addition to the test jars containing the test packaging material, a jar was prepared at the same humidity containing a beaker of water, but without the packaging material, and stored in a similar way. The water sample in this jar was used as the control or reference sample.

Organoleptic evaluation of samples took place in a room, at 20°C with average humidity (40-60%), that was free from external odours such as solvents or chemicals, and other distractions such as noise and visual phenomena.

A panel of 10 assessors were then offered small samples of the water from the test sample jars, each sample being presented on a plastic tea spoon. Panel members were asked to evaluate each sample of water relative to the control sample, using a ranking system. Panel members were allowed to re-taste the control sample between test samples. However, no additional water was supplied for panellists to cleanse their palate, between samples.

Panellists were asked to rank samples, relative to the control, based on the following scale:

1 = Very bad taint

2 = Considerable taint

3 = Distinct taint

4 = Acceptable taint

5 = Perfect (control and sample have identical taste)

In addition to panellists being asked to score test samples on a ranking system, they were also asked to describe their perception of any taint in terms of simple descriptive words.

Originally, no list of sensory descriptors was supplied and panellists were free to use any descriptive terms they thought appropriate. When sufficient data had been collected from subsequent tests, consensus profiling, based on a procedure termed quantitative descriptive analysis (QDA), was used. This generated a list of sensory descriptors/attributes, that had been used frequently in previous tests, from which panellists were asked to make a choice when trying to verbalise their perception of any taint. The list of descriptors (Set A) used is shown in Table 2.11.

Table 2.11 Suggestions for possible taint/odour descriptors to be used in laboratory sensory taint and odour testing (Set A).

Gluey	Sour milk	Tomato planty	Spicy
Plasticy	Metallic	Apricoty	Stuffy
Cardboardy	Sour	Drainy	Rancid
Painty	Stale	Burnt rubber	Medicinal
Oily	Inky/varnishy	Herby	Grape-like
Soapy	Rusty	Pungent	Hay
Papery	Starchy	Sharp	Fishy
Bitter	Woody	Solventy	Fruity
Sweet	Fatty	Petroly	Grassy
Rubbery	Sulphury	Vegetably	Catty

2.2.1.2 Method 2 - Test procedure for taint (taste) test

A second taint test was developed based on the 'Stora - Newton Kyme Ltd. Laboratory Test Method 22 *Evaluation of the Presence of Odour and Taint in Cartonboard and Pulp Samples*'. This developed method, denoted 'Method 2 - taint test', was a three day accelerated test carried out at room temperature (21±2°C), the test substrate being water. This method was adopted so that data collected could be compared to those obtained from Method 1 to

determine the effects of increased temperature and humidity on the migration of tainting compounds. In addition, the effects of a considerably shorter exposure time of packaging to the test substrate were assessed.

The carton-board was assembled as described for Method 1. A 25 cm^3 Pyrex beaker containing 20 cm^3 of high purity water (HPLC grade) was then placed in the jar. The jar was then sealed and stored at room temperature for three days, in the dark. A blank was prepared as described in Method 1 and also stored under the same conditions for three days.

Organoleptic testing of the samples took place under the same conditions as laid down in Method 1 using a panel of 10 assessors. Panel members were asked to rank test samples relative to the control using the ranking scale described on page 63. In addition, panellists were asked to supply simple descriptions of any perceived taints. Originally, a free choice of descriptor terms was allowed. However, when sufficient data were available, panellists used the list of descriptors (Set A) illustrated in Table 2.11, as with Method 1.

2.2.1.3 Method 3 - Test procedure for taint (taste) test

A third taint test was developed based on the 'Stora - Newton Kyme Ltd. Laboratory Test Method 22 *Evaluation of the Presence of Odour and Taint in Cartonboard and Pulp Samples*'. This developed method, denoted 'Method 3 - taint test', was a three day accelerated test for the testing of primary packaging systems, carried out at room temperature (21±2°C), the test substrate being milk chocolate. Milk chocolate was used to determine the effects of a more lipophilic test substrate with respect to the selective migration of compounds from the packaging material.

740 cm^2 of carton-board to be tested was cut into small pieces. These pieces were then placed in a 3 dm^3 Kilner jar, with the food contact side facing upper most. If the carton-board was printed then samples of board were used with similar ink colour types and ink coverage for fair comparison. 10 regular size chunks of 'Cadbury's Dairy Milk Chocolate' were then evenly distributed within the jar, in contact with the carton-board sample. The jar was then sealed and

stored at room temperature for three days, in the dark. In addition to the test jars containing the test packaging material, a jar was prepared containing just 10 regular size 'Cadbury's Dairy Milk Chocolate' chunks, without the packaging material, and stored in a similar way. The chocolate chunks in this jar were used as the control, or reference samples.

Organoleptic assessment of the chocolate samples was carried out under the same conditions as were applied to the two previous tests. The chocolate samples from each test jar were placed in aluminium foil dishes along with the control samples. Individual panellists were asked to consume one chunk of chocolate from each test jar and evaluate it relative to the control sample. Water was supplied for panel members to cleanse their palates between test samples and reduce the effects of flavour carry-over. Panellists were allowed to take nibbles from the control sample and refer back to it between test samples, in order to keep a clear perception of the reference point from which judgements were made. Evaluation was carried out using the ranking scale described above. Panel members were also asked to give descriptions of any taints perceived in terms of simple sensory descriptors, as in Methods 1 and 2.

2.2.1.4 Method 4 - Test procedure for odour test

An odour test was developed based on the 'Stora - Newton Kyme Ltd. Laboratory Test Method 22 *Evaluation of the Presence of Odour and Taint in Cartonboard and Pulp Samples*'. This developed method of odour assessment, known as 'Method 4 -odour test', was a 4 hours accelerated test carried out at 40°C.

The test samples were prepared as in Method 1 - taint test, with the exception that no additional water was placed in the jars.

Organoleptic assessment was carried out under the conditions described previously. A panel of 10 assessors were presented individually with the jars containing the test packaging material and asked to open the jars briefly and smell the air within, promptly replacing the lids afterwards. They were asked to evaluate the general smell perceived relative to the smell perceived from the control jar using a ranking scale. Panellists were allowed to smell the control

sample and test samples only once, since repeated opening of the vessels would result in changes of odour profiles through compounds being lost due to volatilisation.

Panellists were asked to rank samples, relative to the control based on the following scale:

1 = Very bad odour
2 = Considerable odour
3 = Distinct odour
4 = Acceptable odour
5 = Perfect (control and sample have identical odour)

In addition, panellists were asked to describe any odour perceived in sample jars using simple descriptive terms. No list of sensory descriptors was supplied originally, and panel members were free to use any terms they thought appropriate. However, when sufficient data were collected, the list of descriptors (Set A) described in Method 1 - taint Test was generated, and used for panellists to choose which term most fitted the odour they sensed.

2.2.1.5 Method 5 - Test procedure for odour test

A second odour test was developed based on the 'Stora - Newton Kyme Ltd. Laboratory Test Method 22 *Evaluation of the Presence of Odour and Taint in Cartonboard and Pulp Samples*'. This developed method, known as 'Method 5 - odour test', was a three day accelerated test carried out at room temperature (21±2°C).

740 cm^2 of carton-board to be tested was cut into small pieces. These pieces were then placed in a 3 dm^3 Kilner jar. The jar was then sealed and stored at room temperature for three days, in the dark. In addition to the test jars containing the test packaging material, an empty jar was stored in a similar way. This jar was used as the control or reference.

Organoleptic assessment was carried out using a panel of 10 assessors and the ranking scale described above, relative to the control. Panellists were again

asked to describe any odour perceptions in terms of simple sensory descriptors.

2.2.2 TAINT AND ODOUR TESTS - SERIES II

A second series of tests was designed, these tests adopting a more hedonic nature than that possessed by the previous tests described. Panellists were supplied with 2-4 sensory descriptors/attributes for a particular sample under test. For each individual attribute, the panel members were asked whether, or not, they agreed that the attribute applied to the sample in question, using an agreement scale from 1-4. Unlike in earlier tests, no control sample was used. Panellists were asked to judge samples on face value, and not relative to a control sample. As with the first series of tests, panellists' liking or disliking for any taint perceived was not questioned.

2.2.2.1 Method 6 - Test procedure for taint test

The test preparation was, again, derived from Stora - Newton Kyme Laboratory Test Method 22. However, the sample assessment procedure and information sought from the test were different.

25 grams of carton-board sample were cut into small pieces. These pieces were then placed in a 1.5 dm^3 glass jar with a 25 cm^3 Pyrex beaker, containing 20 cm^3 of high purity water (HPLC grade). 2 cm^3 of high purity water (HPLC grade) was then sprayed in to the jar. The jar was then sealed with a ground glass stopper and placed in an oven at 40°C, for 4 hours.

A panel of 10 assessors were then asked to taste samples of the water that had been exposed to the packaging under test and compare their perceived tastes with the taint descriptors/attributes supplied. The tests were carried out under the conditions described previously.

Panellists were asked to give their level of agreement with the given sensory descriptor/attribute using the following scale:

1 - Strongly disagree

2 - Disagree

3 - Agree

4 - Strongly agree

The scale gives no opportunity for panel members to give an 'undecided' level of agreement. Panellists have to either agree or disagree that a particular attribute is present. It was hoped that this would assist in interpretation of results by removing an element of uncertainty.

2.2.2.2 Method 7 - Test procedure for taint test

The preparation for the test were based on Method 3 - taint test for the evaluation of primary packaging systems. 25 grams of carton-board were cut into small pieces. These pieces were then placed in a 1.5 dm^3 glass jar with the surface intended for food contact upper most. 10 'Cadbury's Chocolate Buttons' were placed in the jar in direct contact with the test packaging. The jar was sealed with a ground glass stopper and stored for 3 days at room temperature, in darkness.

Chocolate buttons from each test jar were placed in aluminium foil dishes and the 10 assessors were asked to consume one chocolate button from each sample jar, compare their perceptions with the taint descriptors/attributes supplied and score their agreement using the scale described in Section 2.2.2.1.

2.2.3 TAINT AND ODOUR TESTS - SERIES III

A third series of tests as designed with sample preparation based on the German Standard DIN 10 995 *Testing of Container Materials and Containers for Food Products*. These tests were hedonic in nature as were Method 6 and Method 7. In these tests the emphasis was placed on the effect of the structure of the packaging system with respect to taint and odour, i.e. was the packaging

material used as secondary packaging, or primary packaging with the unprinted and/or printed surface in contact with the food product.

During the tests, panellists were supplied with a sensory descriptor/attribute for each particular test sample. Panel members were then asked whether they greed that the attribute applied to the sample in question, using the agreement scale described in Section 2.2.2.1.

2.2.3.1 Method 8 - Test procedure for taint evaluation with respect to secondary packaging

25 grams of carton-board were cut in pieces and placed in a 1.5 dm^3 glass jar. A petri dish was then placed inside the jar on top of the packaging sample and 10 'Cadbury's Chocolate Buttons' evenly placed within the dish. The jar was then sealed with a ground glass stopper and stored at room temperature for 3 days, in darkness. No control samples were prepared as with Methods 6 and 7.

Assessment of samples was carried out under the conditions described previously using 10 assessors. Chocolate button samples were presented to panellists in aluminium foil dishes.

2.2.3.2 Method 9 - Test procedure for taint evaluation with respect to primary packaging - printed surface in contact with food product.

25 grams of carton-board were cut in pieces and half placed at the bottom of a 1.5 dm^3 glass jar with the printed surface upper most. 10 'Cadbury's Chocolate Buttons' were then evenly placed on top of packaging material within the jar. The other half of the packaging material was then placed on top the chocolate buttons with the printed surfaces facing downwards to create a sandwich. For each test jar, the printed area of the carton in contact with the test substrate was the same, with respect to the different ink colours and ink coverage. The jar was then sealed with a ground glass stopper and stored at room temperature for 3 days, in darkness. Again, no control samples were prepared. Assessment was carried out as in Method 8.

2.2.3.3 Method 10 - Test procedure for taint evaluation with respect to primary packaging - unprinted surface in contact with food product.

25 grams of carton-board were cut in pieces and half placed at the bottom of a 1.5 dm^3 glass jar with the unprinted surface upper most. 10 'Cadbury's Chocolate Buttons' were then evenly placed on top of packaging material within the jar. The other half of the packaging material was then placed on top the chocolate buttons with the unprinted surfaces facing downwards to create a sandwich. The jar was then sealed with a ground glass stopper and stored at room temperature for 3 days, in darkness. Assessment was carried out without the use of control samples, as in Method 8.

2.2.4 TAINT AND ODOUR TESTS - SERIES IV

Up to now, only the migration of odorous compounds from the packaging to the food product has been considered. However, the phenomenon of reverse-migration can occur, i.e. the migration of flavour volatiles from the food product to the packaging material. This effect is known as 'flavour scalping' and can be responsible for a significant reduction in the flavour of a packaged food.

A series of test procedures was developed to measure the effects of this phenomenon. These tests were hedonic in nature with panellists required to assess the flavour of a milk chocolate sample relative to a control on a four point scale. In addition, panellists were asked to described any additional flavours perceived in terms of simple sensory terms. A choice of sensory descriptors/attributes was supplied in the form of a revised list (Set B). Panel members were only permitted to use terms that appeared on that list.

2.2.4.1 Method 11 - Test procedure for evaluation of reverse-migration of flavour volatiles from the food product to the secondary packaging

25 grams of clean Chemi-thermomechanical pulp (CTMP) sheet were cut in to pieces and placed in the bottom of a 1.5 dm^3 glass jar. A petri dish was then placed on top of the CTMP pieces and 10 'Cadbury's Chocolate Buttons' evenly distributed within the dish. The jar was then sealed with a ground glass stopper

and stored at room temperature for up to a week, in darkness. An empty jar containing 10 chocolate buttons was prepared as a control and stored in a similar way to the test sample jars.

The test was also carried out under conditions of increased humidity during which 2.0 cm^3 of high purity water (HPLC grade) was sprayed into the test jars before the chocolate was added.

Assessments of samples were carried out under the conditions described previously using 10 assessors. Chocolate button samples were presented to panellists in aluminium foil dishes. Panel members were asked to assess the flavour of each of the test chocolate buttons, relative to the control sample, using the following scale:

1 - No flavour
2 - Weak flavour
3 - Strong flavour
4 - Very strong flavour

Panellists were given water to cleanse their palate between samples, and were also allowed to refer back to the control between samples. If an additional flavour perception was experienced, panellists were encouraged to state this in terms of the descriptors provided. The list of descriptors provided for this series of tests was revised from that used in earlier tests in that some of the least used descriptor terms had been removed (Table 2.12).

2.2.4.2 Method 12 - Test procedure for evaluation of reverse-migration of flavour volatiles from the food product to the primary packaging

25 grams of clean Chemi-thermomechanical pulp (CTMP) sheet was cut in to pieces and half placed in the bottom of a 1.5 dm^3 glass jar. 10 'Cadbury's Chocolate Buttons' were then evenly distributed on top of the CTMP in the jar. The other half of the CTMP was then placed over the chocolate buttons.

Table 2.12 Suggestions for possible taint/odour descriptors to be used in laboratory sensory taint and odour testing (Set B).

Green	Oily	Fatty	Rancid
Citrusy	Soapy	Sulphury	Medicinal
Gluey	Bitter	Herby	Grape-like
Plasticy	Sweet	Pungent	Fishy
Musty	Sour	Sharp	Fruity
Cardboardy	Stale	Solventy	Grassy
Painty	Woody	Vegetably	Catty

The jar was then sealed with a ground glass stopper and stored at room temperature for 3 days, in darkness. An empty jar containing 10 chocolate buttons was prepared as a control and stored in a similar way to the test sample jars.

Assessment of samples was carried out under the conditions described in Method 11 using 10 assessors. Panel members were asked to assess the flavour of each of the test chocolate buttons relative to the control sample using the scale described above, and supply simple descriptions of any additional flavours encountered, using the list of descriptors (Set B), illustrated above.

2.2.5 ODOUR CHARACTERISATION TESTS

There are much published data regarding the sensory characteristics of volatile compounds associated with carton-board taints. However, this sensory information is useless if the panellists involved in subsequent sensory tests were unable to recognise these sensory attributes. A series of tests was carried out in which samples of cleaned CTMP flat sheet were impregnated with low amounts of known odorous compounds and panellists asked to describe the odour perceived in terms of the list of Taint/Odour Descriptors (Set B).

10 grams of CTMP sheet, which had been pre-cleaned in a vacuum oven at 120°C for 2 hours, were placed in a clean 2 dm^3 desiccator. A known volume of odorous compound was then injected in to the sheet by inserting a needle syringe between layers of the sample, and the lid placed on the desiccator. This sealed vessel was then left to equilibrate for 1 hour at room temperature.

A set of 10 panellists was then asked to smell the contents of the desiccator, promptly replacing the lid afterwards to prevent excess loss of odorous compound vapour. A period of 1 minute was placed between each panellist smelling the sample to allow the vapour to re-equilibrate itself each time.

No more than two samples were supplied to each panellist during each testing session to prevent problems associated with carry-over.

2.3 RESULTS AND DISCUSSION

2.3.1 TAINT TESTS - METHODS 1,2 AND 3

Methods 1, 2 and 3 taint tests demanded a lot from the panel members. Initially, panellists were unfamiliar with the test format and the data collected tended to be erratic and not particularly accurate. As panellists became more familiar with the test procedures and with what was required from them, taint scores were found to be more reproducible. The improved reproducibility was highlighted through more consistent results being obtained in repeat testing and in reduced standard deviations for sets of taint scores. Initially, on starting up sensory testing using Methods 1,2 and 3, standard deviations were in the range 0.98-1.04, 1.25-1.34, and 0.65-0.71, respectively. As panellists gained experience and as poor panel performers were discarded, standard deviations fell to the ranges of 0.65-0.68, 0.46-0.49, and 0.40-0.43, respectively, for Methods 1, 2 and 3.

The variation in sensitivity, between panellists, was found to be significant. Some panellists gave persistently high taint and odour scores and were unable to distinguish the difference between taints and odours. Since individuals gave unrepresentative information and were of little use to this type of test, they were discarded. Certain panellists were extremely sensitive, giving harsh taint and

odour scores for the majority of test samples. These individuals, representing the most sensitive packaged food consumers, were of much greater use and gave valuable information. As mentioned previously, the variation in sensitivities of the most sensitive and least sensitive people can be of the magnitude of 10^6 (Kilcast, 1993).

For this type of test, in which information regarding the character of any taint was sought and not a pass/fail judgement, it was clear that only the more sensitive panellists should be used. A panel with a broad range of sensitivities representing the consumer population would not be effective. However, the more sensitive panellists were not always available and a compromise had to be reached regarding the people available.

Despite more consistent taint scoring, as panellists became familiar with the test procedures, verbalisation of the perception of taints using simple descriptors still proved difficult for the majority of panellists. This was indicated by the large number of different descriptors/attributes generated that did not tend to follow any particular pattern. If any useful information was to be gained from the generation of descriptors for a particular taint, fewer descriptors needed to be used. If information concerning the nature of the taint was sought, panel members needed to produce descriptors that were in agreement with one another.

In later tests a list of 45 suggested taint/odour descriptors was supplied to panellists in the form of 'Taint/Odour Descriptors (Set A)', to aid their verbalisation of the perceived taint/odour. This appeared to have a limited effect in reducing the number of different descriptors being produced. Such problems of obtaining comparable descriptions between individual panellists are widely recognised, and have been reported by a number of authors (Dravnieks and Bock, 1978; Söderhjelm and Pärssinen, 1985).

Samples of Carton Silkia (480μm thickness), some of which were virgin unprinted samples and some freshly printed samples, were evaluated using Method 1,2 and 3 taint tests. The printed samples were produced on a Field Packaging production line, using UV-cured offset lithographic inks and a coater varnish. Both unprinted and printed board were from the same batch.

The tests were conducted at a stage when panellists were familiar with the test format. These panellists were selected on previous test performance, and had acquired a certain degree of experience in quantifying taint and perception verbalisation. Each board sample type was evaluated four times and the taint scores collected, using the 1-5 scale, averaged over all the tests. Table 2.13 shows the average taint scores obtained for samples, using all three tests. Figure 2.7 represents the scores in the form of a three dimensional bar graph.

Table 2.13 Average taint scores obtained for Carton Silkia using Method 1,2 and 3 taint tests.

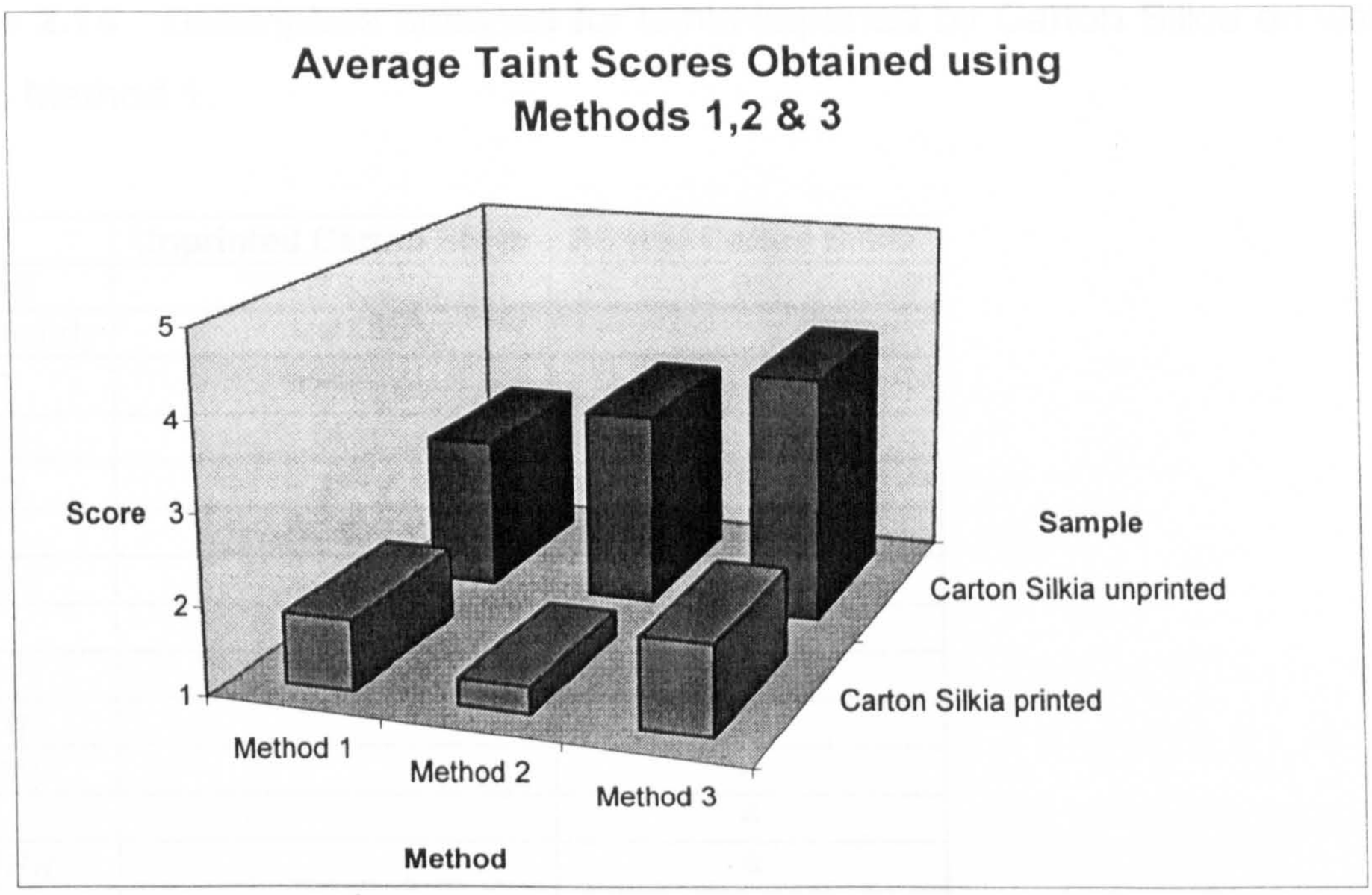
	Method 1	Method 2	Method 3
Carton Silkia printed	1.8	1.3	2.0
Carton Silkia unprinted	2.7	3.2	3.8

All three methods show that the printed Carton Silkia imparted more of a taint on the test substrate. Average scores ranged from 1.3 for Method 2 to 2.0 for Method 3. Therefore, the taint could be regarded as 'very bad' (score of 1.3) to 'considerable' (score 2.0).

Average scores for the unprinted Carton Silkia ranged from 2.7 to 3.8, indicating that the taint imparted to the test substrate was 'considerable/distinct' to virtually 'acceptable'.

It is apparent from the tests that milk chocolate, as used in Method 3, was less sensitive to the taint imparted by the test packaging sample, indicated by the higher scores obtained for Method 3 compared to Methods 1 and 2. This difference in sensitivity can be explained by the presence of numerous sensory competitors in the chocolate that are absent in the water, water being essentially tasteless. These sensory competitors have the effect of hiding tainting compounds to varying degrees, depending on the nature and the presence of the tainting compounds.

Figure 2.7 Graphical representation of average taint scores obtained for Carton Silkia using Method 1,2 and 3 taint tests.



Method 1 appeared to produce a higher average score for the printed samples compared to Method 2. However, a lower score was seen for the unprinted samples using Method 1 compared to Method 2. This would suggest that taint formation in the water substrate, resulting from components in the printed film, may be relatively slow, occurring over a longer period, i.e. 3 days as opposed to 4 hours. This could be due to the volatile compounds of interest having a low affinity for water, resulting in the rate of diffusion through the system being slow. Such volatile compounds are likely to be non-polar in character. If this is so, mobility would not be enhanced by the humid conditions existing during the Method 1 test. The volatile compounds associated with the unprinted samples may be more polar in nature as indicated by the lower score, i.e. the higher degree of taint, seen for samples tested using Method 1 compared to Method 2. In this case, despite a significantly shorter exposure time of test packaging to test substrate, a higher degree of taint was imparted under the conditions of higher humidity and temperature.

A number of taint descriptions were obtained from panellists during the testing of the unprinted Carton Silkia samples and printed Carton Silkia samples, using Methods 1, 2 and 3. Descriptors that were chosen more than

three times, during the four tests carried out on each sample type, are listed in Table 2.14, Table 2.15 and Table 2.16.

Table 2.14 Descriptors obtained for taints imparted by Carton Silkia on water using Method 1.

	Unprinted Carton Silkia	Printed Carton Silkia
Plasticy	12	8
Cardboardy	8	
Green	4	
Rancid	4	
Papery	6	8
Bitter	10	12
Metallic	4	
Oily	8	
Stale	4	4
Woody		6
Starchy		4
Inky		4
Varnishy		4
Fishy		4
Solventy		4

Table 2.15 Descriptors obtained for taints imparted by Carton Silkia on water using Method 2.

	Unprinted Carton Silkia	Printed Carton Silkia
Plasticy		12
Metallic		6
Stale	6	
Woody	4	4
Starchy	4	
Inky		4
Varnishy		4
Sulphury	4	4
Chemically		4
Sweet	6	
Gluey		8

For the unprinted board, the largest number of descriptors was obtained using Method 1 and the fewest obtained from Method 3. This supports the theory that sensory competitors present in the chocolate have the ability to hide, to an extent, taint imparted from external sources.

Table 2.16 Descriptors obtained for taints imparted by Carton Silkia on milk chocolate using Method 3.

	Unprinted Carton Silkia	Printed Carton Silkia
Plasticy		8
Stale	4	
Starchy		4
Inky		10
Varnishy		6
Sulphury		4
Woody		4
Rancid		4
Cardboardy		4
Bitter		4
Solventy		4

The only flavour defect found in the chocolate samples, using Method 3, was a 'staleness'. This may not be a taint resulting from the migration of volatiles from the packaging to the chocolate, but more of a case of reverse migration of flavour volatiles in the chocolate to the packaging, i.e. the phenomenon known as 'flavour scalping'.

Interestingly, more taint descriptors were picked out using Method 1 than Method 2, for the unprinted samples. This supports the theory that the compounds responsible for taints imparted during Method 1 may be more polar in nature and have higher mobilities at the elevated temperature that existed during the test. At the ambient temperature, associated with Method 2, these compounds may have much lower vapour pressures and, thus, be less mobile.

The number of descriptors obtained for the printed samples did not vary significantly with test method. The most pronounced 'inky/varnishy' taints, picked out by panellists, were imparted to samples of the milk chocolate, during Method 3. The number of panellists picking these descriptors was similar for both Methods 1 and 2. This is likely to indicate that the compounds responsible for taint in the ink and varnish are lipophilic, possessing a higher affinity for the fat-containing chocolate, compared to the lipophobic water.

A distinct 'plasticy' taint was picked out in all tests, suggesting that the compound(s) responsible possess relatively high mobility and high affinity for both lipophobic and lipophilic substrates.

2.3.2 ODOUR TESTS - METHODS 4 AND 5

Like Methods 1,2 and 3 taint tests, Methods 4 and 5 odour tests created similar problems in perception verbalisation. It was found that after sufficient experience of the test format, panellists supplied more consistent odour scores in line with their taint scoring.

Samples of Carton Excel carton-board with reported poor odour quality were evaluated using Methods 4 and 5 Odour tests. Samples of this board were supplied in unprinted and printed forms along with samples of unprinted board from a different batch having no reported odour problems. The printed samples were produced using UV offset lithography and over-coated with coater varnish.

Samples of each board-type were also evaluated using the Method 2 taint test and the results compared to the odour scores obtained. Each odour and taint test was carried out twice and the results averaged. Table 2.17 shows the averaged odour and taint scores obtained for the various samples of Carton Excel. Figure 2.8 shows these scores graphically.

Table 2.17 Averaged odour and taint scores obtained for samples of unprinted and printed Carton Excel.

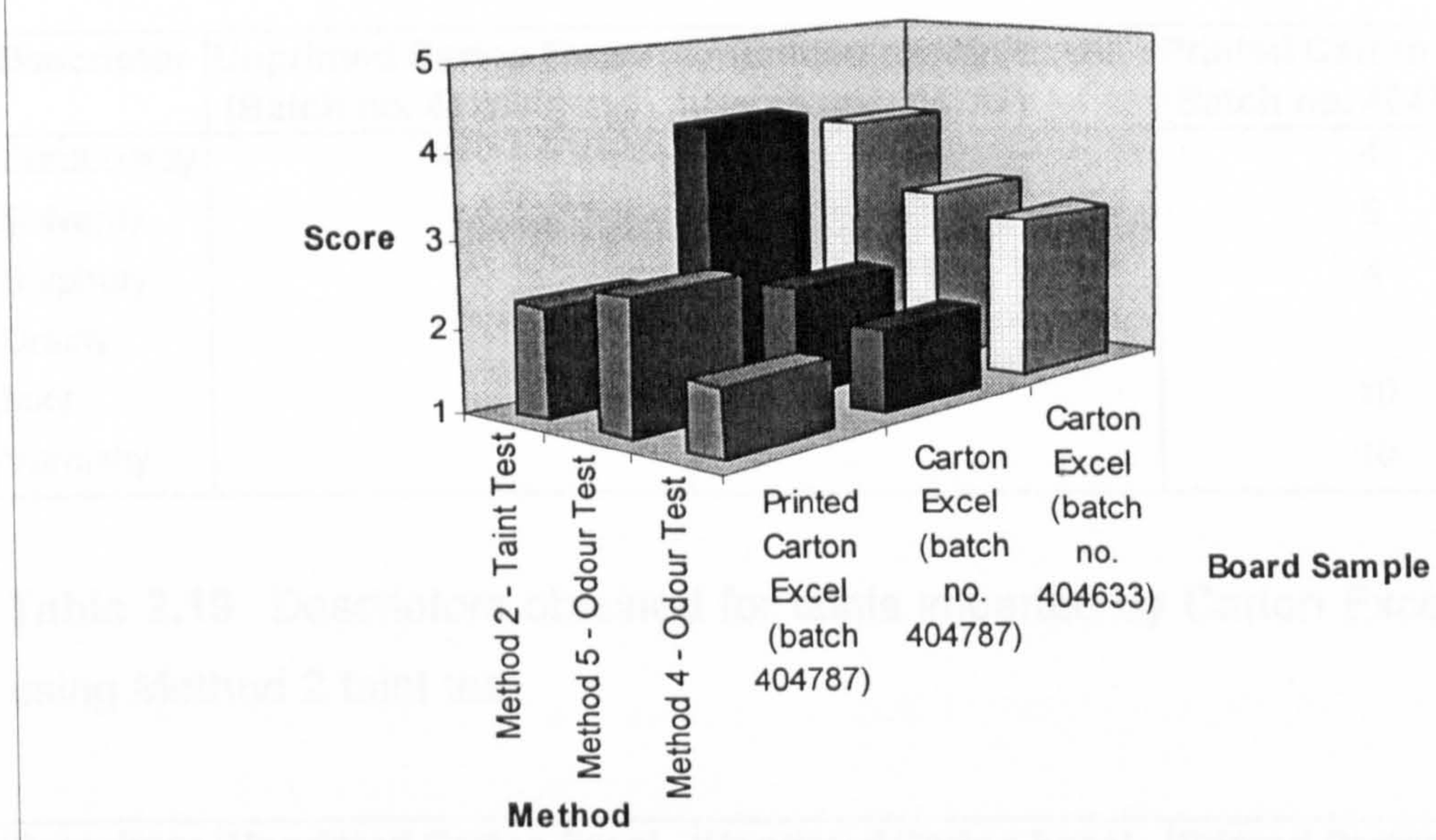
Test Method	**Sample**		
	Carton Excel (batch no. 404633)	Carton Excel (batch no. 404787)	Printed Carton Excel (batch 404787)
Method 4 - Odour Test	2.9	2.0	1.8
Method 5 - Odour Test	3.1	2.3	2.6
Method 2 - Taint Test	3.9	4.1	2.3

The odour tests produced lower scores, generally, than the taint test. In addition, the Method 4 odour test produced lower values than Method 5 odour test.

The odour tests' results indicated that the Carton Excel (batch no.: 404787) had the poorer odour quality. The unprinted samples produced averaged odour scores of 2.0 and 2.3 compared to 2.9 and 3.1 for the samples from the other batch. The printed samples produced an even lower averaged score for Method 4, but a slightly higher one for Method 5. The differences in scores, between the odour methods indicates the high dependency of volatile compound mobility on humidity and temperature. Polar compounds with lower

volatility have higher mobility when Method 4 is used, whereas Method 5 is likely to favour the mobility of highly volatile compounds of both non-polar and polar character.

Figure 2.8 Graphical representation of the averaged odour and taint scores obtained for samples of Carton Excel.



Comparing the odour scores obtained with the taint scores, it was interesting to see significantly higher taint scores, using the Method 2 taint test, for both unprinted samples. However, the taint score obtained for the printed sample was not too different to those of the two odour scores. In addition, a slightly higher taint score was obtained for the unprinted sample with the poorer odour quality.

These conflicting scores may highlight the possibility of odour quality being perceived in a different way to taste quality. As discussed, odour sensations are brought about by the same basic mechanisms as taste sensations. However, differences exist, where certain foods such as Limburger cheese have a pleasant flavour, but do not smell good (Maruniak and Mackay-Sim, 1984). The differences between panellists' acceptance of odour and taste, associated with samples of the Carton Excel, may suggest that the compounds

responsible for the unfavourable odour contribute to a favourable taste. This discrepancy further complicates sensory testing, which aims to achieve taint and off-odour identification.

Those odour descriptors picked out more than three times by panellists for the samples of Carton Excel, using the Method 5 odour test and the Method 2 taint test are listed in Table 2.18 and Table 2.19.

Table 2.18 Descriptors obtained for odours originating from Carton Excel using Method 5 odour test.

Descriptor	Unprinted Carton Excel (Batch no. 404633)	Unprinted Carton Excel (Batch no. 404787)	Printed Carton Excel (Batch no. 404787)
Cardboardy	4	4	4
Solventy			6
Sulphury		16	4
Drainy		4	
Inky			10
Varnishy			10

Table 2.19 Descriptors obtained for taints imparted by Carton Excel on water using Method 2 taint test.

Descriptor	Unprinted Carton Excel (Batch no. 404633)	Unprinted Carton Excel (Batch no. 404787)	Printed Carton Excel (Batch no. 404787)
Cardboardy	6		
Oily	4		
Plasticy		4	4
Solventy			8
Gluey			4
Sweet			4

The majority of panellists picked out a 'sulphury' odour within the samples from batch no. 404787. This 'sulphury' sensation was not picked out during the taint testing. In addition, a significant number of panellists found an 'inky' / 'varnishy' odour, but not taint, associated with the printed sample. Conversely, there was a number of taint descriptors that were not picked up as odours, for example, 'oily', 'plasticy', 'gluey', and 'sweet'.

It is clear that the nature of the off-odour could be described as 'sulphury' and to a lesser extent as 'drainy'. These sensations produced objection when perceived as odours, but were not perceived during tasting.

It is possible that the sulphurous compounds responsible for the off-odour and the compounds associated with the 'inky/ varnishy' odours may have low affinity for the water substrate and, thus, not migrate in sufficient quantities to be perceived during tasting.

2.3.3 TAINT TESTS - METHODS 6 AND 7

Method 6 and Method 7 taint tests were developed to produce useful data from sensory analysis that did not rely so heavily on the individual's ability to verbalise his/her perception of a taint or odour.

The tests adopted a hedonic nature in which panellists were supplied with a sensory descriptor/attribute for a sample. Panellists were then asked to record their level of agreement that this descriptor/attribute was perceived. The test protocol is a hybrid between descriptive and hedonic procedure relying on the ability of panellists to recognise a particular sensory attribute and to supply a level of agreement of its presence.

Averaged scores from the sensory panel, for the agreement with a given sensory attribute, were interpreted in the following way:

Score	Interpretation
1.0 - 2.2	The panel did not find the sensory attribute present in the sample.
2.3 - 2.9	The panel was not certain that a sensory attribute was present in the sample.
3.0 - 4.0	The panel was certain that a sensory attribute was present in the sample.

Samples of fresh Stora Kopparwhite carton-board and samples of Stora Koppargloss carton-board were evaluated using this type of sensory testing procedure. Two grades of each type of board were used:

- Kopparwhite 350μm
- Kopparwhite 440μm
- Koppargloss 440μm
- Koppargloss 705μm

The taint descriptors/attributes supplied to panellists were:

- plasticy
- green
- musty/stale

'Plasticy' was chosen due to it being used frequently in previous tests for the description of taints imparted to water samples from board surface binders and inks/varnishes. 'Green' was chosen due to it being associated with the formation of certain oxidation products from unsaturated species within carton-board. 'Musty/stale' was chosen due to it being frequently used in previous tests for the description of taints imparted to chocolate and water samples from carton-board.

Separate tests were carried out in which only one of the above descriptors/attributes was supplied for each of the carton-board samples at a time. This was designed to eliminate any confusion between attributes and allow panellists to concentration solely on the attribute for the duration of the test. Tests were carried out three times and the agreement scores averaged.

Figure 2.9 shows the average agreement scores (scale 1-4) for the four samples of board, when 'plasticy' was supplied as the descriptor/attribute.

The values of 2.8 and 2.3 for 350μm and 440μm Kopparwhite board, respectively, indicated that panellists were undecided whether the water samples had been imparted with a 'plasticy' taint during the test. The values of 3.0 and 3.4 for 440μm and 705μm Koppargloss board, respectively, indicated that panellists were more certain that the water had been imparted with a 'plasticy' taint.

Figure 2.9 Agreement scores obtained for Method 6 - taint test using the descriptor: 'plasticy'.

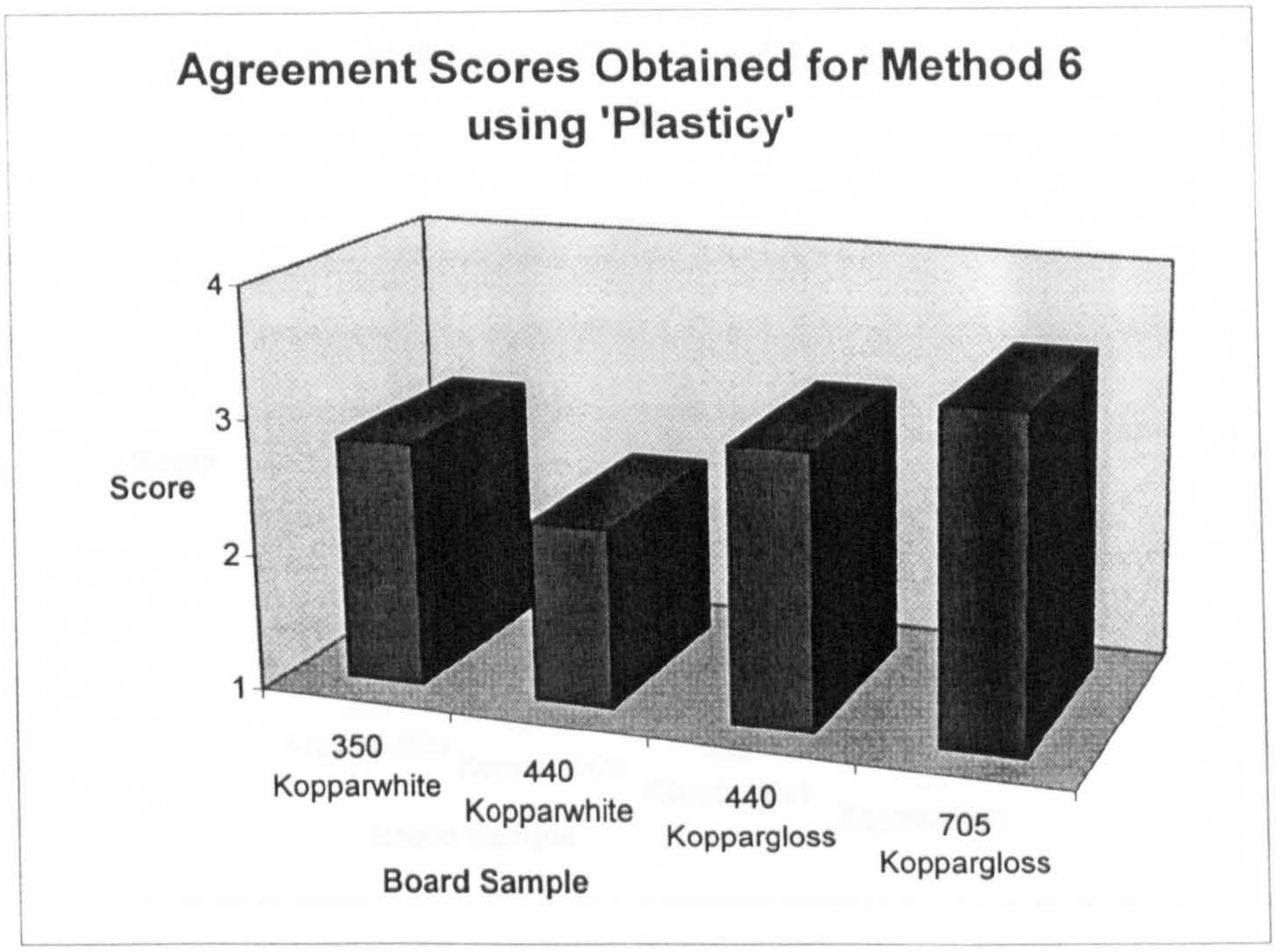


Figure 2.10 shows the average agreement scores (scale 1-4) for the four samples of board when 'green' was supplied as the descriptor/attribute. The definition of 'green' supplied to panellists was the sensation created by freshly cut grass.

350μm and 440μm Kopparwhite board produced values of 2.2 and 1.8, respectively; and the 440μm and 705μm Koppargloss board produced values of 2 and 2.2, respectively. The low agreement scores indicated that panellists did not perceive a 'green' sensation within the water samples and, therefore, that the board samples, under test, had not oxidised to any great extent.

Figure 2.11 shows the average agreement scores (scale 1-4) for the four samples of board when 'musty/stale' was supplied as the descriptor/attribute.

350μm and 440μm Kopparwhite board both produced agreement values of 2.6, indicating panellists were unable to pick out any distinctive taint of this nature. The 440μm and 705μm Koppargloss board produced values of 3.1 and 3.5, respectively, indicating that there was general agreement that the Koppargloss board imparted a 'musty/stale' taint on the water samples.

Figure 2.10 Agreement scores obtained for Method 6 - taint test using the descriptor: 'green'.

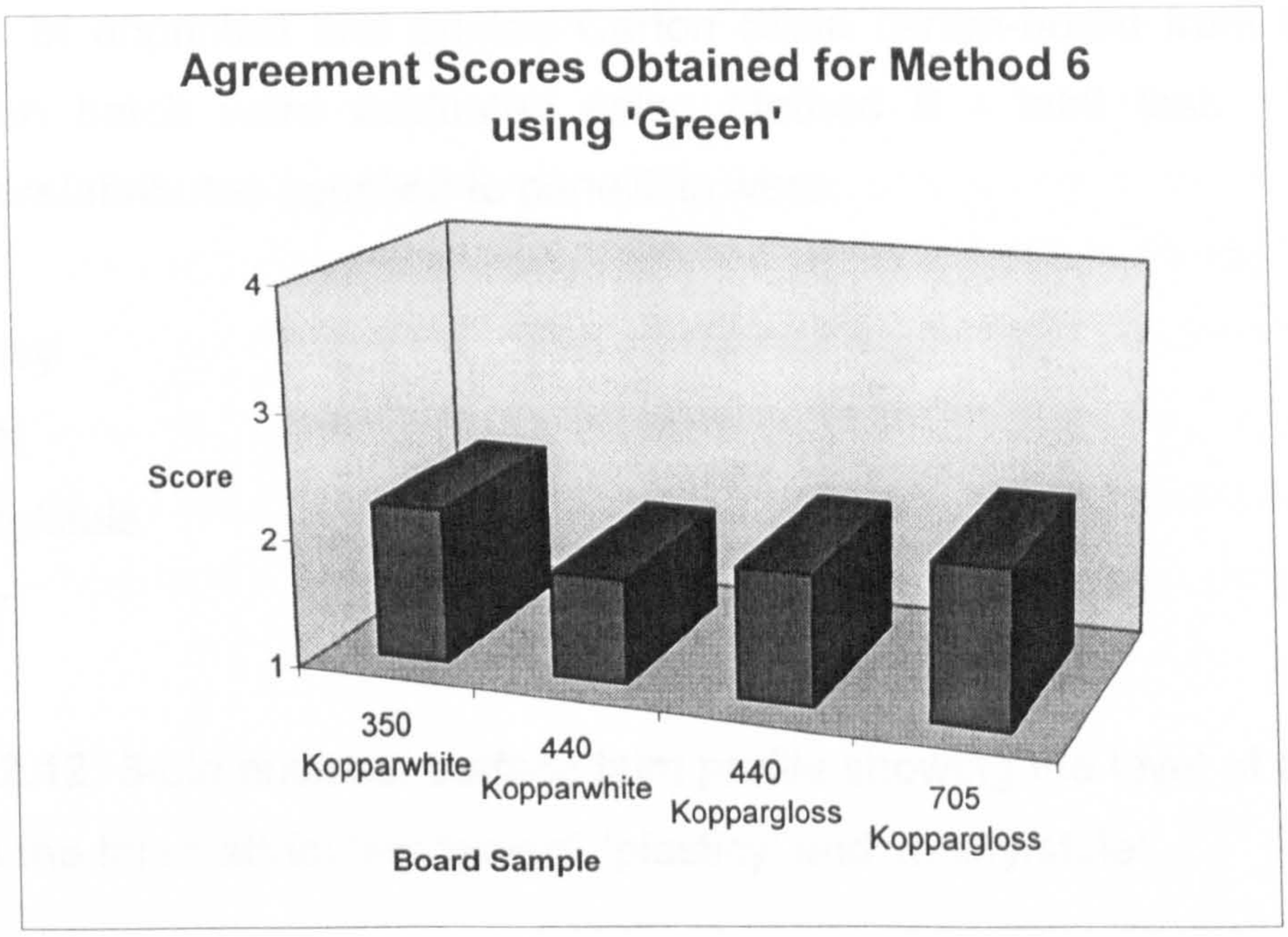


Figure 2.11 Agreement scores obtained for Method 6 - taint test using the descriptor: 'musty/stale'.

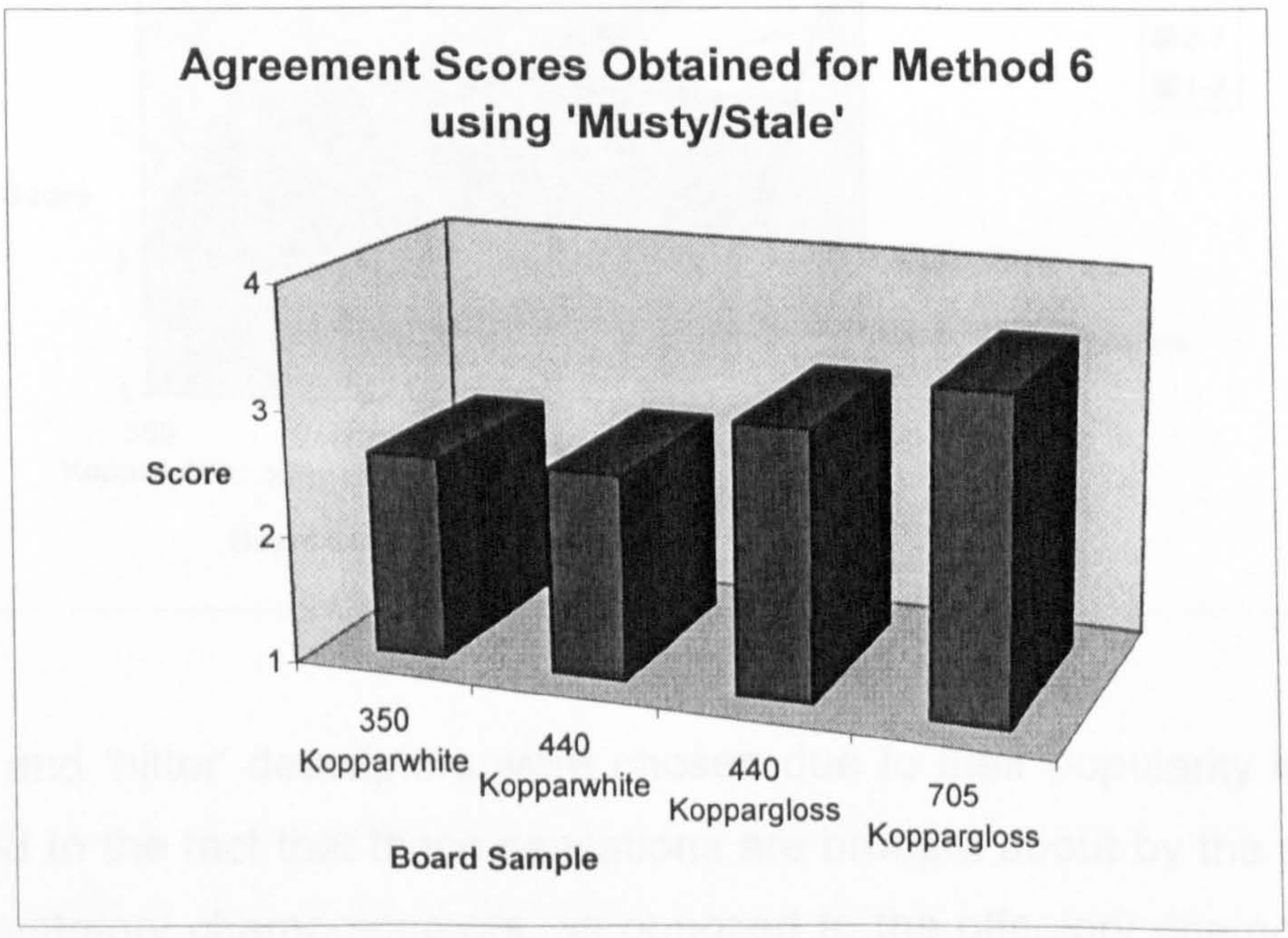


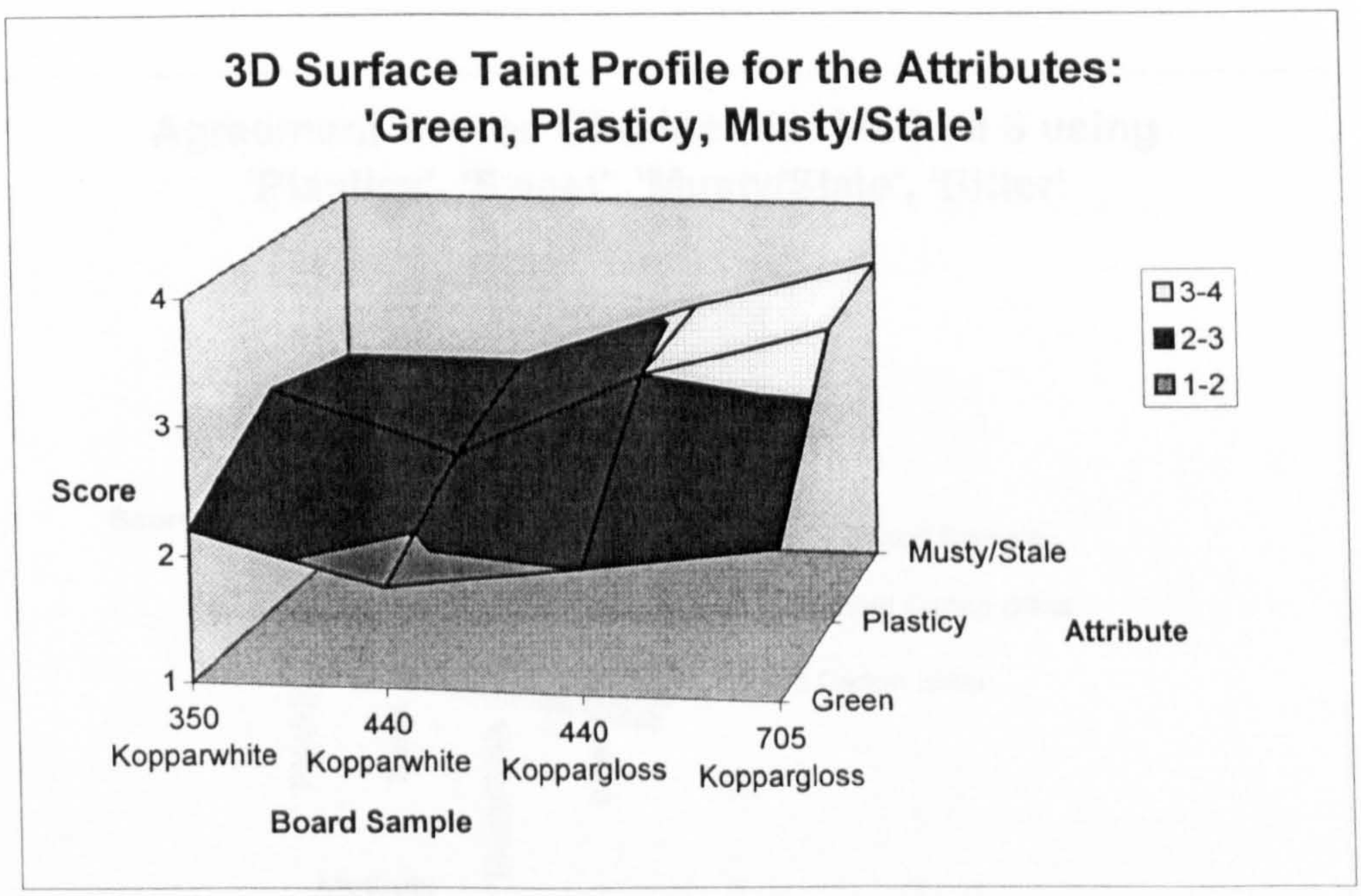
Figure 2.12 shows the 3-dimensional surface profile generated by the level of agreement towards the three sensory attributes assessed on the four types of carton-board. The profile gives a visual indication of the taint characteristics of the boards, with respect to the three attributes. The 705μm Koppargloss

appears to impart the highest level of taint and the 440μm Kopparwhite the lowest level of taint, with respect to these attributes.

Samples of unprinted and printed Carton Silkia carton-board from the same production batch were evaluated using Method 6 - taint test. The taint descriptors/attributes supplied to panellists were:

- plasticy
- sweet
- musty/stale
- bitter

Figure 2.12 3-dimensional surface taint profile showing the level of agreement towards the three attributes: 'green', 'plasticy' and 'musty/stale'.

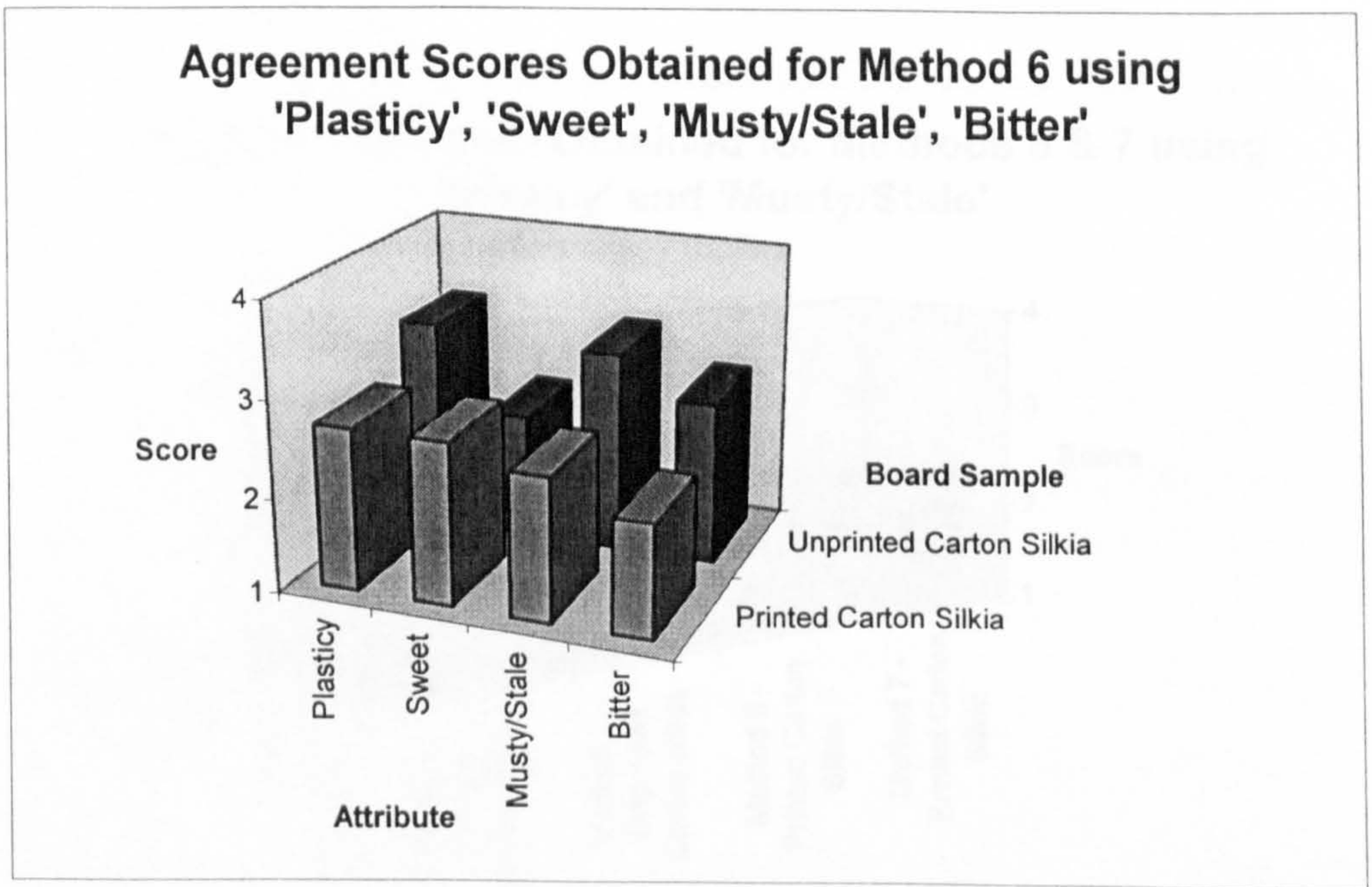


'Sweet' and 'bitter' descriptors were chosen due to their popularity in previous tests and to the fact that these sensations are brought about by the stimulation of the gustatory chemoreceptors, as opposed to the olfactory chemoreceptors. Unlike in the previous tests described above, the four attributes were supplied in a single test session to four identical samples of the carton-board sample. Thus, only one board type was assessed per test session. Figure 2.13 shows the average agreement scores obtained from samples of unprinted Carton

Silkia and of printed Carton Silkia, obtained over three tests using the attributes: 'plasticy', 'sweet', 'must/stale', 'bitter'.

Panellists, generally, agreed that a lower degree of taint was imparted by the printed samples to the water than was imparted by the unprinted samples, with respect to the four attributes. However, panellists agreed that samples of water exposed to the printed samples were slightly sweeter than those water samples exposed to unprinted samples. This could be due to the presence of small amounts of the characteristically sweet compound, benzophenone, in the water samples. Such benzophenone could be present, as residual photoinitiator, in the ultra-violet (UV) irradiated cured ink film on the printed carton-board surface.

Figure 2.13 Agreement scores obtained for Method 6 - taint test using the descriptors: 'plasticy', 'sweet', 'musty/stale', 'bitter'.

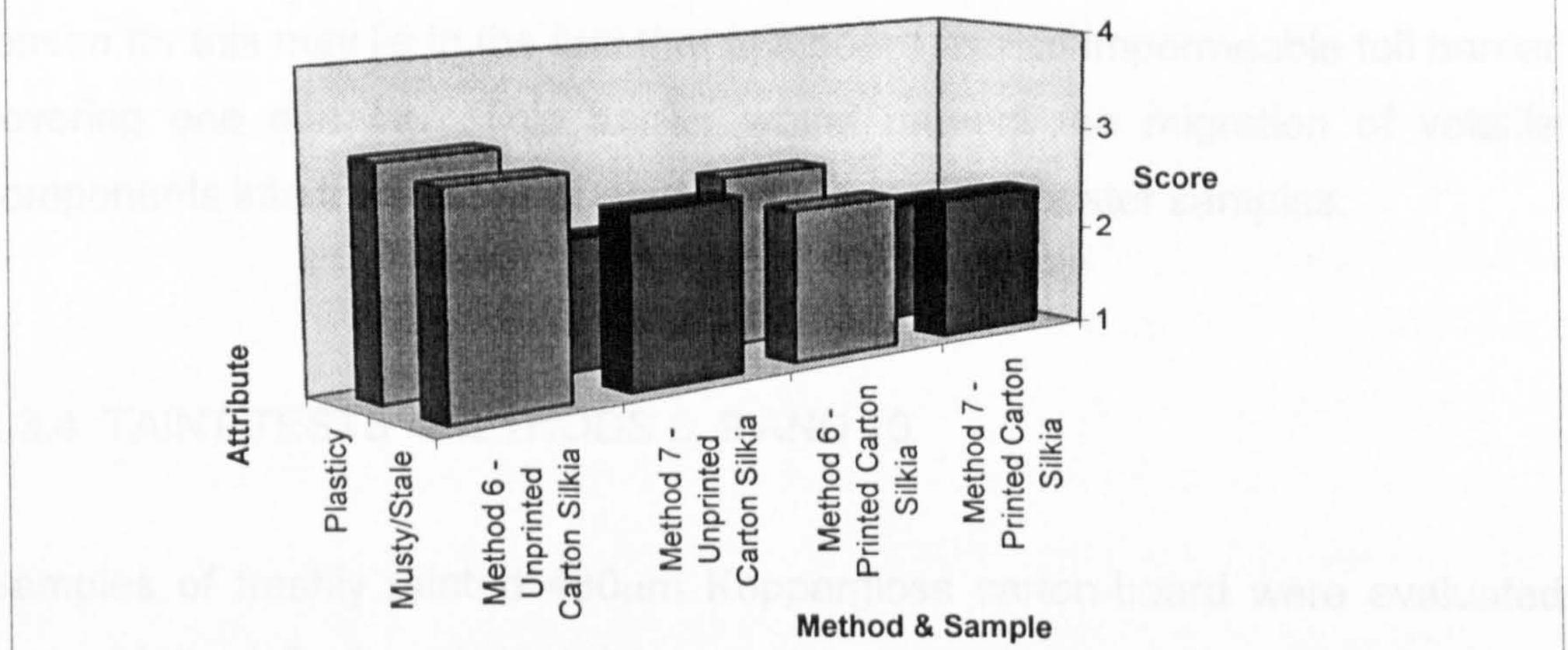


The higher level of agreement for the presence of the other taint descriptors, in the water samples exposed to unprinted board samples indicates that the cured ink/varnish film might be acting as a barrier, restricting the movement of compounds from the carton-board to the water, via the airspace between.

Samples of unprinted and printed Carton Silkia were assessed using taint test - Method 7, which adopted milk chocolate as the test substrate. The board was tested with the chocolate in contact with the unprinted surface, as it would be in the actual carton-package. Taint descriptors supplied to panellists were 'plasticy' and 'musty/stale'. Three tests were carried out and the average agreement scores compared with those from Method 6 on the same batch of carton-board. Figure 2.14 shows the average agreement scores obtained from samples of unprinted and printed Carton Silkia using both Method 6 and Method 7.

Slightly higher levels of agreement are seen for Method 6, using water as the test substrate, compared to Method 7, using milk chocolate as the test substrate.

Figure 2.14 Agreement scores obtained from Methods 6 & 7 using the descriptors 'plasticy' and 'musty/stale'.



This difference was not as pronounced as that seen for the taint intensity scores between Method 2 and Method 3, these methods being used to evaluate the total taint impression imparted by the board samples, and not just a single attribute at a time, as with Method 6 and Method 7.

It would appear that there was agreement that the chocolate was more susceptible to a 'musty/stale' taint than to a 'plasticy' taint. The opposite was true for the water which was more susceptible to the 'plasticy' taint. The difference for this is unclear, though the differences in hydrophobic/hydrophilic nature of the two substrates may be significant. Alternatively, the possibility of reverse migration from the chocolate to the carton-board of flavour volatiles, producing a stale sensation cannot be discounted.

Samples of Kraft board, Gold-Foil Backed Board, and recycled board were assessed using the Method 6 - taint test. The taint descriptors supplied were 'plasticy', 'musty/stale', 'painty', and 'bitter'. 'Painty' was chosen since it has been linked with the presence of certain compounds associated with taint (Kilcast, 1993). The test was carried out three times and the agreement scores averaged, the results of which are shown in Figure 2.15.

There is general agreement that all three samples impart a 'plasticy' taint on the water. With the exception of the Kraft board, panellists were not so sure that the samples imparted a 'musty/stale' taint, and panellists generally disagreed that 'painty' or 'bitter' taints were transferred to the water.

The lowest values, overall, were associated with the Foil-Backed board. The reason for this may lie in the fact that this board has an impermeable foil barrier covering one surface. This barrier would prevent the migration of volatile components into the airspace between the board and water samples.

2.3.4 TAINT TESTS - METHODS 8, 9 AND 10

Samples of freshly printed 480μm Koppargloss carton-board were evaluated using Method 8, 9 and 10 taint tests to determine the effect of the carton structure on the development of taint, using milk chocolate buttons as the test substrate.

Two tests were performed, one in which panellists were supplied with the descriptors: 'plasticy' and 'musty/stale' and one in which panellists were supplied with the descriptors: 'sweet' and 'green'. The tests were repeated three times and the agreement scores averaged.

Figure 2.15 Agreement scores obtained from Method 6 using the descriptors 'plasticy', 'musty/stale', 'painty' and 'bitter'.

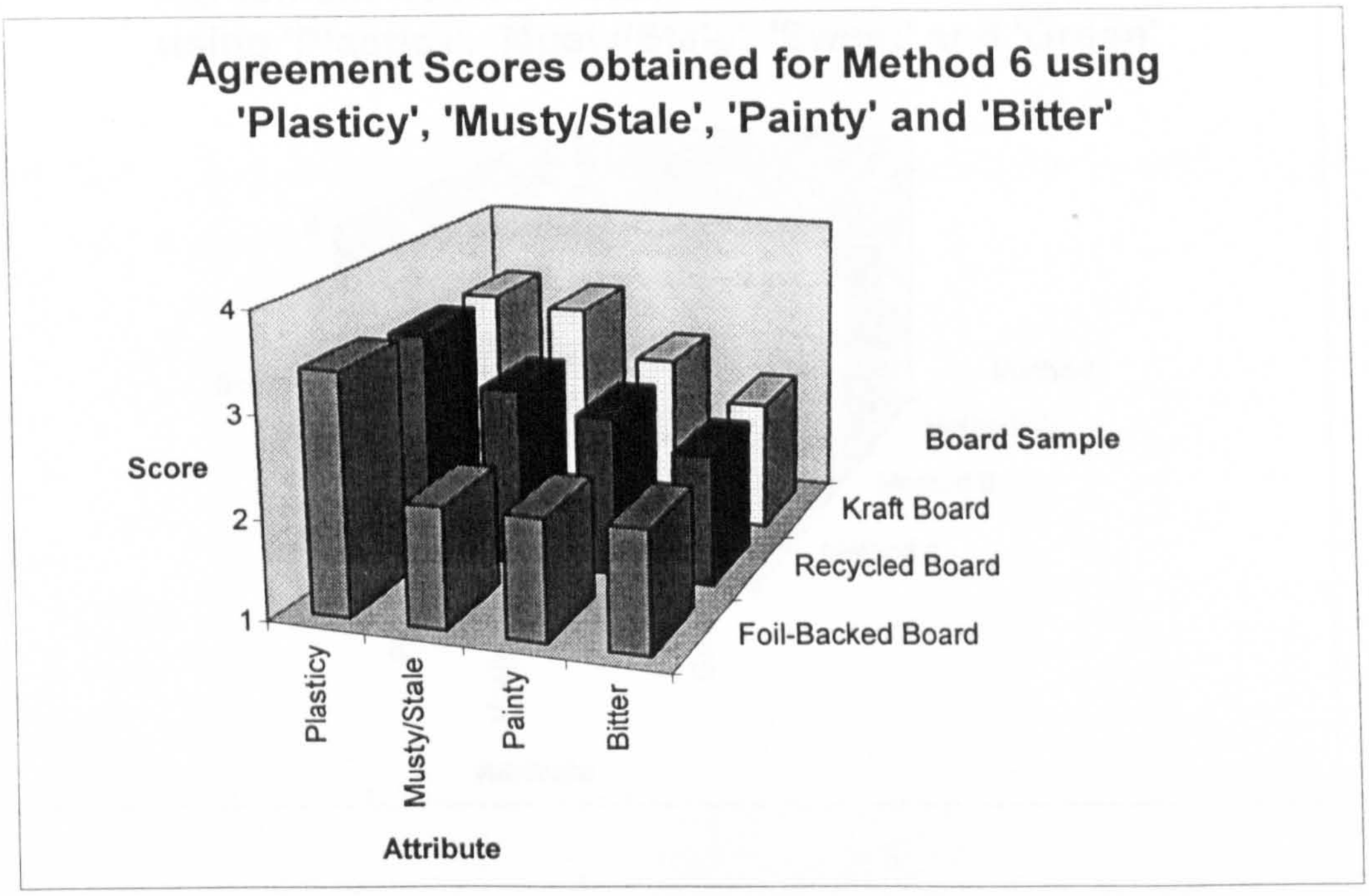
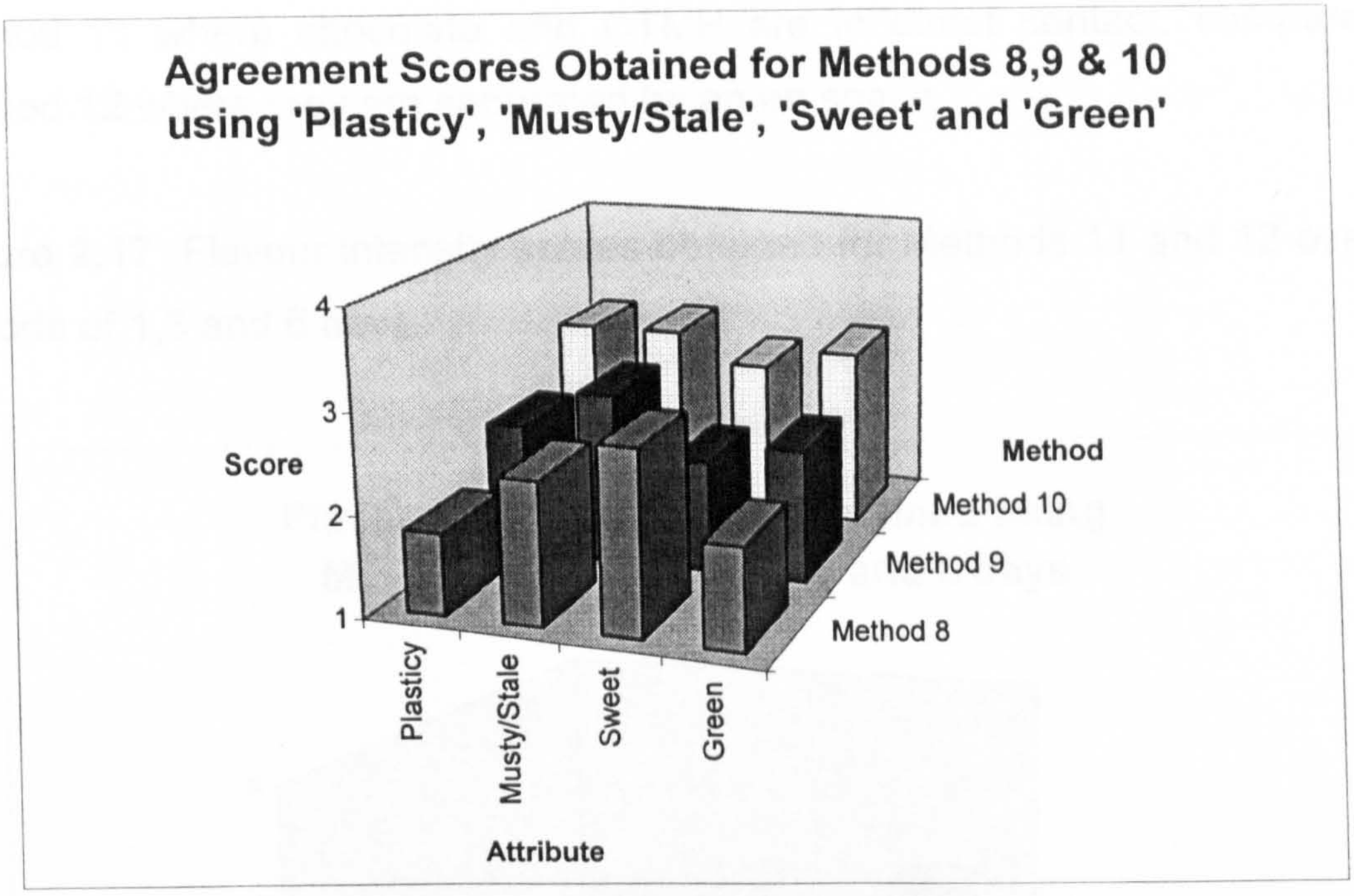


Figure 2.16 shows the average agreement scores obtained, using Methods 8, 9 and 10, for samples of printed Koppargloss carton-board. The descriptors supplied were 'plasticy', 'musty/stale', 'sweet' and 'green'.

The highest agreement levels were seen for the attributes supplied using Method 10, suggesting that panellists agreed that all four taint attributes were present in the board samples tested, including the 'green' attribute which is associated with the oxidation of unsaturated species within the board itself. During this test, the chocolate test substrate was placed in direct contact with the unprinted side of the carton-board. Lower agreement scores were seen for Method 9 in which the printed side of the carton was placed in direct contact with the food. The lowest scores were seen for Method 8, with the exception of the agreement score for 'sweet'.

In the case of primary food packaging, the lower scores seen for Method 9, compared to Method 10, support the theory that the cured print/varnish surface acts as a barrier to the migration of odorous compounds to the food substrate.

Figure 2.16 Agreement scores obtained using Method 8, 9 and 10 using the descriptors 'plasticy', 'musty/stale', 'sweet' and 'green'.



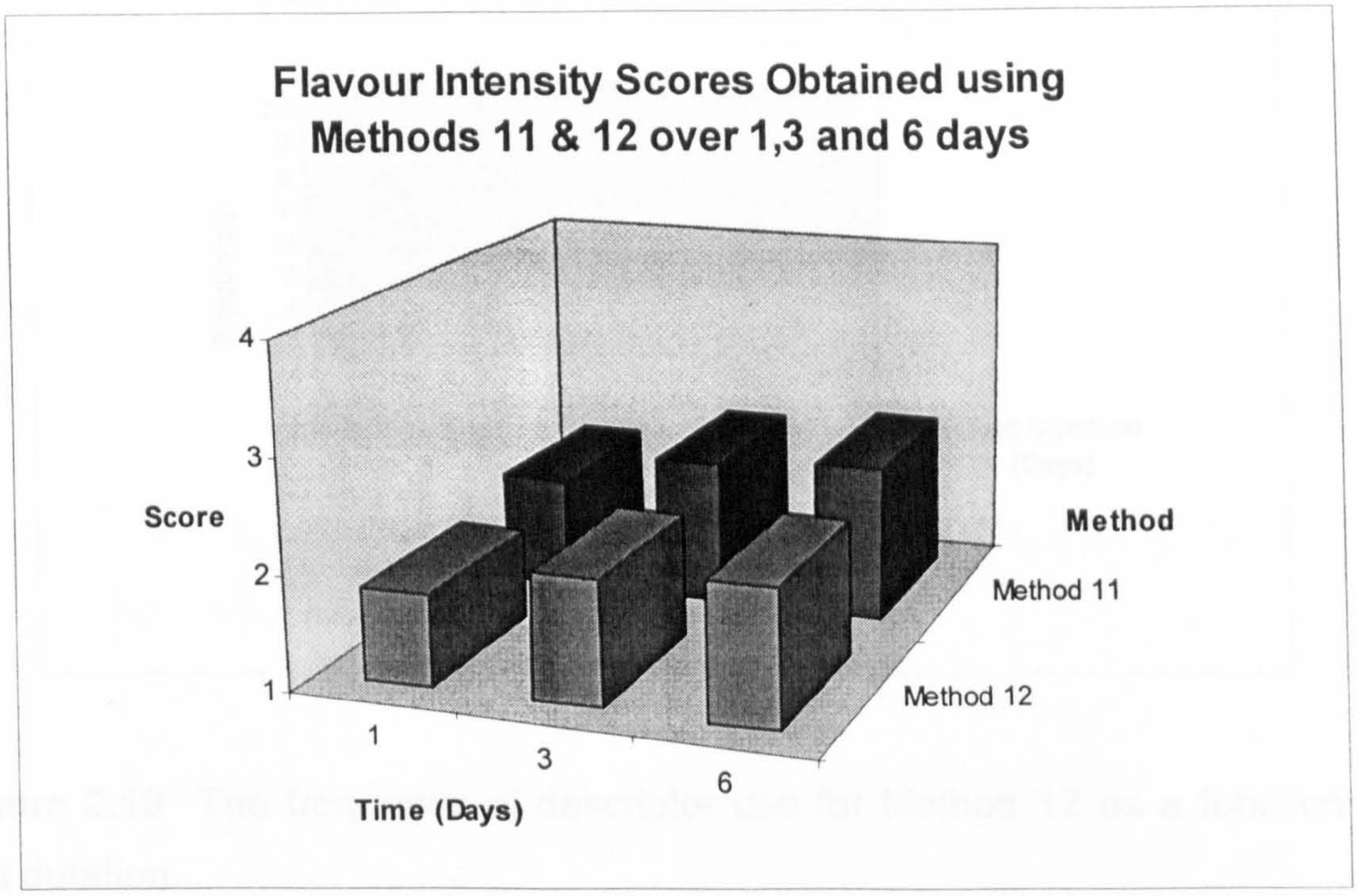
Scores obtained from Method 8, with the exception of those obtained for the 'sweet' attribute would suggest that migration of volatiles in secondary packaging systems occurs to a lower extent, imparting no significant taint and, thus, it can be assumed that diffusion across an air space between the packaging and the food does not occur as readily as diffusion between the packaging and the food in direct contact.

2.3.5 TAINT TESTS - METHODS 11 AND 12

Methods 11 and 12 were carried out to determine the effect of 'flavour scalping' on primary and secondary packaging systems. Samples of flat sheet CTMP were evaluated, with chocolate, over the periods of 1,3 and 6 days at room temperature and standard relative humidity. Flavour intensities of chocolate were scored on the 1-4 scale, relative to the control, and the test repeated twice. Figure 2.17 shows the average flavour intensity scores obtained for Methods 11 and 12 over the periods of 1,3 and 6 days.

The chart shows that the flavour of the chocolate does decline during the test and the extent of this decline is proportional to the duration of the test. The chart, also, shows that slightly more flavour is lost from the chocolate using Method 11 where chocolate and CTMP are in direct contact, compared to Method 12 where they are separated by an air space.

Figure 2.17 Flavour intensity scores obtained for Methods 11 and 12 over the periods of 1,3 and 6 days.



Panellists were, also, asked to supply a description of any additional tastes perceived, using the lists of descriptors provided (Set B). Figures 2.18 and 2.19 show the frequency of the descriptors chosen by the panel members.

Method 11 did not produce significant descriptions of any taints present other than a 'stale' description from a number of panellists at 3 days. Method 12 produced three popular taint descriptors, that of 'cardboardy' and 'stale', which developed between 3-6 days of the test duration, and a 'flat' description, which developed at 3 days. A number of descriptors were picked by only one or two panel members and were not considered to be statistically significant.

The nature of the descriptors chosen, particularly those of 'stale' and 'flat' indicate the occurrence of 'flavour scalping'. As the chocolate lost flavour

volatiles to the CTMP, it became blander (flatter) and gave the impression of being less fresh.

Figure 2.18 The frequency of descriptor use for Method 11 as a function of test duration.

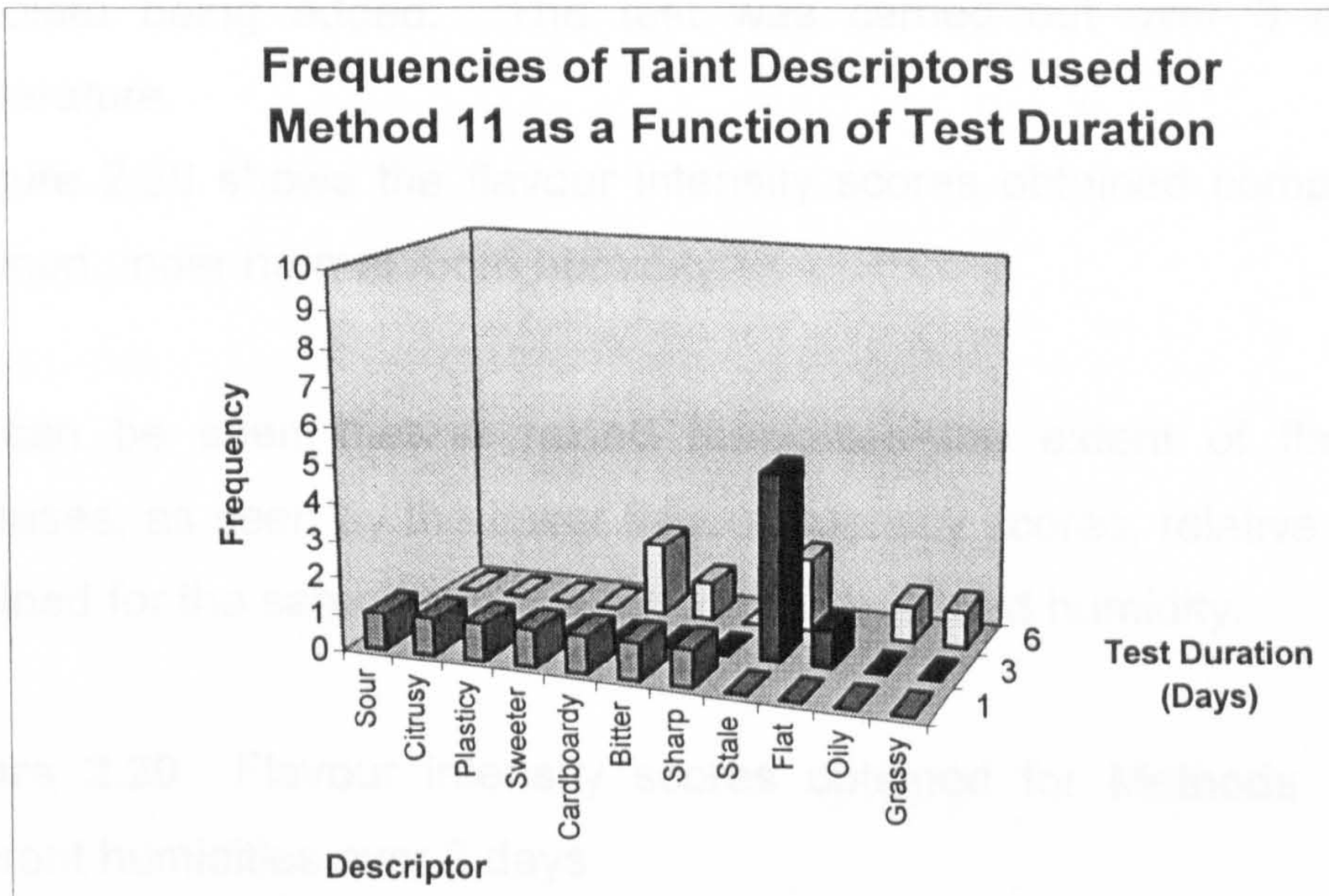
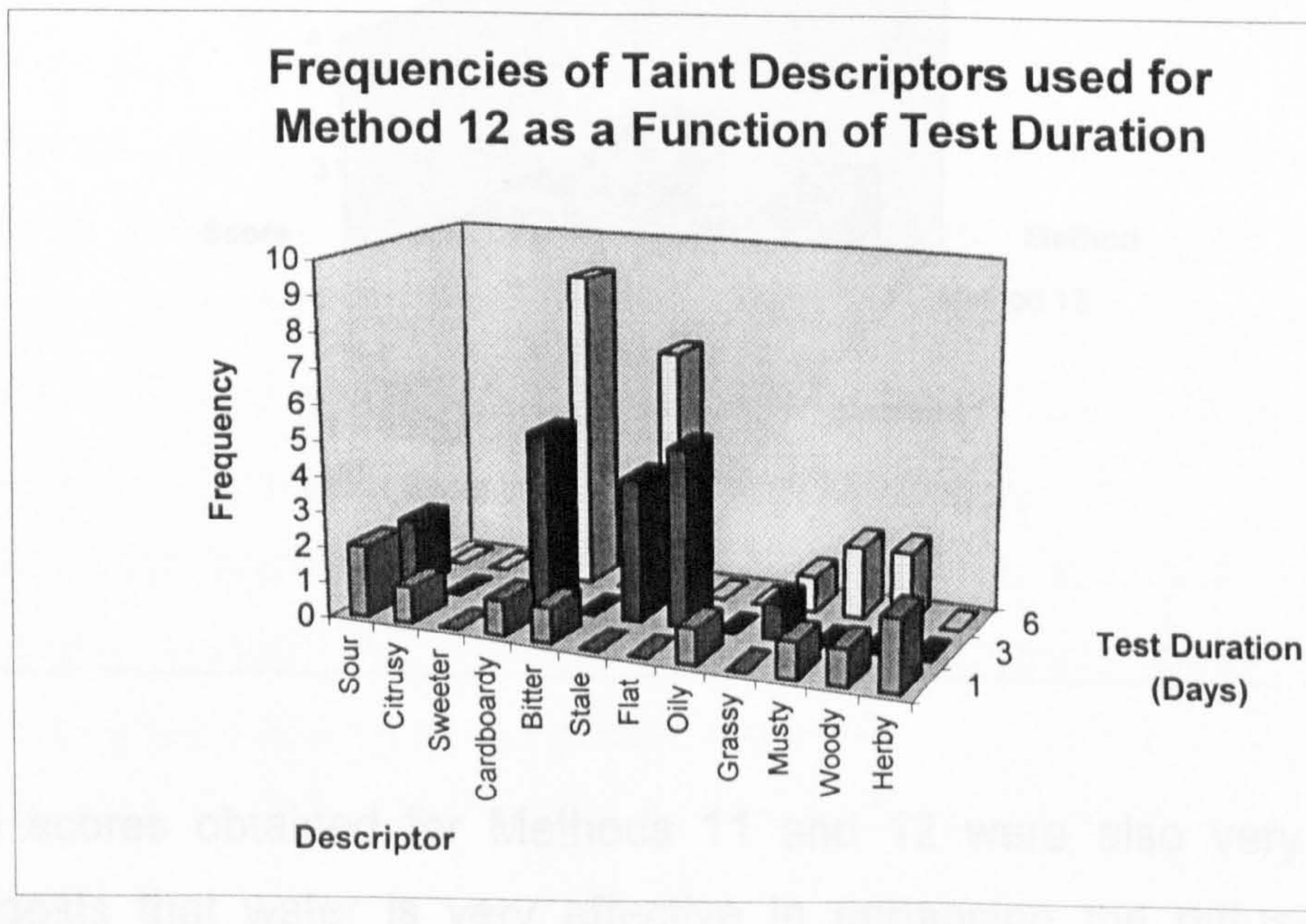


Figure 2.19 The frequency of descriptor use for Method 12 as a function of test duration.



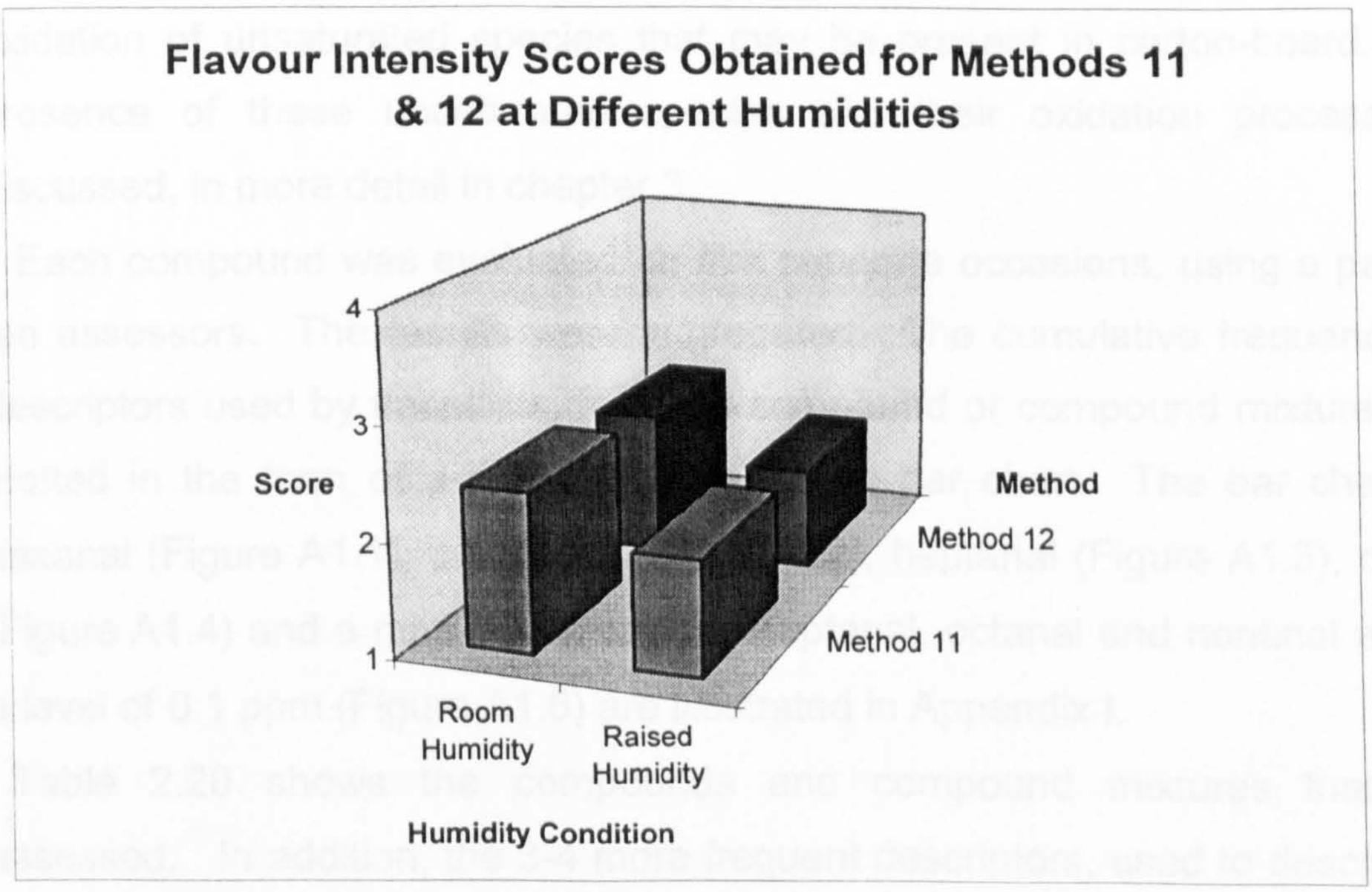
Panel members probably associated this with ageing and perceived the chocolate as being stale.

Methods 11 and 12 were carried out at raised humidity to determine the effect of humidity on flavour scalping. Samples were set up as in previous tests. However, 2.0 cm^3 of water was added to the test jars, via a spray, prior to the chocolate being added. The test was carried out over 3 days at room temperature.

Figure 2.20 shows the flavour intensity scores obtained compared to those obtained under normal room humidity.

It can be seen that at raised humidities the extent of flavour scalping increases, as seen by the lower flavour intensity scores, relative to the control, obtained for the samples subjected to the increased humidity.

Figure 2.20 Flavour intensity scores obtained for Methods 11 and 12 at different humidities over 3 days.



The scores obtained for Methods 11 and 12 were also very similar. This suggests that water is very effective in enhancing the diffusion of volatiles

across the air space, as in the case of Method 9 where chocolate and CTMP were not in direct contact.

2.3.6 ODOUR CHARACTERISATION TESTS

A number of individual compounds and mixtures of compounds were evaluated at a level of 0.1 ppm in 10 grams of CTMP sheet. These are listed below:

- Pentanal
- Hexanal
- Heptanal
- Octanal
- 2-Pentyl furan
- Hexanal, Heptanal, Octanal. Nonanal
- Hexanal, Nonanal, Decanal
- Pentanal, Hexanal, Heptanal, Octanal, Nonanal, 2-Pentyl furan

The compounds were chosen because they can form as a result of the oxidation of unsaturated species that may be present in carton-board. The presence of these unsaturated species and their oxidation processes is discussed, in more detail in chapter 3.

Each compound was evaluated on five separate occasions, using a panel of ten assessors. The results were aggregated. The cumulative frequencies of descriptors used by panellists, for each compound or compound mixture, were plotted in the form of a cumulative frequency bar chart. The bar charts for hexanal (Figure A1.1), pentanal (Figure A1.2), heptanal (Figure A1.3), octanal (Figure A1.4) and a mixture of hexanal, heptanal, octanal and nonanal each at a level of 0.1 ppm (Figure A1.5) are illustrated in Appendix I.

Table 2.20 shows the compounds and compound mixtures that were assessed. In addition, the 3-4 more frequent descriptors, used to describe the sensations experienced by panellists, are listed.

Table 2.20 The more frequently used sensory descriptors associated with a number of odorous compounds/compound mixtures, as determined by sensory panel.

Compound/Compound Mixture	Sensory Description
Pentanal	rancid, green, fatty
Hexanal	green, grassy, fruity
Heptanal	green, grassy, cardboardy, soapy
Octanal	citrusy, sweet, fruity
2-Pentyl furan	fruity, sweet, soapy
Hexanal, Heptanal, Octanal, Nonanal	citrusy, soapy, fruity
Hexanal, Nonanal, Decanal	citrusy, soapy, sweet, pungent
Pentanal, Hexanal, Heptanal, Octanal, Nonanal, 2-Pentyl furan	citrusy, oily, fruity, sweet

Some compounds and compound mixtures were evaluated at a level of 0.5 ppm in 10 grams of CTMP. These are listed below:

- Hexanal
- Hexanal, Nonanal, Decanal

Table 2.21 shows the 3-4 more frequently employed terms to describe the above compound/compound mixture at levels of 0.5 ppm.

Table 2.21 The more frequently used sensory descriptors associated with the odorous compound/compound mixture at a level of 0.5 ppm, as determined by sensory panel.

Compound/Compound Mixture	Sensory Description
Hexanal	green, pungent, musty
Hexanal, Nonanal, Decanal	citrusy, plasticy, musty

Of the sensory attributes described, 'green' and 'citrusy' appear to be the more abundant descriptions, through the range of compounds and compound mixtures.

At the higher concentrations, panellists picked out a 'musty' odour which was not as apparent when the compounds were present at a lower level. In addition, panellists found hexanal to pungent at the higher concentration.

The above sensory descriptions may not give a picture of the overall odour of carton-board. However, in the event of a taint resulting from any of the above compounds, panellists should be able to recognise the compound's sensory characteristics and to supply useful information, aiding its identification.

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APPENDIX I

Figure A1.1

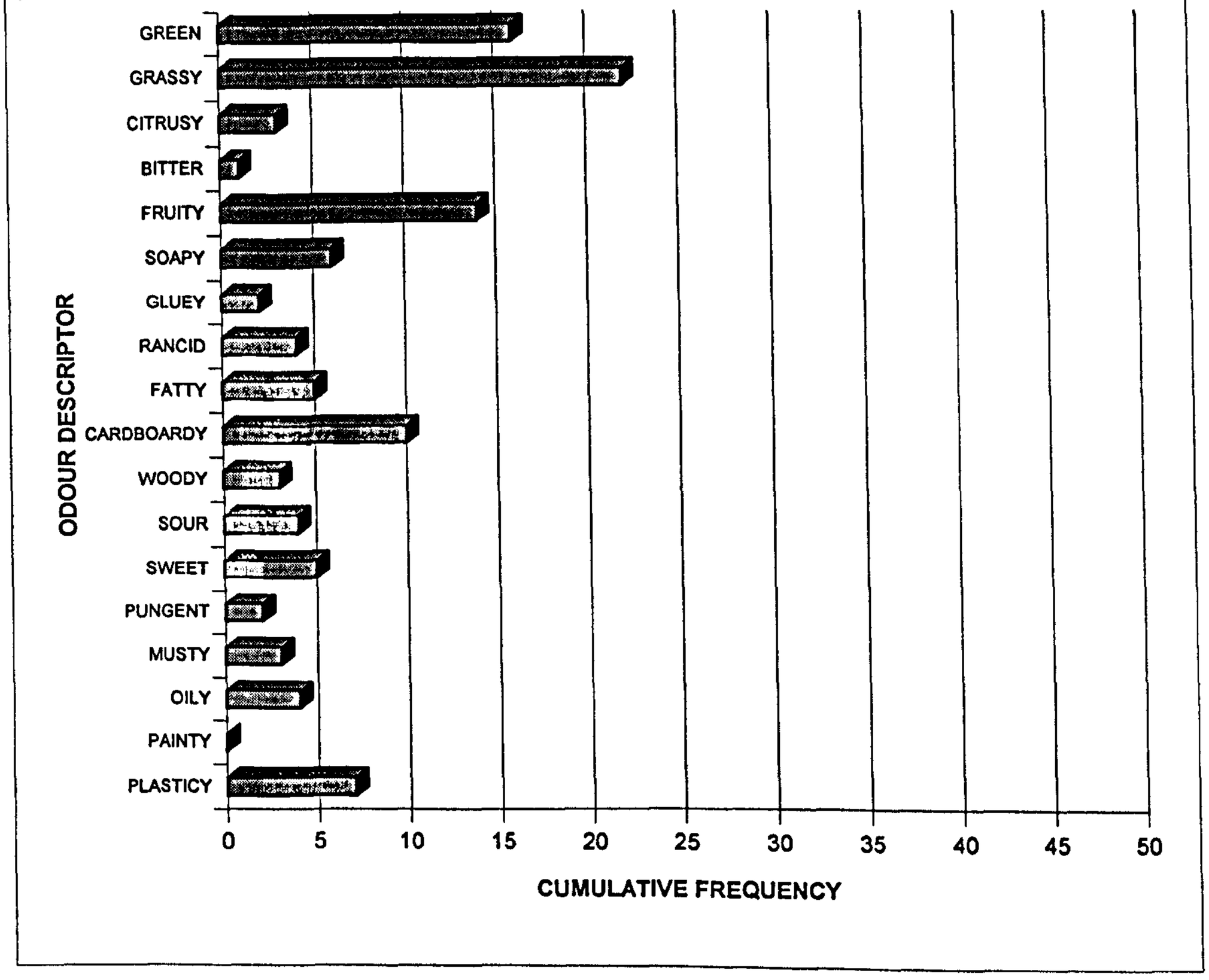


Figure A1.2

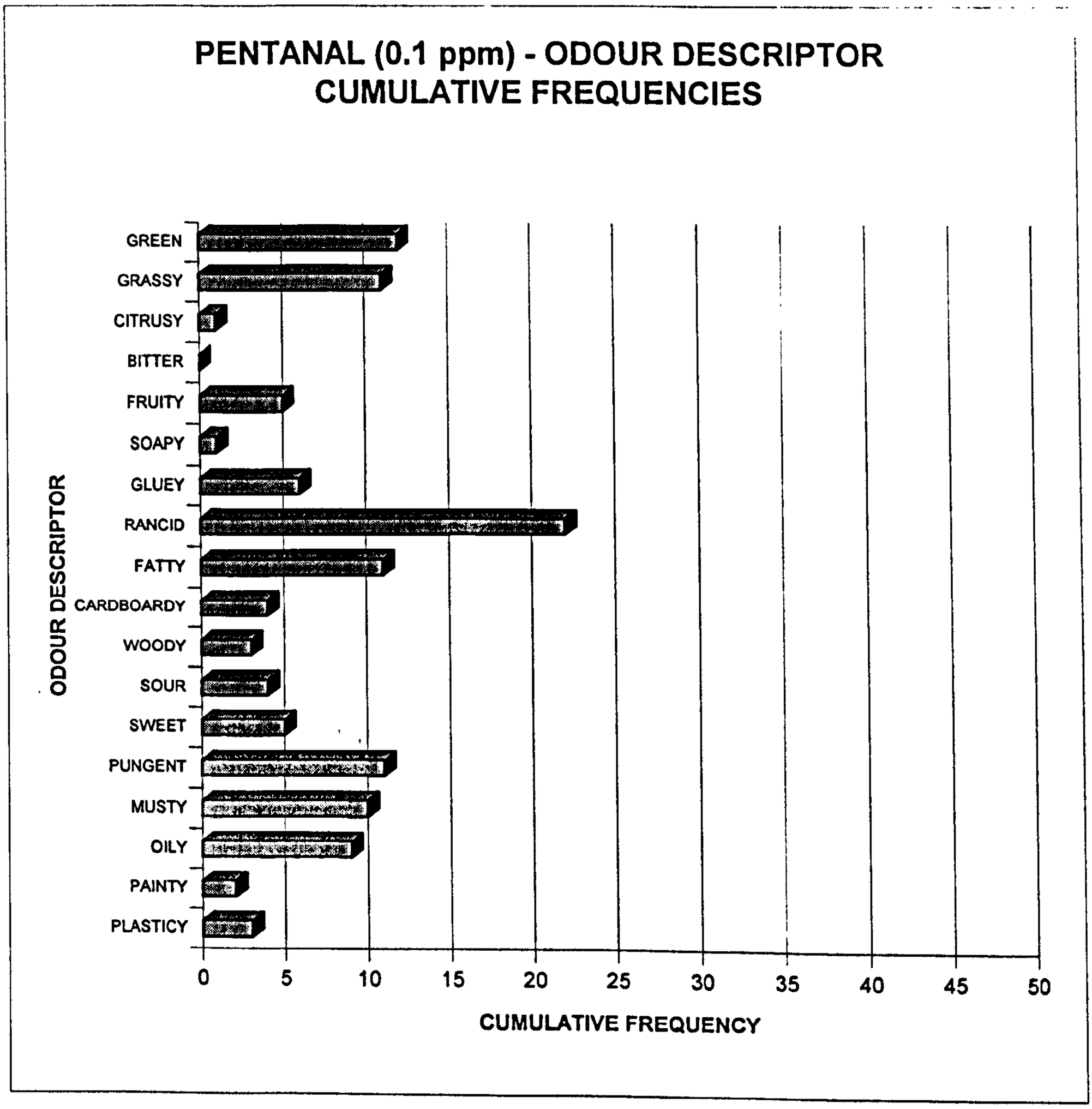

PENTANAL (0.1 ppm) - ODOUR DESCRIPTOR
CUMULATIVE FREQUENCIES
ODOUR DESCRIPTOR
GREEN
GRASSY
CITRUSY
BITTER
FRUITY
SOAPY
GLUEY
RANCID
FATTY
CARDBOARDY
WOODY
SOUR
SWEET
PUNGENT
MUSTY
OILY
PAINTY
PLASTICY
0
5
10
15
20
25
30
35
40
45
50
CUMULATIVE FREQUENCY

Figure A1.3

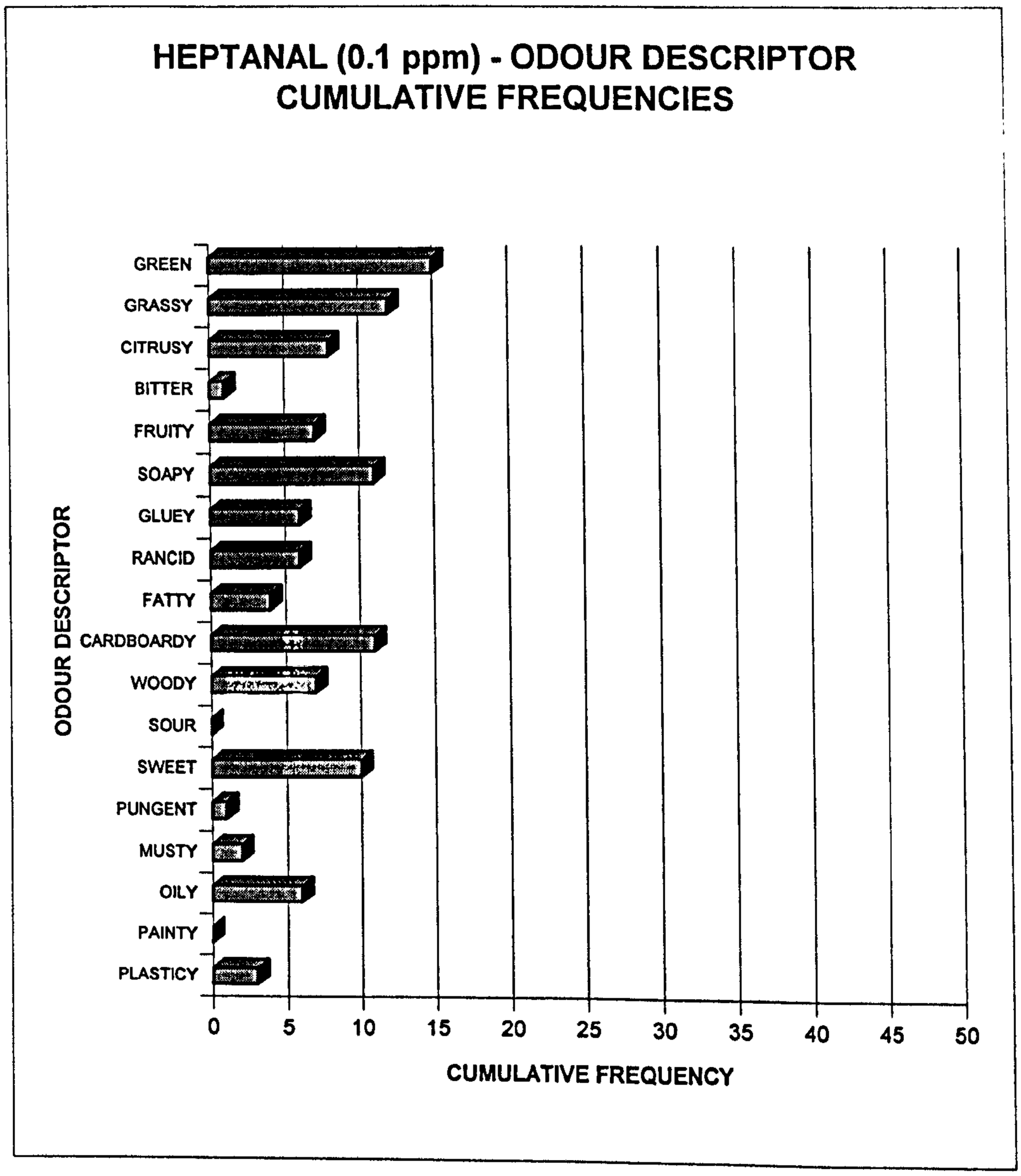

HEPTANAL (0.1 ppm) - ODOUR DESCRIPTOR
CUMULATIVE FREQUENCIES
ODOUR DESCRIPTOR
GREEN
GRASSY
CITRUSY
BITTER
FRUITY
SOAPY
GLUEY
RANCID
FATTY
CARDBOARDY
WOODY
SOUR
SWEET
PUNGENT
MUSTY
OILY
PAINTY
PLASTICY
0
5
10
15
20
25
30
35
40
45
50
CUMULATIVE FREQUENCY

Figure A1.4

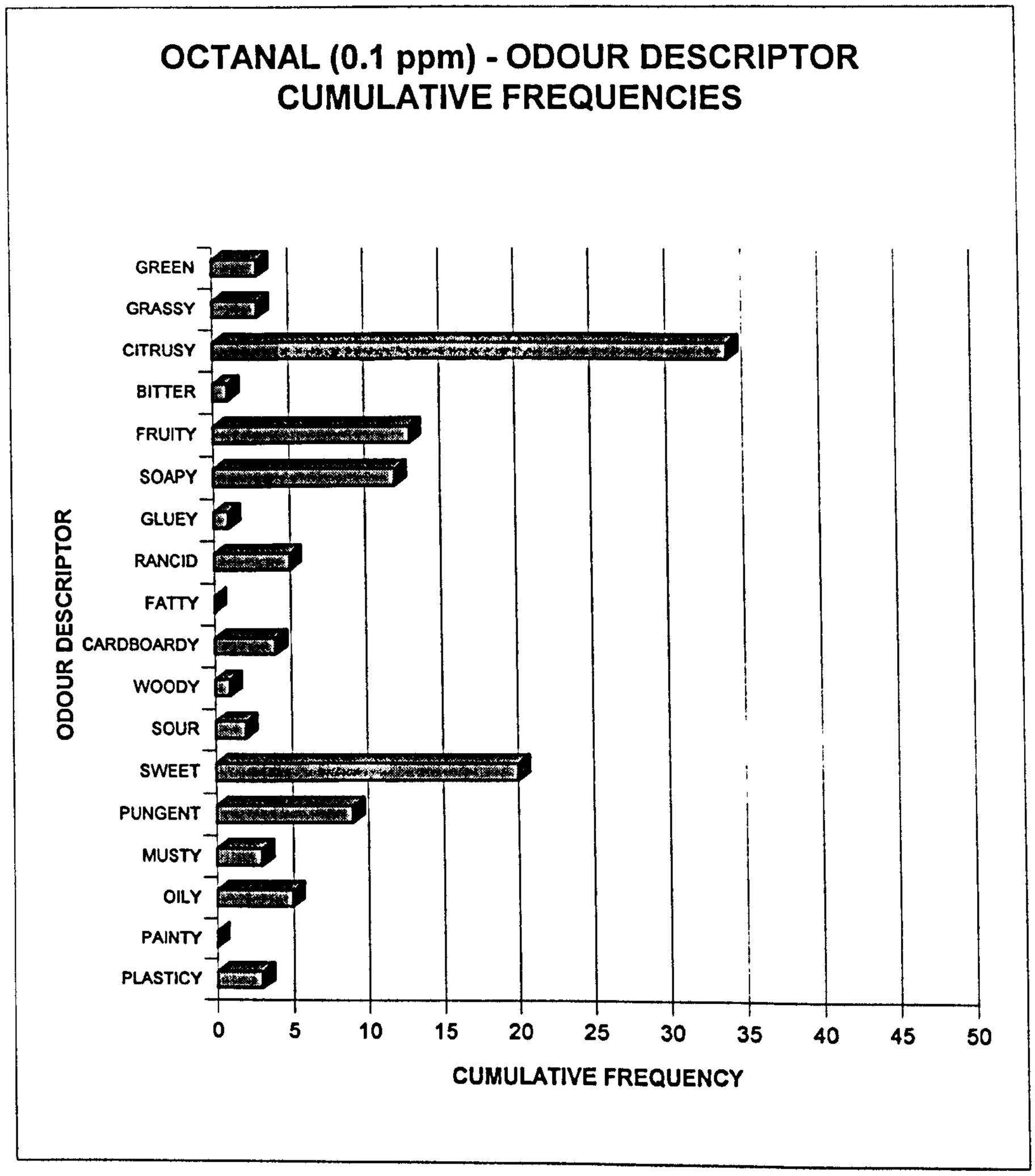
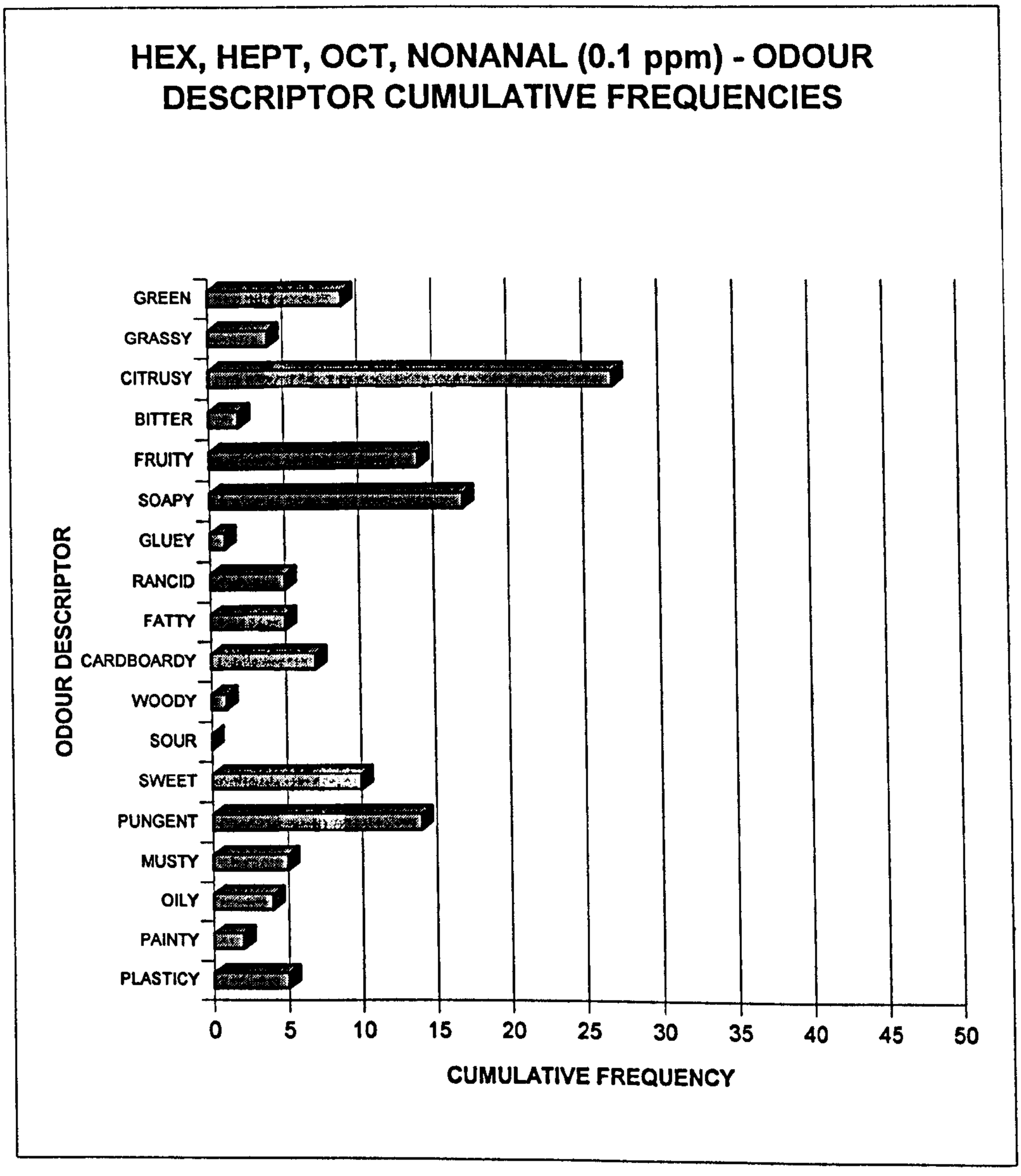

OCTANAL (0.1 ppm) - ODOUR DESCRIPTOR
CUMULATIVE FREQUENCIES
ODOUR DESCRIPTOR
GREEN
GRASSY
CITRUSY
BITTER
FRUITY
SOAPY
GLUEY
RANCID
FATTY
CARDBOARDY
WOODY
SOUR
SWEET
PUNGENT
MUSTY
OILY
PAINTY
PLASTICY
0
5
10
15
20
25
30
35
40
45
50
CUMULATIVE FREQUENCY

Figure A1.5


HEX, HEPT, OCT, NONANAL (0.1 ppm) - ODOUR DESCRIPTOR CUMULATIVE FREQUENCIES
ODOUR DESCRIPTOR
GREEN
GRASSY
CITRUSY
BITTER
FRUITY
SOAPY
GLUEY
RANCID
FATTY
CARDBOARDY
WOODY
SOUR
SWEET
PUNGENT
MUSTY
OILY
PAINTY
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CUMULATIVE FREQUENCY

3 CARTON SYSTEM CHARACTERISATION AND IMPLICATIONS FOR TAINT AND ODOUR

3.1 INTRODUCTION

A printed carton-board package is a complex system consisting of many different components within which are numerous individual chemical species. A large number of these species can be classed as being volatile.

Stora Kopparwhite and Koppargloss carton-board have a laminate structure consisting of a number of different layers. Kopparwhite is made up of two layers of peroxide-bleached chemical pulp sandwiching a layer of mechanical pulp or peroxide-bleached chemi-thermomechanical pulp (CTMP), depending on the grade of the board. On one side of the board laminate is an additional double coating. On the other side is a matt coating. The chemical pulp layers consist of 40% pine fibres and 60% birch fibres and make up 18-20% of the board by mass. The pine softwood produces long fibres, whilst the birch hardwood produces short fibres . The mechanical pulp or CTMP layer consists of 86% spruce fibres and 14% Mill Broke and makes up 60-64% of the board by mass.

Koppargloss has a similar structure to Kopparwhite except that the top layer has a different surface preparation. The mechanical pulp or CTMP layer is made from the same fibre types. However, the bottom chemical pulp layer is made from 100% pine fibres.

Wood contains a large number of organic and inorganic compounds resulting from the biosynthesis of a living system. Some of these compounds remain after processing within carton-board materials and are known as extractives.

Organic extractives include: resin acids, free fatty acids, neutral compounds, hydrocarbons, waxes, triglycerides, higher alcohols, diglycerides, monoglycerides and oxidised compounds (Fengel and Wegener, 1984). Some extractives are referred to as resins, a term that does not characterise the chemical compounds but rather their physical condition. Terpenes, lignans, stilbenes, flavonoids and other aromatics can be termed 'resins'.

Fatty acid content of softwood is approximately 0.3-0.4% (based on dry wood mass), as determined for Picea abies and Pinus sylvestris (Fengel and Wegener, 1984). Fatty acids are commonly esterified with glycerol in the form of glycerides. In addition, free fatty acids are also present as components of the extractives. Among the glycerides, the triglycerides are dominant in relation to mono- and diglycerides. The percentage of free fatty acids is considerably higher in heartwood than in sapwood.

More than 20 different fatty acids have been identified in softwoods (Fengel and Wegener, 1984). They tend to be saturated, monoenic, dienic and trienic acids with 16-22 carbon atoms. However, lower acids (C_{10}-C_{14}), higher acids (C_{24}-C_{30}) and tetraenic acids have also been detected.

The dominant fatty acids in various conifers appear to be oleic acid (C_{18}:1(9)), linoleic acid (C_{18}:2(9,12)) and 5,9,12-octadecatrienic acid (C_{18}:3(5,9,12)). Smaller amounts of palmitic acid (C_{16}:0), stearic acid (C_{18}:0), 14-methyl hexadecanoic acid (C_{17}) and 5,11,14-eicosatrienic acid (C_{20}:3(5,11,14)) have been found.

Much work has been carried out dealing with the relevance of the presence of unsaturated fatty acids on food oils and fats, with regard to taint and odour effects (Labuza, 1971; Selke *et al.*, 1980; Schieberle and Grosch, 1981; Grosch, 1987; Kochhar, 1993).

In the processing of food oils and fats, undesirable minor components such as coloured compounds, free fatty acids, trace metals, etc. are removed. However, major neutral lipids, such as triglycerides, are retained. Triglycerides may, therefore, be retained during the refining and pulping of wood.

Unsaturated fatty acids, present in esterified form, as triglycerides are susceptible to oxidation by atmospheric oxygen. Polyunsaturated fatty acids (PUFAS), containing two or more double bonds, particularly if they are methylene-interrupted double bonds in the *cis* configuration, are especially vulnerable to attack by atmospheric oxygen.

Oxidative deterioraton, as oxidative rancidity, leads to the development of objectionable flavours and odours in oils and in lipid-containing materials.

Söderhjelm and Sipiläinen-Malm (1996) state that odorous volatiles within different carton-board types decrease in the order: bleached mechanical and bleached chemical pulp composites > unbleached chemical and bleached

chemical pulp composites > unbleached chemi-mechanical and bleached chemical pulp composites > bleached chemical pulps.

Carton-board materials may be printed with ultraviolet (UV)-curable inks and overcoated with UV-curable varnishes (Holman, 1984; Oldring, 1991; Todd, 1994) .

On activation of these inks by exposure to UV radiation in the 200-400 nm waveband, free radicals are formed by excitement and breakdown of the photoinitiator(s) in the formulation. These free radicals react with the alkenic double bonds of the ink vehicle to form propagating chain species. Further reaction of the propagating chain species lead to oligomer chains that have the ability to cross-link, if more than one double bond is present in the propagating species. The result of these cross-linking, chain growth reactions is the formation of a cured ink film.

3.2 ANALYSIS OF VOLATILE COMPONENTS OF THE CARTON-BOARD PACKAGING SYSTEM

3.2.1 GAS CHROMATOGRAPHIC ANALYSIS OF CARTON-BOARD PACKAGING

The separation and resolution of volatile mixtures can be carried out effectively using the technique of gas chromatography (GC). Like all chromatographic techniques, GC separates mixtures by taking advantage of the analyte components' differential distribution between two phases - one stationery and the other moving past it. GC uses a gas as the moving, or mobile, phase. A sample of the mixture to be separated is introduced into this carrier gas stream just before it encounters the stationery phase; the components are separated by elution and are detected as they emerge within the carrier gas at the other end of the chromatography column. Components are distinguished by the different times which they take to pass through the column. This time lapse is known as the retention time.

The retention time of a substance is dictated by the position of its distribution equilibrium between the two phases. Separation, therefore, depends on the

components of a volatile mixture having significantly different distribution equilibria. If there is little interaction between molecules in the gas phase, the gaseous mobile phase plays a mainly passive role in the separation, serving merely to carry the components through the system. The distribution equilibria are effectively controlled by the components' vapour pressures and their sorption by the stationery phase.

It is important that separation is carried out at a temperature at which the components' vapour pressures are high enough to allow a realistically short analysis time, but at which differences between their vapour pressures are proportionally high.

The stationery phase has to be chosen so that it forms a stronger attraction for one component than for another. The stationery phase usually makes the greatest contribution to a separation, since interactions between it and the analyte components can be strong and varied. The most widely used stationery phases are liquid phases. GC, using a liquid stationery phase, is sometimes termed gas-liquid chromatography (GLC). Popular liquid stationery phases include silicone polymers and polyamides that will withstand the high temperatures used for the separation of higher molecular weight species.

Irregularities in the stationery phases of the original packed chromatographic columns, resulting in analyte peak broadening and low resolution, led to the use of capillary columns, or open tubular columns. In these, the stationery phase of the capillary column is coated on to the internal wall of a very narrow bore tube of considerable length. The capillary is normally made from fused silica that is up to 50 metres in length. The internal bore diameters range from 250μm to 530μm.

A further aspect of complex mixture analysis is that the components may have a very wide range of boiling points, so that no one analysis temperature would be suitable for all of them. For this type of mixture, a low temperature, that is appropriate to the most volatile component, is used at the start of the analysis. The temperature is then increased during the analysis until the least volatile component has been eluted at an appropriately high temperature. This technique is known as temperature programming.

The volatile analyte mixture is introduced to the GC via an injection port. Liquid samples are injected using a glass syringe, fitted with an appropriate needle, through a pierceable rubber septum. The area around the needle tip

consists of a replaceable glass liner which has been chemically deactivated. This part of the injector assembly is heated so that the liquid analyte rapidly vaporises and enters the carrier gas stream as a narrow band.

Only a fraction of the vaporised analyte mixture is introduced to the column, the rest may be vented to the atmosphere. This type of injection technique is known as split injection and is used for mixtures that have a high solvent content. During the splitting procedure, a larger amount of the more volatile solvent than the solute analyte components of interest is vented. Thus, a degree of sample concentration is achieved. The splitless injection technique involves the introduction of the majority of the sample onto the column. Only the very small proportion of residual vapours within the injector is vented, or swept clean, to the atmosphere after the bulk of the sample has entered the column. This technique can be used for trace analysis where the analytes of interest are present at very low levels.

Although the earliest gas chromatographic separations monitored elution from the column by titration, modern detection methods involve monitoring some physical, rather than chemical, property of the effluent gas stream. This is because most physical changes can be converted into an electrical signal which can then be amplified and recorded or manipulated in some other way. The physical property chosen must be one which changes significantly when the carrier gas becomes contaminated by a component eluting from the column. Many such properties have been exploited and over 50 different types of detector have been used.

In its simplest form, qualitative analysis is performed by means of retention data. The retention of a compound will be determined by the position of its distribution equilibrium between the stationery and mobile phases, i.e. the distribution ratio. Its retention data will, therefore, be characteristic of it, though not uniquely so. It is possible for several compounds, especially in a complex mixture, to have the same retention time. Some knowledge of the nature of the analyte compounds makes identification possible using retention time comparisons with standard compounds. The possibility of coincidence can not be ruled out since retention time is only one piece of information.

3.2.2 GAS CHROMATOGRAPHY COUPLED TO MASS SPECTOMETRY (GC/MS)

GC does not tell the analyst very much about a completely unknown compound. Molecular spectrometry, in its various forms, is far more informative. The benefits from combining the two techniques are quite clear, unfortunately, problems were encountered initially. GC produces separated components that emerge as very small amounts mixed with a very large amount of carrier gas in a fast moving stream at a little above atmospheric pressure. Mass spectrometry (MS), in particular, requires a small sample that has to be introduced into the instrument without reducing the vacuum significantly. The simplest way of removing the large amount of carrier gas from the eluted analytes is to introduce an outlet splitter to the column and pass the diverted gas stream into the mass spectrometer inlet system by way of a suitable flow restrictor. The restrictor can then be adjusted to reduce the gas flow into the mass spectrometer to a level at which its pumping system could cope. Only a small proportion of the separated component will, thus, find its way into the spectrometer. Therefore, the sensitivity would not be very high. However, the widespread use of capillary columns, with their low carrier gas flow rates, and with improvement in both the sensitivity and pumping systems of modern mass spectrometers means that the entire carrier gas flow can now be passed to the spectrometer.

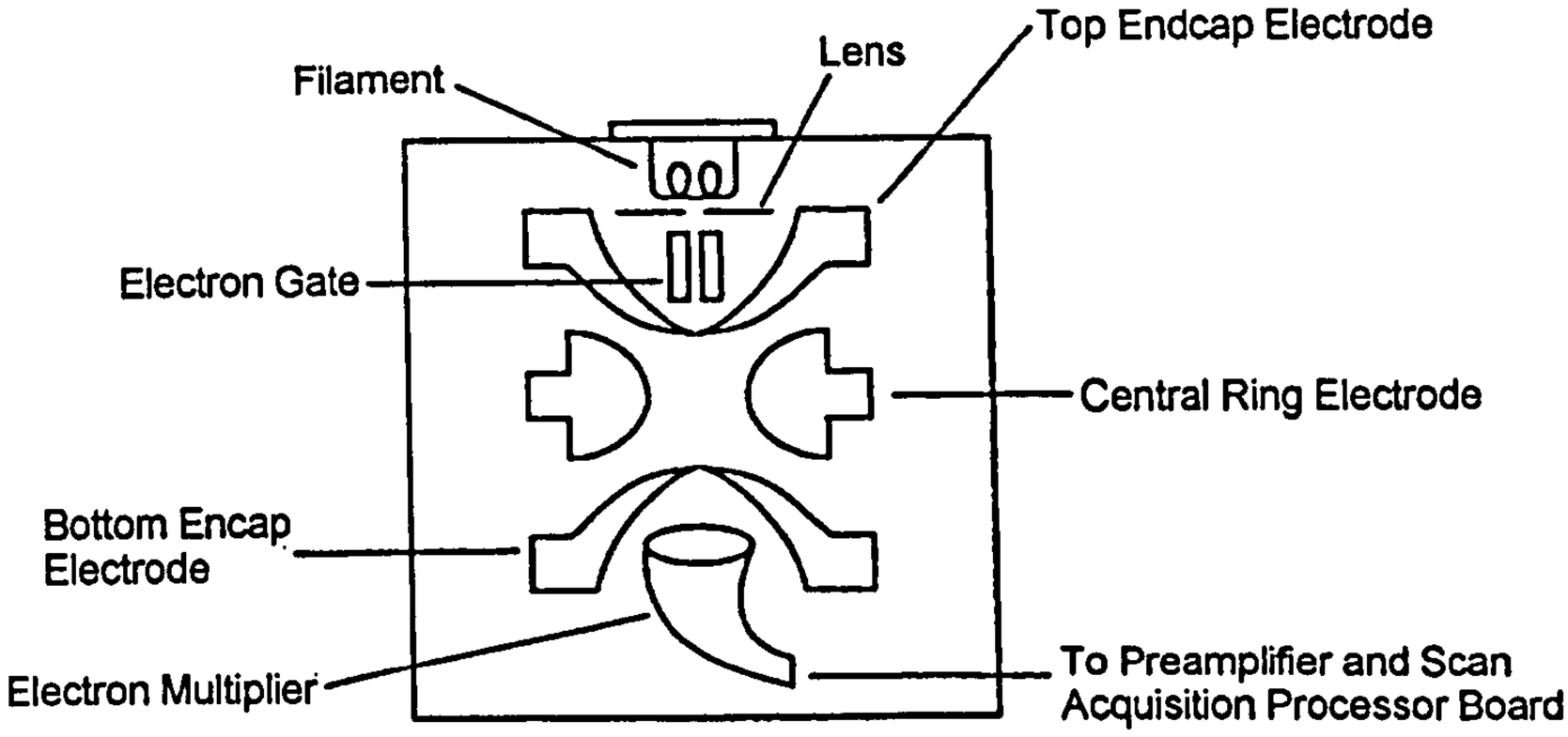
3.2.3 MASS SPECTROMETRY

Mass spectrometry (MS) was developed in 1920 and was used for the separation of isotopes. A mass spectrometer is an instrument that can establish the molecular weight and the structure of compounds.

Analyte molecules entering the mass spectrometer are ionised. The ions, once formed, undergo fragmentation and are collected, according to their mass/charge (m/z) ratios, by an electrical detector. The ion currents corresponding to the different ion species are amplified and stored within a computer to produce a mass spectrum for that compound. This mass spectrum is a plot of m/z against ion intensity and can be used to calculate the molecular structure of a compound and, thus, its identity.

One type of mass spectrometer is the ion trap mass detector. The configuration of the ion trap detector is shown (Figure 3.1). It consists of a central ring electrode, secured in the horizontal plane, with two domed end caps placed above and below it.

Figure 3.1 The ion trap mass spectrometer.



Source: Varian (1989)

The end caps are held at ground potential. The ring electrode has a radio frequency (RF) voltage applied to it. Electrons, from the filament, positioned above the top end cap, are allowed into the trap via an electron gate, wherein they can effect the ionisation of eluted analyte compounds allowed into the trap via the GC. Fragmentation occurs in the ion trap cavity. The RF voltage applied to the central ring electrode, keeps the ions circulating in a stable 'figure of eight' path. This storage of ions is the reason for the name 'ion trap' since the ions can be stored and trapped in this way indefinitely. Detection is not necessarily performed immediately after ionisation. After storage, the RF voltage is ramped, thus destabilising the ions in a vertical direction. 50% are neutralised on the top end cap and 50% travel through holes in the bottom end cap, to be detected. The signal from the ions is detected and amplified by an electron multiplier.

Destabilisation of all the ions does not occur at the same time. Ions of lower m/z are destabilised before those of higher m/z.

Ionisation occurs by the process of electron impact in which high energy electrons, produced by thermionic emission from a tungsten filament, are fired at the analyte molecules. On being bombarded, the molecular species loses an

electron to gain a net positive charge and form a radical $M^{\cdot}+$, the molecular ion. The molecular ion has the same molecular weight as the parent molecule. If the bombarding electrons have sufficient energy, the additional energy may be released by breaking certain chemical bonds of the molecular ion, producing fragments that are detected in the same way as the molecular ion. With electron impact, or electron ionisation (EI), predominantly positively charged ions are formed.

An ion striking the electron multiplier detector results in the release of an electron from the detector cathode, which subsequently collides with the inner surface of the multiplier, releasing more electrons, resulting in a cascade effect. These electrons are collected at the detector anode. The resulting ion current signal is passed on to the MS electronics for amplification and processing. The ion current signal is proportional to the total number of ions exciting the mass analyser.

The mass spectrometer produces a chromatogram by plotting the total ion current against time. As mentioned before, total ion current is proportional to the number of electrons in the ion trap at any one time. Thus, as a component is eluted from the column and enters the ion trap, the total ion current increases in proportion to the amount of the component present. A peak is produced of which the area is proportional to the amount of analyte it represents.

The total ion current (TIC) chromatogram is made up of individual scans, taken at various points throughout the GC separation. The mass spectrometer can be set up to acquire in the range of one scan every two seconds to ten scans per second. One scan per second is usually suitable for full scanning mass spectrometry. Each scan, represented by a scan number, consists of a complete mass spectrum for the contents of the ion trap cavity at a particular moment in time.

The mass spectrum produced for an analyte can supply enough information for identification of that component. Interpretation of the mass spectrum can be carried out by calculating the molecular structure of an analyte from the fragmentation pattern, the molecular ion, and the base peak. The base peak is the most intense peak within a mass spectrum to which all other peaks are normalised. The base peak usually represents formation of a stable ion fragment during ionisation and can be characteristic of a particular class of compound.

Analyte identification may, alternatively, be carried out by running a computer library search to find a suitable spectrum match with a data base of stored mass spectra. The computer conducts a library search based on three attributes. These are (i) purity; the signal to noise ratio of the spectrum peaks and the presence of interfering peaks, (ii) fit; how well the standard spectrum fits into the sample spectrum when they are overlaid, and (iii) reverse fit; how well the sample spectrum fits into the standard spectrum when they are overlaid. Each of these attributes is given a score out of 1000. The computer is set up to prioritise purity, fit, or reverse fit within a search. High values of three attributes indicate that the sample spectrum and the standard spectrum are a good match, i.e. positive identification has been achieved.

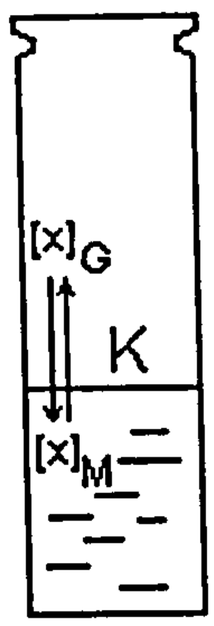
3.2.4 STATIC HEADSPACE SAMPLING OF VOLATILE COMPOUNDS

In an open system, volatile compounds existing in solid mixtures and liquid mixtures tend to evaporate to the atmosphere to varying degrees, depending on their volatility, substrate/volatile interactions and the temperature. In a closed system these volatiles are trapped in the air space above the sample. These volatiles will continue to migrate out of the liquid/solid matrix into the air space until the volatiles in the air space (or headspace) reach saturated vapour pressure. The migration of volatiles into the headspace is counteracted by the diffusion of volatiles back into the liquid/solid. When a condition of saturated vapour pressure is reached within the headspace, the migration of volatiles into the headspace is completely balanced by the diffusion of volatiles back into the liquid/solid matrix. At this point, the system is in an equilibrium state. This process is illustrated in Figure 3.2.

At equilibrium, the concentration of volatiles in the gas phase ($[x]_G$) is at its highest for a given temperature. As the temperature of the system is increased, the equilibrium of the system favours an increase in $[x]_G$ and a lower distribution coefficient.

During static headspace sampling, the sample of interest is sealed within a vial using a rubber septum cap. This sample is then equilibrated for a set period of time. To increase $[x]_G$, and reduce the time needed for the system to reach equilibrium, the sample is heated. At the end of this equilibration period, the vial is pressured with helium carrier gas.

Figure 3.2 The static headspace system.



$[x]_G$ = concentration of analyte in gas phase

$[x]_M$ = concentration of analyte in the sample matrix (liquid or solid)

$[x]_M/[x]_G$ = Distribution coefficient = K

A vent valve is then opened and the pressure within the vial displaces the headspace through a sample collecting loop to the atmosphere, filling the loop with the headspace contents. The vent valve is then closed. Injection of the headspace sample occurs when the sample loop is placed in line with the column carrier gas sweeping the vapours, via a transfer line, to the GC.

3.2.5 METHODS OF ANALYSIS FOR THE CARTON-BOARD SYSTEM

Static headspace sampling was carried out using a Varian Genesis Headspace Autosampler. Samples were introduced to the system within 22 cm^3 glass vials, sealed with PTFE-coated silicone rubber septa. Headspace acquisition was achieved using the developed protocol shown in Figure 3.3.

Figure 3.3 Static headspace acquisition method.

SAMPLE EQUIL. TEMP	50-105	°C
SAMPLE EQUIL. TIME	60	min.
SAMPLE LOOP TEMP.	130	°C
SAMPLE TRANSFER LINE TEMP.	130	°C
VIAL PURGE PRESSURE	15	psi

The sample to be analysed was equilibrated within the headspace unit for 60 minutes, at temperatures ranging from 50°C to 105°C. The sample loop and transfer line to the GC injector port were kept at 130°C, to prevent condensation and adsorption on to the internal walls of the system. The headspace vapours were purged from the vial into the sample loop for 30 seconds using helium at a pressure of 15 psi. Prior to purging, and after equilibration, solid and liquid samples were agitated within the headspace unit for 10 seconds to encourage the formation of vapours above the sample.

A Varian Saturn 3 GC/MS system was used for volatile compound analysis. The gas chromatograph was a Varian 3400CX fitted with a Varian 1077 split/splitless injector. The injector was fitted with a splitless injector liner and used in splitless mode. An injection temperature of 150°C was considered appropriate for analysis since the majority of compounds of interest would be vaporised at this temperature. The use of a higher temperature possibly results in an increase in the decomposition of volatiles within the injection port.

The chromatographic column used was a 30 metre DB-1 fused silica capillary with an internal bore diameter of 320μm. The liquid stationery phase was dimethylpoly(siloxane) which is non-polar in character. The stationery phase film thickness was 1μm. This particular column was chosen due to its robustness and its stability at higher column temperatures. The carrier gas used was helium (99.996% purity).

Figure 3.4 shows the developed GC acquisition method used for the analysis of components of the carton-board system. The method was developed over period of time to achieve optimum separation of volatiles in a convenient time cycle.

The mass spectrometer profile was developed over time and is shown in Figure 3.5. The instrument was set up to scan for ions with a m/z ratio range between 35 and 190. A background mass of 33 m/z was adopted to remove the possibility of interference and the higher baseline resulting from atmospheric oxygen and nitrogen, forming molecular ions at 32 m/z and 28m/z, respectively. In using a background mass of 33 m/z, all ions of lower m/z are ejected from the ion trap during the pre-scan stage of the full scan. A full scan

was conducted once a second. The mass defect can be defined as the difference between the nominal mass of an ion and its exact mass.

Figure 3.4 GC acquisition method

INJECTOR TEMP.	150	°C
COLUMN OVEN INITIAL TEMP.	40	°C
COLUMN OVEN FINAL TEMP.	229	°C
COLUMN RUN TIME	42	min.
TEMP.RISE	4.5	°C/min.
GC/MS TRANSFER LINE TEMP.	240	°C

The mass spectrometer reports masses to the nearest integer mass unit only. If the exact mass of an ion happens to fall close to the dividing line between integer masses, it may be assigned the wrong mass. This is more likely for ions with higher masses. Normally the mass defect is set to 0 in order to avoid mass misassignments.

Figure 3.5 Mass spectrometer acquisition method

Mass range	35	to	190	m/z
Scan time	1	second		
Segment length	42	minutes		
Fil/Mul Delay	0	minutes		
Peak threshold	1	count		
Mass Defect	0	mu/100u		
Background Mass	33	m/z		

Using static headspace analysis in the absence of high concentrations of solvents, a filament/multiplier delay was not needed. This delay is used during

liquid injections, or some headspace analyses, where a high elution of solvent may damage the filament and multiplier assemblies. It is set to coincide with the complete elution of the solvent band from the system.

3.2.6 CHARACTERISATION OF VIRGIN CARTON-BOARD

Figure 3.6 and Figure 3.7 show the chromatograms acquired from the headspace vapours of a 2 gram sample of Stora Koppargloss (440μm thickness) carton-board and a 2 gram sample of Iggesund Carton Excel carton-board (400μm thickness), respectively, both equilibrated at 105°C for 60 minutes. The X axis of the chromatogram represents the retention time of the analyte component. The X axis labels show the retention time in minutes (top) and the scan number (number of seconds) (bottom). The Y axis shows the total ion count response of the instrument with respect to time.

Figure 3.6 Chromatogram acquired from the headspace vapours of a sample of Stora Koppargloss (440 μm thickness).

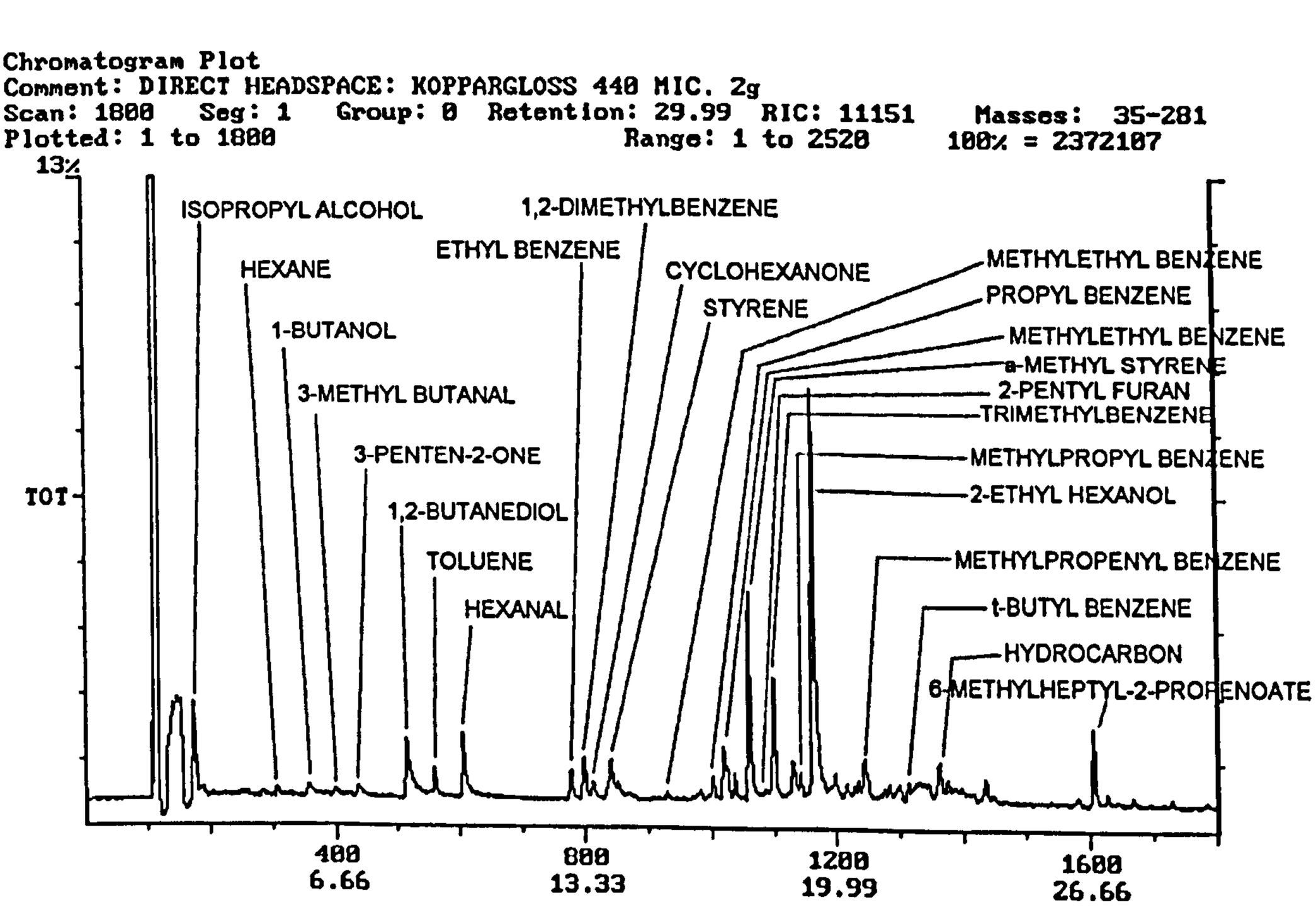
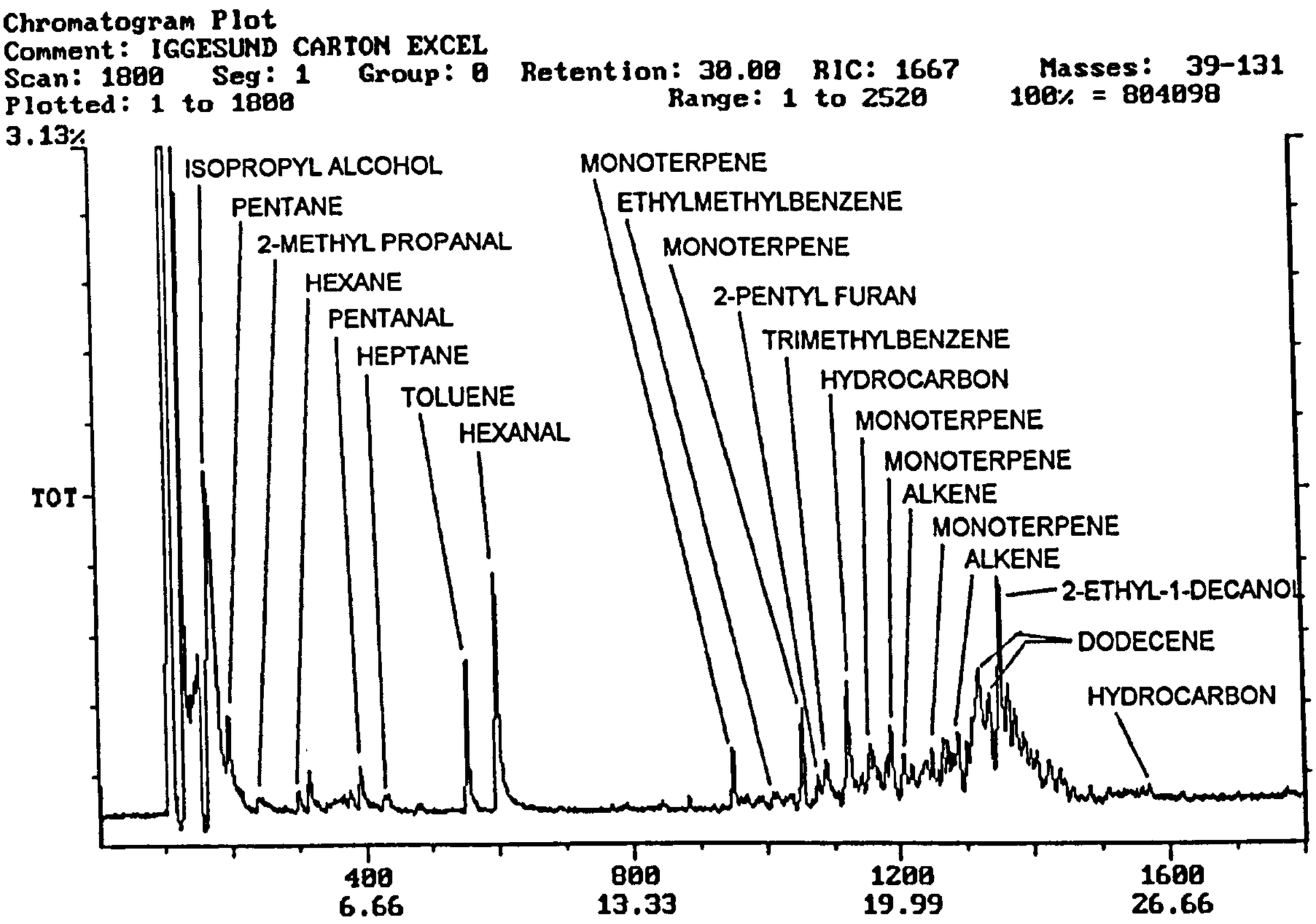


Figure 3.7 Chromatogram acquired from the headspace vapours of a sample of Iggesund Carton Excel (400 μm thickness).



The chromatograms show that there is a large number of volatile compounds present in the carton-board, of which many are odorous.

The nature of the compounds present can be broken down into two different sources:

(i) compounds associated with wood pulp such as the monoterpenes, aldehydes, ketones, esters, heterocyclics (furans) and some hydrocarbons.

(ii) compounds associated with the synthetic pigment binder used in the carton-board surface preparation. Examples include acyclics (substituted benzenes), hydrocarbons (alkanes and alkenes) and alcohols.

Small amounts of monoterpenes present in the carton-board system may remain after pulping and processing, or they may result from the breakdown of higher terpenes. Donetzhuber (1981) identified five monoterpenes and eight sesquiterpenes within pulp and paper samples.

The presence of aldehydes, ketones, esters, heterocyclics (furans) and some hydrocarbons, of which only a relatively small proportion can be found in fresh wood samples, can be explained by the reactions of the original resin compounds within the wood. These reactions occur by the attack of oxygen which can include activated singlet oxygen, hydroxyl radicals, ozone, superoxide anions, (perhydroxyl radicals at low pH) and hydrogen peroxide (Donetzhuber, 1981).

Autoxidation reactions of unsaturated species within the fresh wood occur by free-radical mechanisms. The processes involve an initiation, a propagation and a termination step. These will be discussed in detail at a later stage.

Many of the volatile oxidation products, particularly those containing functional groups such as carbonyl groups, have a strong odour and low detection thresholds. They need only be present in small amounts to cause a taint problem in a packaging system. The most dominant compound, resulting the oxidation of unsaturated species within the carton-board, is hexanal. This is apparent from both chromatograms above.

The presence of acyclics (substituted benzenes), hydrocarbons (alkanes and alkenes) and alcohols, can be attributed to the synthetic binder used in the surface preparation of the board. These compounds arise from impurities within the binder formulation and from the presence of residual monomers after polymerisation. The binders used can include a styrene/butadiene copolymer system or a styrene-acrylate system. Several volatile products have been identified where the binder is of the styrene/butadiene type (Tice, 1993). These include:

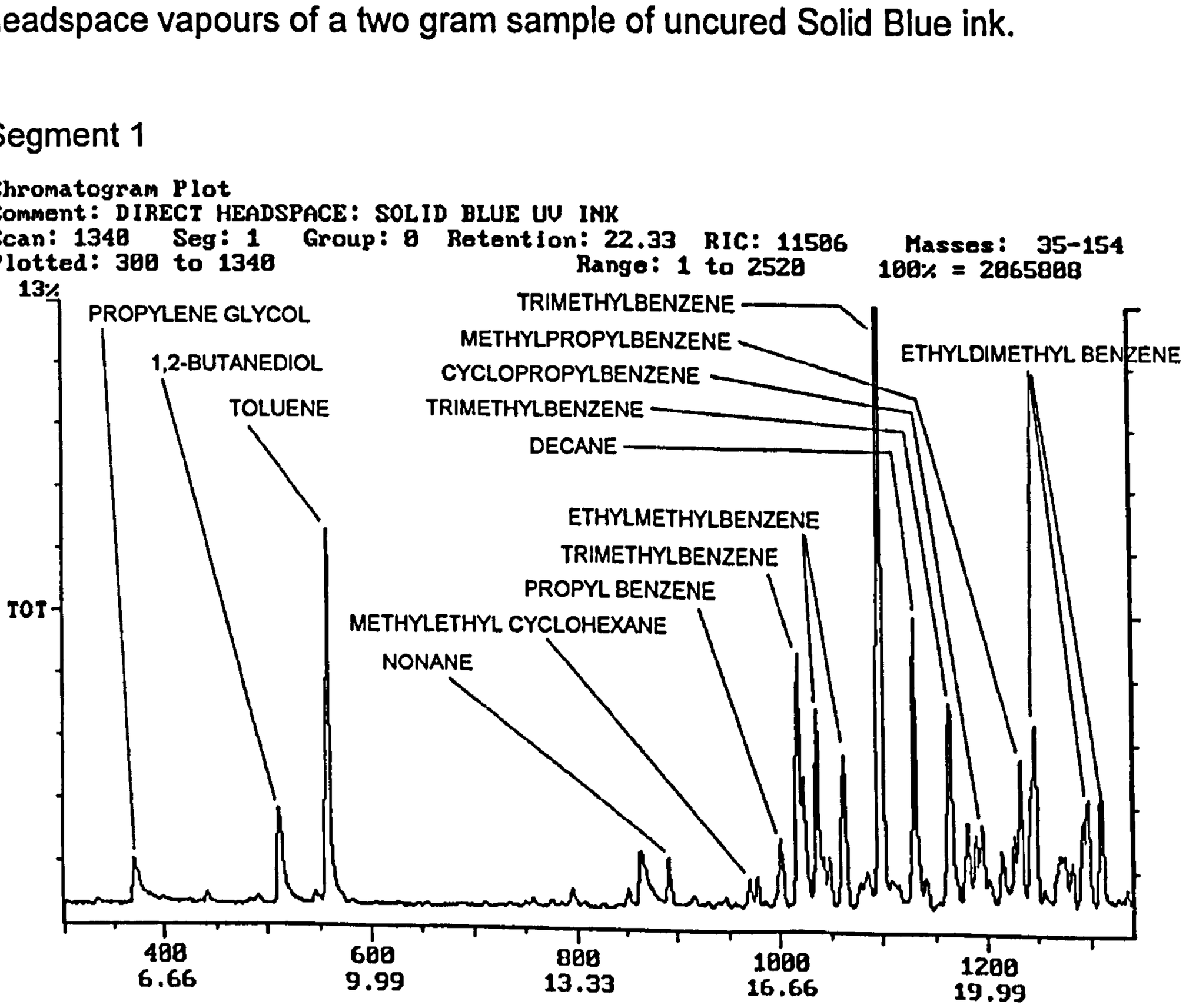
- vinyl cyclohexane
- ethyl benzene
- styrene
- cumene (isopropyl benzene)
- *n*-propyl benzene
- α-methyl styrene
- 4-phenyl cyclohexene
- other alkylated substituted benzenes

The chromatogram for the sample of Koppargloss carton-board (shown in Figure 3.6) shows the presence of styrene, ethylbenzene, α-methylstyrene, *n*-propyl benzene, and numerous other alkylated benzenes, indicating that the synthetic binder consists of a styrene copolymer. It should be noted that since production of the this carton-board sample, Stora have stopped using a styrene based copolymer binder in their Koppargloss products. The chromatogram for the Carton Excel (Figure 3.7) shows an absence of styrene and lower numbers of other acyclic compounds, indicating that a styrene binder system has not been used.

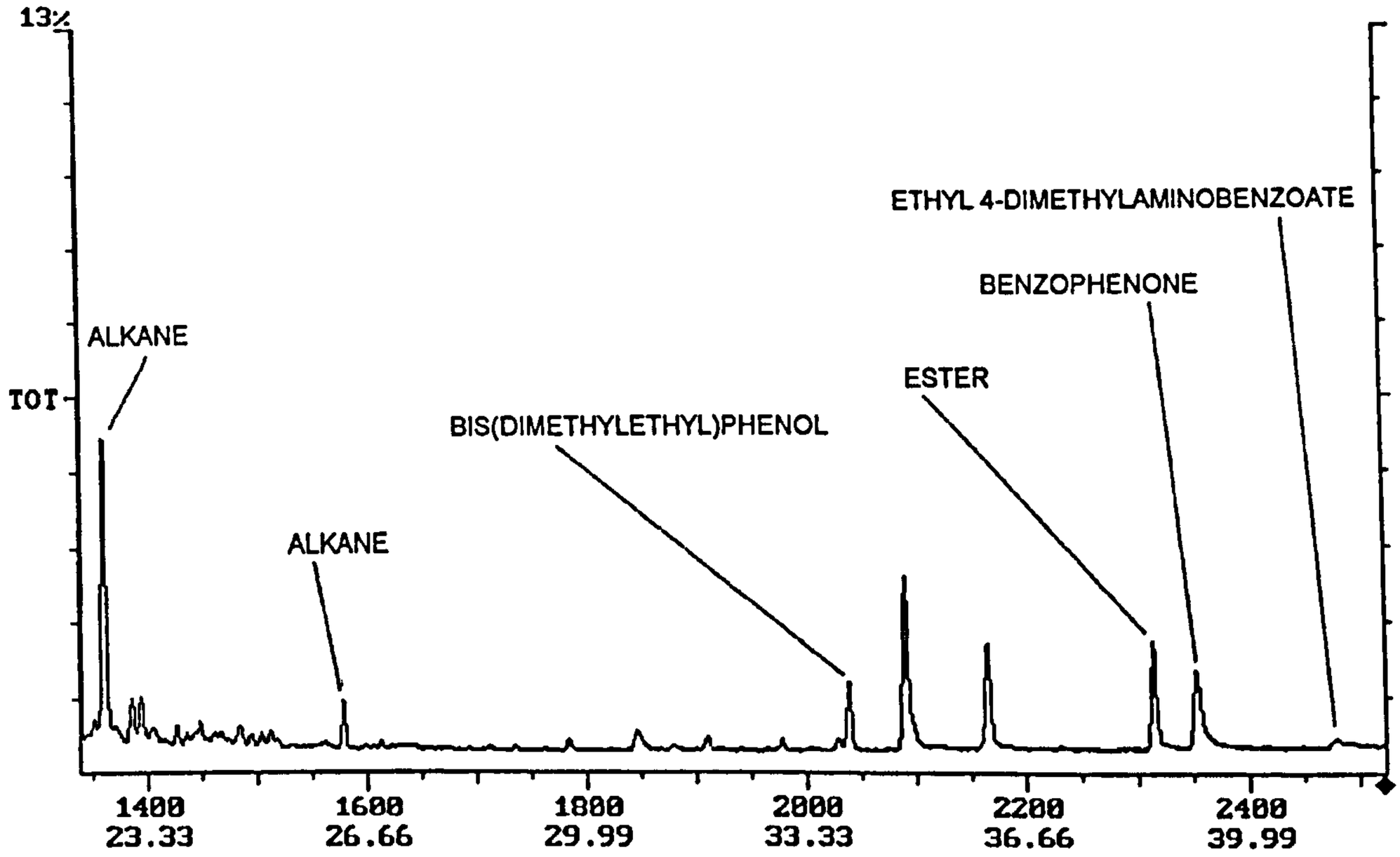
3.2.7 CHARACTERISATION OF UV-CURABLE OFFSET LITHOGRAPHIC INKS

Figure 3.8 shows the chromatogram of the headspace vapours acquired from a two gram sample of uncured Solid Blue ink.

Figure 3.8 Chromatogram (segment 1 and segment 2) acquired from the headspace vapours of a two gram sample of uncured Solid Blue ink.



Segment 2



Solid Blue is a blend of two Unicure inks made by Coates Lorilleux, Orpington, Kent. It is used to print cartons with the characteristic blue of Cadbury's Dairy Milk products.

The uncured ink contains a number of volatile compounds. These include substituted benzenes, alkanes and alcohols that are present in residual amounts after the manufacture of the ink prepolymer and diluent components of the ink formulations. The prepolymers and diluents are synthesised in solvent systems. These solvents are not entirely removed after processing.

The presence of an ester and of bis(dimethylethyl)phenol is likely to result either from unreacted species associated with the production of the prepolymers or from by-products of the production process.

The presence of benzophenone clearly indicates the use of this compound as a photoinitiator in the ink formulations. The presence of ethyl 4-(dimethylamino) benzoate indicates the use of this compound as a photoactivator.

The chromatogram shows no evidence of reactive diluents, suggesting that these compounds are absent or that they have relatively low volatility and

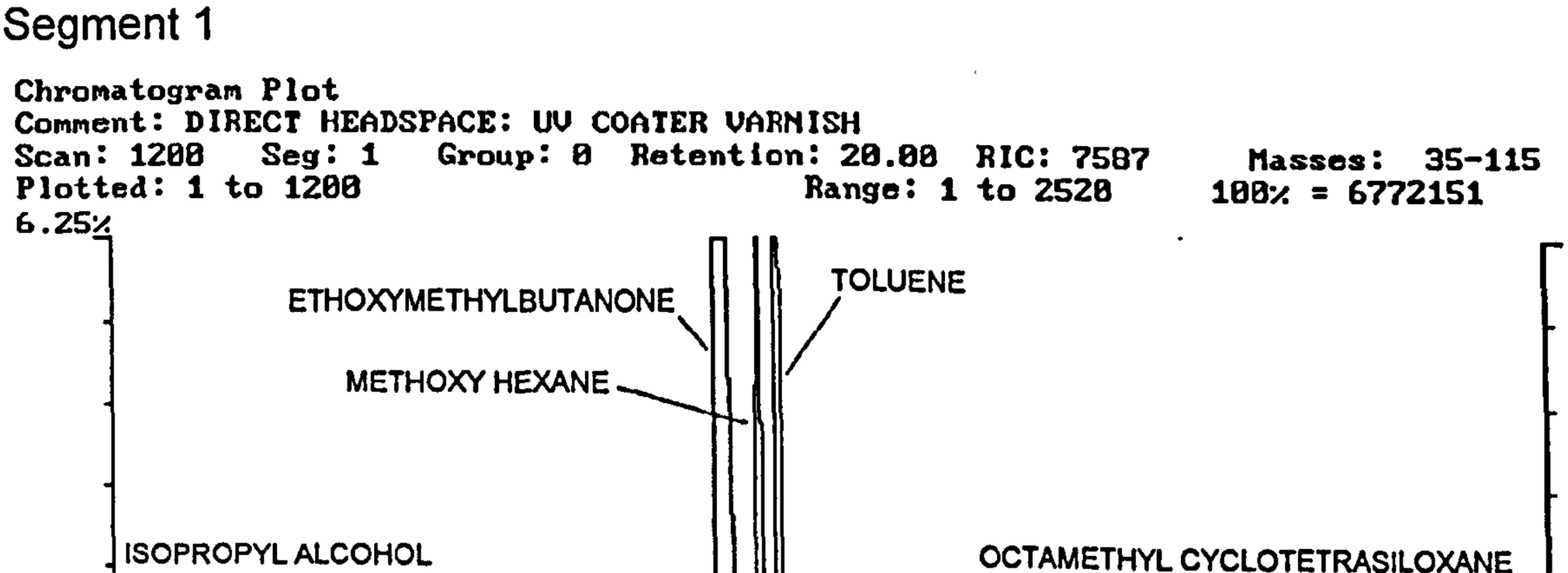
insufficient vapour pressure, at the headspace equilibration temperature used, to be detected.

3.2.8 CHARACTERISATION OF THE UV-CURABLE OVER-VARNISHES

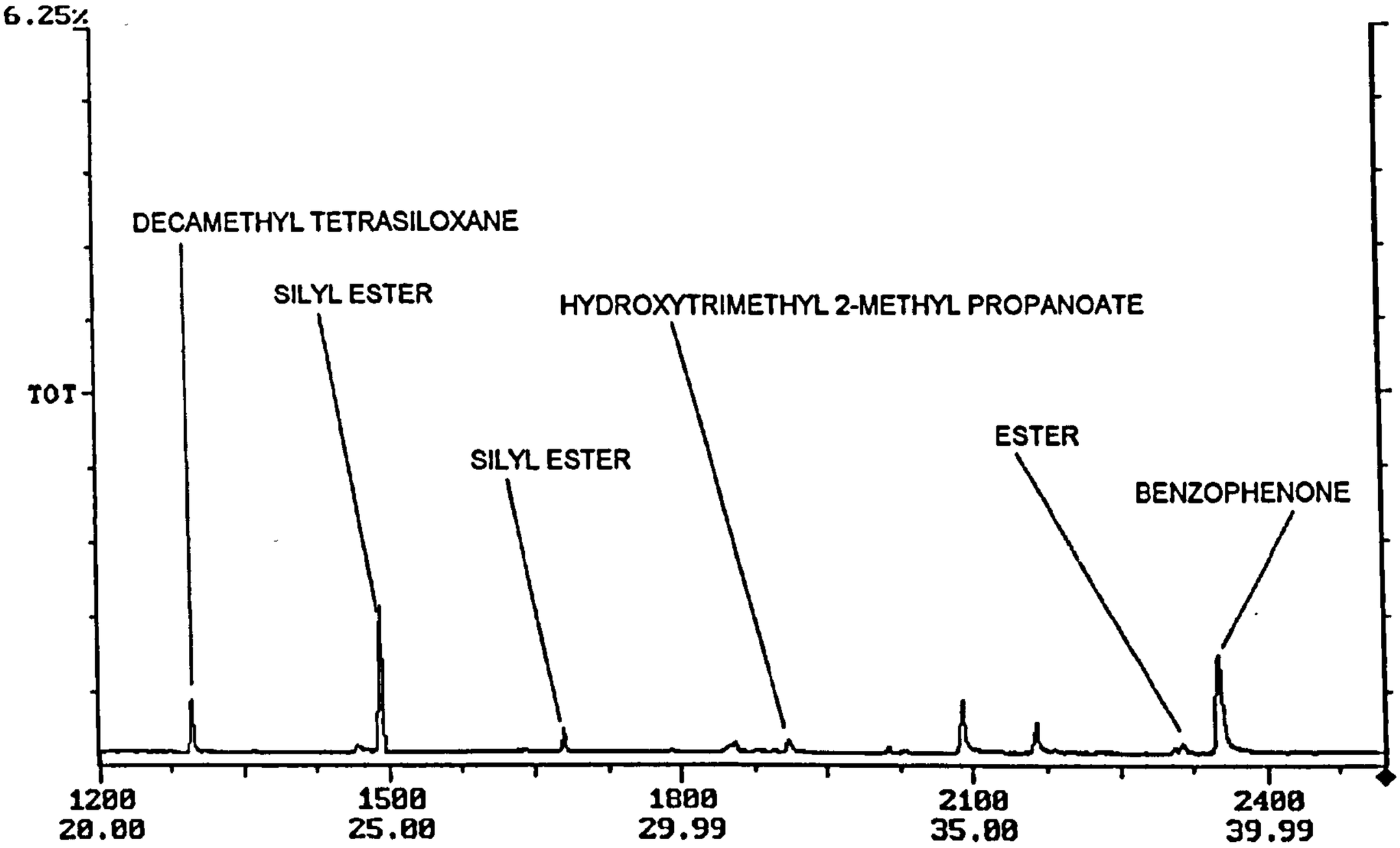
Figure 3.9 shows the chromatogram acquired from a two gram sample of uncured UV-curable coater varnish.

The volatile components of the varnish are somewhat different to those of the inks. This indicates a difference in nature between the two systems. The three more dominant volatiles appear to be two ethers and toluene. The ethers may be present as impurities or by-products of the inclusion of polyether acrylate reactive monomers in the formulation. Toluene may be a residual solvent, remaining after monomer synthesis.

Figure 3.9 Chromatogram (segment 1 and segment 2) acquired from the headspace vapours of a two gram sample of UV-coater varnish.



Segment 2



A number of silicon containing compounds is present. They were incorporated probably to improve the slip and abrasion resistance characteristics of the cured varnish film. Benzophenone is present as the photoinitiator. However, no amine was evident. This suggests that the formulation may contain a higher molar mass amine. For example an acrylated amine which has a higher molecular weight and lower volatility.

3.2.9 CHARACTERISATION OF THE FOUNTAIN SOLUTION

During the lithographic printing of carton-board, a fountain solution is used. This solution serves two major functions:

- to keep non-image areas of the printing plate clean and free from ink
- to help minimise the amount of water needed to obtain a clean print and so-assist in the maintenance of ink/water balance during printing

The main component of the fountain solution is a desensitiser that regenerates the non-image areas of the printing plate, making them very hydrophilic. The

hydrophobic ink, therefore, has very little affinity for these areas, maintaining non-image areas free from ink. A commonly used desensitiser is litho gum.

Other components of fountain solution include: a pH buffer system and preservatives to prevent growth of fungi and bacteria. The pH is important. At too high a pH value excessive ink/water emulsfication can occur. With too low a pH ink drying difficulties can result. The ideal running pH is generally regarded as being between 5 and 7.

One fountain solution concentrate that is widely used in the lithographic printing industry is Dupont Fountsol F-5. The components of this fountain solution are litho gum, a citric acid/sodium citrate buffer, tetrasodium EDTA, Bacteron B6 and linamine turquoise dye.

The fountain solution is buffered to ~pH 5. Tetrasodium EDTA (ethylenediamine tetraacetate) is used as a complexing agent to remove soluble calcium salts. If these salts are not removed, problems with press roller stripping and ink/water emulsification can occur.

Isopropyl alcohol (IPA) is usually added with the fount solution. The alcohol speeds up the evaporation of moisture from the printing plate surface between dampening and inking and results in less ink/water emulsification.

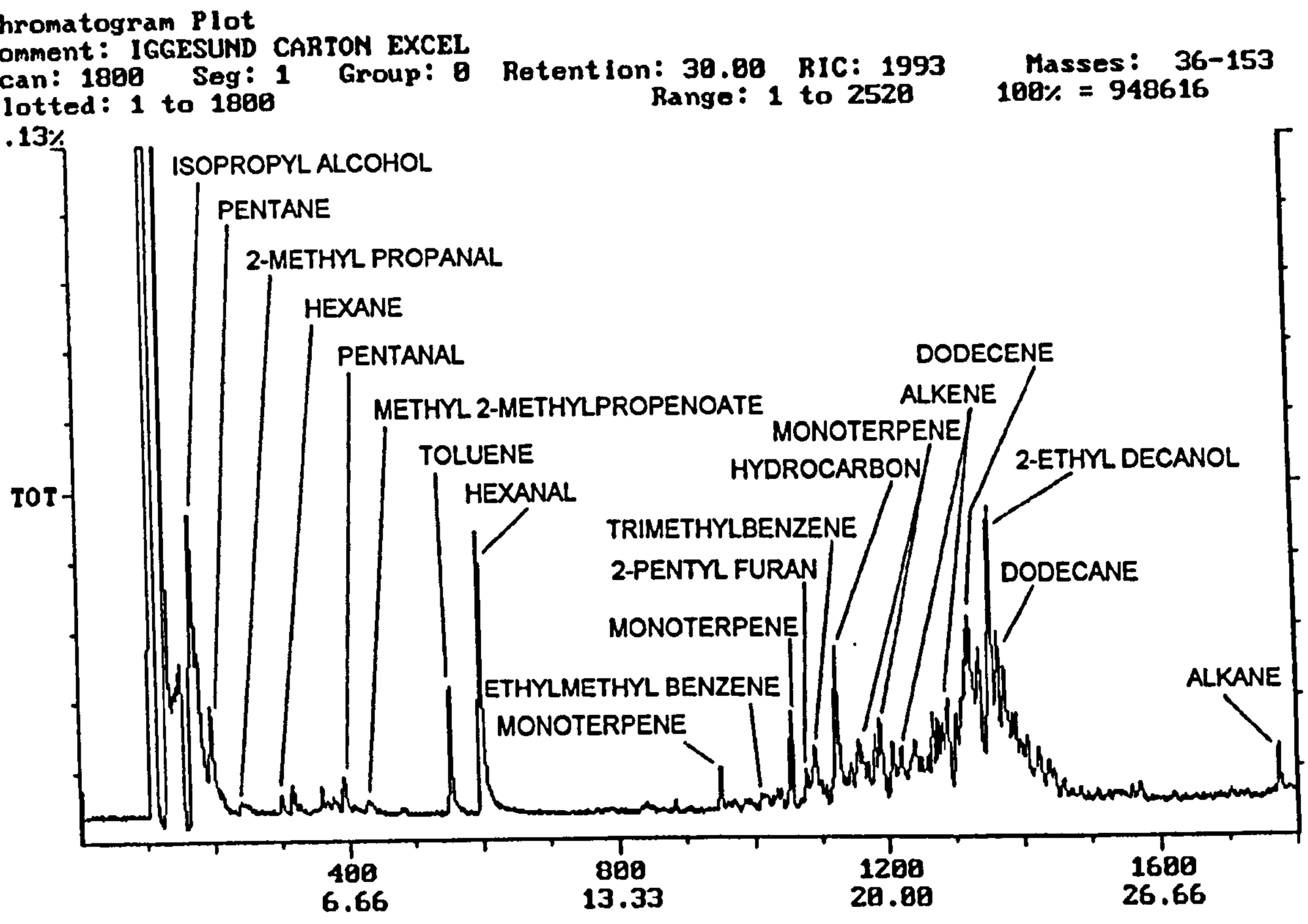
Fountain solution concentrate may be added to the press reservoir, at a loading of 5%(v/v) and IPA at a loading of 4-8%(v/v) in de-ionised water.

It is inevitable that some of the fountain solution/IPA will come into contact with the carton-board during printing. Factors such as press geometry and printing speed will determine the degree of contact. Headspace analysis of the fount solution concentrate showed that no significant volatiles were present within the fount formulation.

3.2.10 CHARACTERISATION OF THE PRINTED CARTON-BOARD SYSTEM

The printed carton-board system consists of the carton-board, the cured lithographic inks, the coater varnish and, possibly, a small amount of fountain solution and IPA. Figure 3.10 shows the chromatogram acquired from the headspace vapours of a two gram sample of printed Iggesund Carton Excel carton board. Figure 3.11 shows the chromatogram acquired from the headspace vapours of a two gram sample of printed Carton Silkia carton-board.

Figure 3.10 Chromatogram acquired from the headspace vapours of a two gram sample of printed Carton Excel.

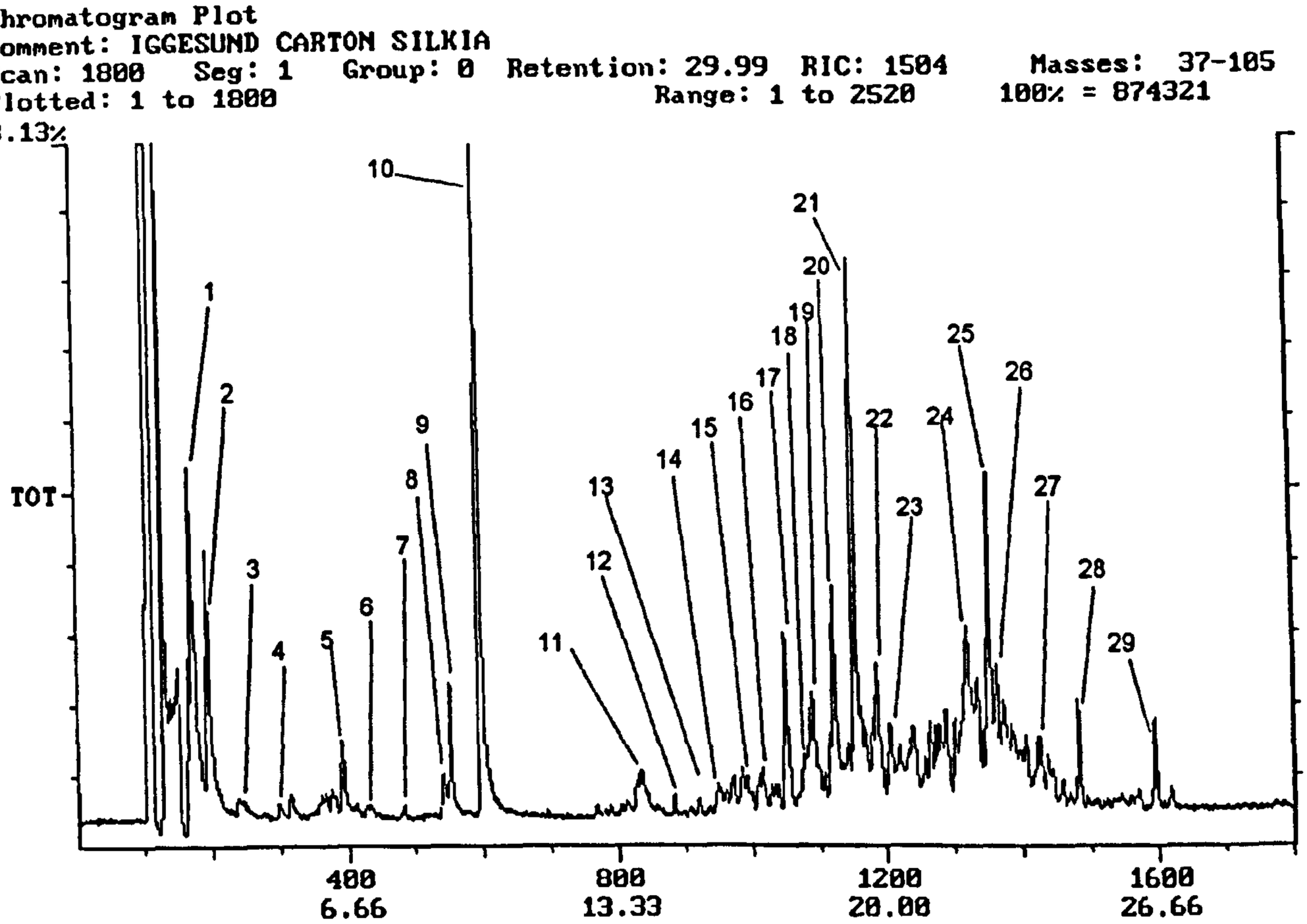


The chromatograms acquired from printed board samples do not show significant amounts of volatile compounds that might have originated from the inks and varnish. This suggests that these compounds are effectively bound up during the curing process and are not free to enter the vapour phase.

One of the more dominant volatiles in both printed samples of board appears to be hexanal. There are variations in the recoveries of other volatiles from each board type, indicating their diverse nature.

The technique of static headspace GC/MS can be used quantitatively to determine the amounts of identified volatiles (Mahungu, 1994a and 1994b). This can be achieved using an external standard calibration series so as to avoid interference of standard peaks with sample peaks, that may occur using internal standard calibration series.

Figure 3.11 Chromatogram acquired from the headspace vapours of a two gram sample of printed Carton Silkia.



Peak assignment

Peak No.	Compound	Peak No.	Compound
1	IPA	16	ethylmethyl benzene
2	pentane	17	α-methylstyrene
3	2-methyl butanal	18	2-pentyl furan
4	hexane	19	trimethylbenzene
5	pentanal	20	hydrocarbon
6	methyl 2-methylpropenoate	21	2-ethyl hexanol
7	methyl cyclohexane	22	monoterpene
8	1-pentanol	23	alkene
9	toluene	24	dodecene
10	hexanal	25	2-ethyl decanol
11	styrene	26	dodecene
12	monoterpene	27	dodecene
13	ethylmethyl benzene	28	alkyl silane
14	butoxypropanol	29	6-methylheptyl 2-propenoate
15	propyl benzene		

The approach involves using high purity samples of the volatile compounds of interest and carefully producing a series of standards of increasing concentration. These standards were produced within headspace vials by injecting a known amount of the standard compound in to an inert substrate that represents the board sample. The substrate used for all quantitation standards was vacuum-purified CTMP.

To prevent loss of volatiles from the vial, the standard compounds were injected through the septum of the sealed vial. Prior to sampling, an internal standard was injected into the vial. This was used to remove any errors associated with variation, from sample to sample, in the amount of headspace vapour trapped within the sample loop of the headspace autosampler. An internal standard was also injected into samples to be quantified, to remove this source of error. The internal standard used was benzene (99.9% purity), at a volume of 1 nL.

Calibration standards were equilibrated under the same conditions as were used for the unknown samples, i.e. 60 minutes at 105°C.

Calibration curves were produced of standard compound peak area versus standard compound concentration. The peak area taken was not that for the total ion chromatogram, i.e. the full mass spectrum, but that for a specified quantitation ion. The m/z value taken for this ion was determined by the abundance of the ion within the mass spectrum for that standard compound and by ions associated with nearby or overlapping component peaks. Ideally, a m/z value is taken for an relatively abundant ion, that is unique to the compound of interest, and does not appear in the mass spectra of components with similar retention times. The use of selected ion monitoring usually removes problems associated with the integration of co-eluting peaks and background interference.

Table 3.1 shows the amounts of volatile compounds (in nL) recovered from a two gram sample of printed Carton Excel using external calibration standard series. The component peak retention times and quantitation ion values are also given. Table 3.2 shows the amounts of volatile compounds (in nL) recovered from a two gram sample of printed Carton Silkia using external calibration standard series.

A large proportion of the volatiles that were sought and quantified are odorous. However, the calculated amounts of the volatiles recovered from the two samples of printed board are very low (1-16 nanolitres, or 1-16 ppm (parts per million)) based on the mass of board. At these levels, the odorous volatiles are not present in sufficient quantities to cause a problem. This finding confirms that the carton-board samples were organoleptically acceptable.

Table 3.1 Amounts of volatile compounds (in nL) recovered from a two gram sample of printed Carton Excel using static headspace GC/MS analysis .

NAME OF COMPOUND	RETENTION TIME (in minutes)	QUANTITATION ION (in m/z)	CALCULATED AMOUNT (in nL)
Pentanal	6.54	58	8
Hexanal	9.93	44	10
Heptanal			
Octanal			
Nonanal	22.21	57	2
trans 2-heptenal	16.03	83	3
trans 2-octenal	20.29	70	6
2-Pentanone			
2-Hexanone			
2-Heptanone	13.64	58	1
6-Undecanone			
1-Butanol			
1-Pentanol			
1-Octen-3-ol	17.25	57	1
1-Butoxy-2-propanol			
Methyl octanoate			
Pentyl pentanoate			
2-Pentyl furan	17.94	138	1
Pentane	2.86	41	2
Heptane	7.25	71	1
Nonane	14.71	57	4
Decane	18.71	57	1
1-Hexene			
1-Heptene			
1-Undecene	22.01	55	4
1-Dodecene			
Toluene	9.20	91	7
Ethylbenzene	12.81	106	4
1,2-Dimethylbenzene	14.11	106	7
Styrene	13.91	104	2
a-Methyl styrene	17.54	118	1
Propylbenzene	16.56	91	4
1,2,3-Trimethylbenzene	19.28	105	8
1,3,5-Trimethylbenzene	17.14	105	1

The choice of particular quantitation standard compounds was based upon the dominant volatiles found in carton-board samples, in addition to the volatile oxidation products found during static headspace analysis of oxidised unsaturated lipid systems. This will be discussed in detail at a later stage.

Table 3.2 Amounts of volatile compounds (in nL) recovered from a two gram sample of printed Carton Silkia using static headspace GC/MS analysis .

NAME OF COMPOUND	RETENTION TIME (in minutes)	QUANTITATION ION (in m/z)	CALCULATED AMOUNT (in nL)
Pentanal	6.51	58	8
Hexanal	9.93	44	16
Heptanal	13.95	70	1
Octanal	18.08	43	1
Nonanal			
trans 2-heptenal	16.01	83	4
trans 2-octenal	20.29	70	6
2-Pentanone			
2-Hexanone	9.66	58	1
2-Heptanone	13.54	58	1
6-Undecanone			
1-Butanol	5.81	56	5
1-Pentanol	8.99	42	8
1-Octen-3-ol	17.34	57	1
1-Butoxy-2-propanol			
Methyl octanoate			
Pentyl pentanoate			
2-Pentyl furan	17.93	138	1
Pentane	2.86	41	2
Heptane	7.24	71	1
Nonane	14.73	57	4
Decane	18.71	57	1
1-Hexene			
1-Heptene	6.93	56	7
1-Undecene	22.11	55	3
1-Dodecene			
Toluene	9.18	91	7
Ethylbenzene	12.83	106	4
1,2-Dimethylbenzene	14.06	106	7
Styrene	13.89	104	3
a-Methyl styrene	17.51	118	1
Propylbenzene	16.54	91	4
1,2,3-Trimethylbenzene	19.28	105	8
1,3,5-Trimethylbenzene	17.13	105	7

3.3 OXIDATION WITHIN THE CARTON-BOARD PACKAGING SYSTEM AND ITS IMPLICATIONS FOR TAINT AND ODOUR CHARACTERISITCS

3.3.1 DETERMINATION OF THE FACTORS AFFECTING CTMP

In order to determine how different factors affect carton board materials, and how they might influence taint and odour characteristics, samples of CTMP (chemi-thermomechanical pulp) were obtained from the Stora Fors Mill, Fors,

Sweden. Samples were supplied in the form of a damp pulp, sealed in black poly(ethylene) bags. On receipt, these samples were stored in a fridge, at 2°C, until they were needed. This was to reduce the rate of any on-going oxidation processes within the pulp. Production of CTMP in a board format, was carried in the following way:

20-100g of damp pulp was placed in a rotary blender with 1dm^3 of deionised water, at 80°C. The pH of the resulting mixture was adjusted to pH 7-8 using small amounts of dilute sodium hydroxide solution. This mixture was then pulverised for 3 minutes. The resulting suspension was poured into an 18.5cm Buchner funnel fitted with a low ash filter of medium flow characteristics. The water was, subsequently, drawn off under reduced pressure and then resubmitted to the funnel to ensure that any residue, still present in the filtrate, was not lost. The resulting filter cake was left to dry on the funnel for 2-3 minutes before it was removed, pressed between two paper towels and placed in an oven at 50°C for approximately 20-30 minutes to dry. Round, flat paper sheets were produced. These were wrapped in aluminium foil and stored at room temperature until needed.

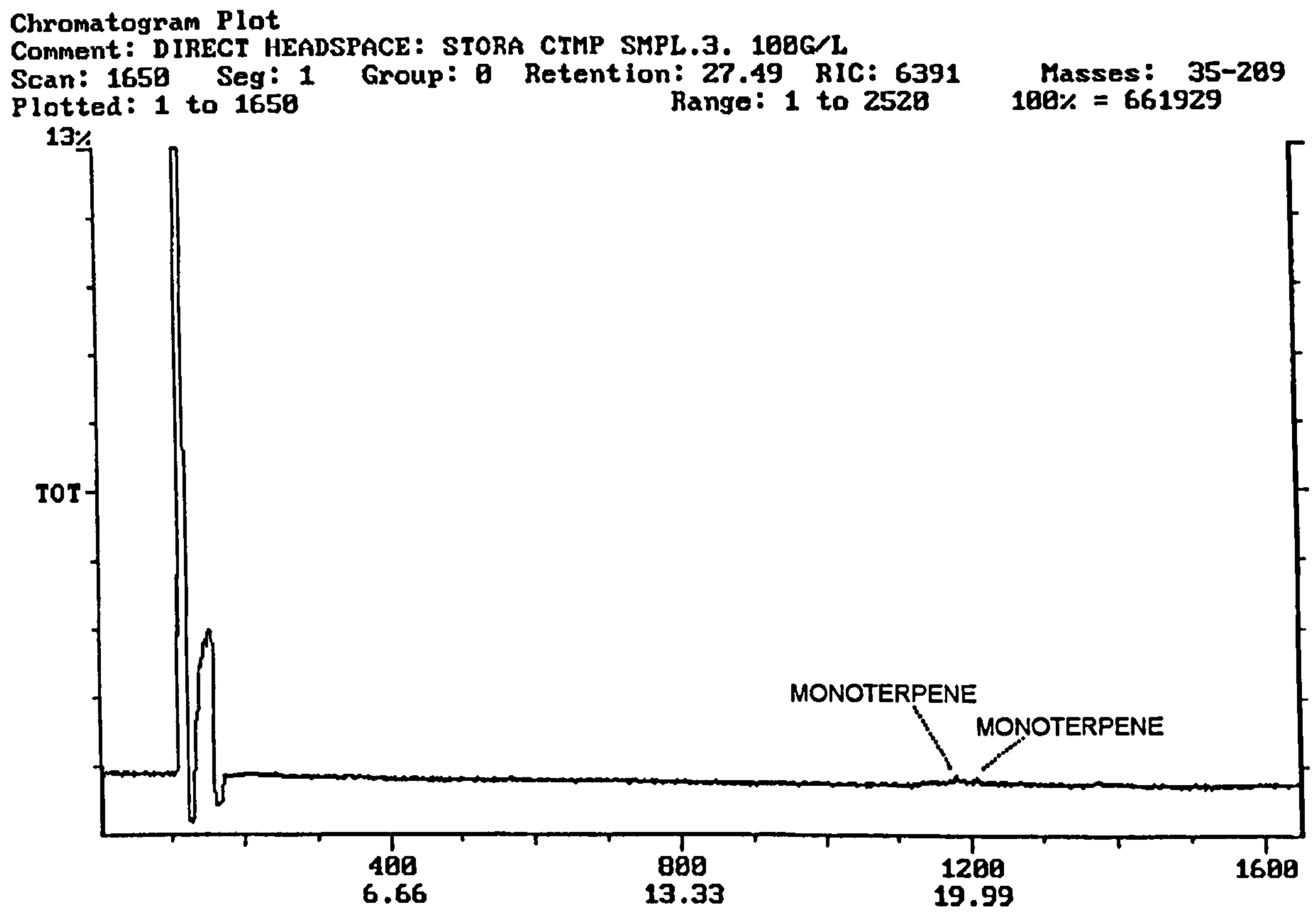
3.3.1.1 The effect of storage time on the volatiles present in CTMP sheet

The initial CTMP paper samples were made using 100g of CTMP, pulverised in the presence of 1 litre of water. The resulting paper sheets were very thick, averaging 6-7mm. Later paper samples were made using only 20g of CTMP. These had an thickness of approximately 2mm. Samples of the former type were used as the benchmark to determine the effects of time on the volatile content of CTMP paper samples.

For the determination of time dependence of volatile compound formation, samples of one particular CTMP paper sheet were taken for static headspace analysis at various times over an extended period. The first sample was taken approximately two hours after the extruded sheet was made. Further samples were taken after 1, 2, 4, 8, 9, 16, 22 and 35 days. Between sampling, the sample of paper sheet was wrapped in aluminium foil and stored at room temperature in a clean atmosphere.

Figure 3.12 shows the chromatogram acquired shortly after the sample was made. No major components are present and the two components identified were monoterpenes, represented by very small peaks, with characteristic molecular ions of m/z= 136.

Figure 3.12 Chromatogram acquired from the headspace vapours of a sample of freshly prepared CTMP sheet.



The chromatogram representing a sample taken 24 hours later shows three broad unresolved 'humps' indicating hexanal, nonanal and decanal, respectively, and is shown in Figure 3.13.

The presence of three monoterpenes was also seen as three very small peaks. The sample taken after two days showed slightly larger and more highly resolved peaks for hexanal, nonanal and decanal. After four, eight and sixteen days storage, the peaks for hexanal, nonanal and decanal appear to have remained essentially unchanged. However, at 22 days significant developments were seen.

Figure 3.13 Chromatogram acquired from the headspace vapours of a sample of laboratory prepared CTMP sheet stored for 24 hours at room temperature.

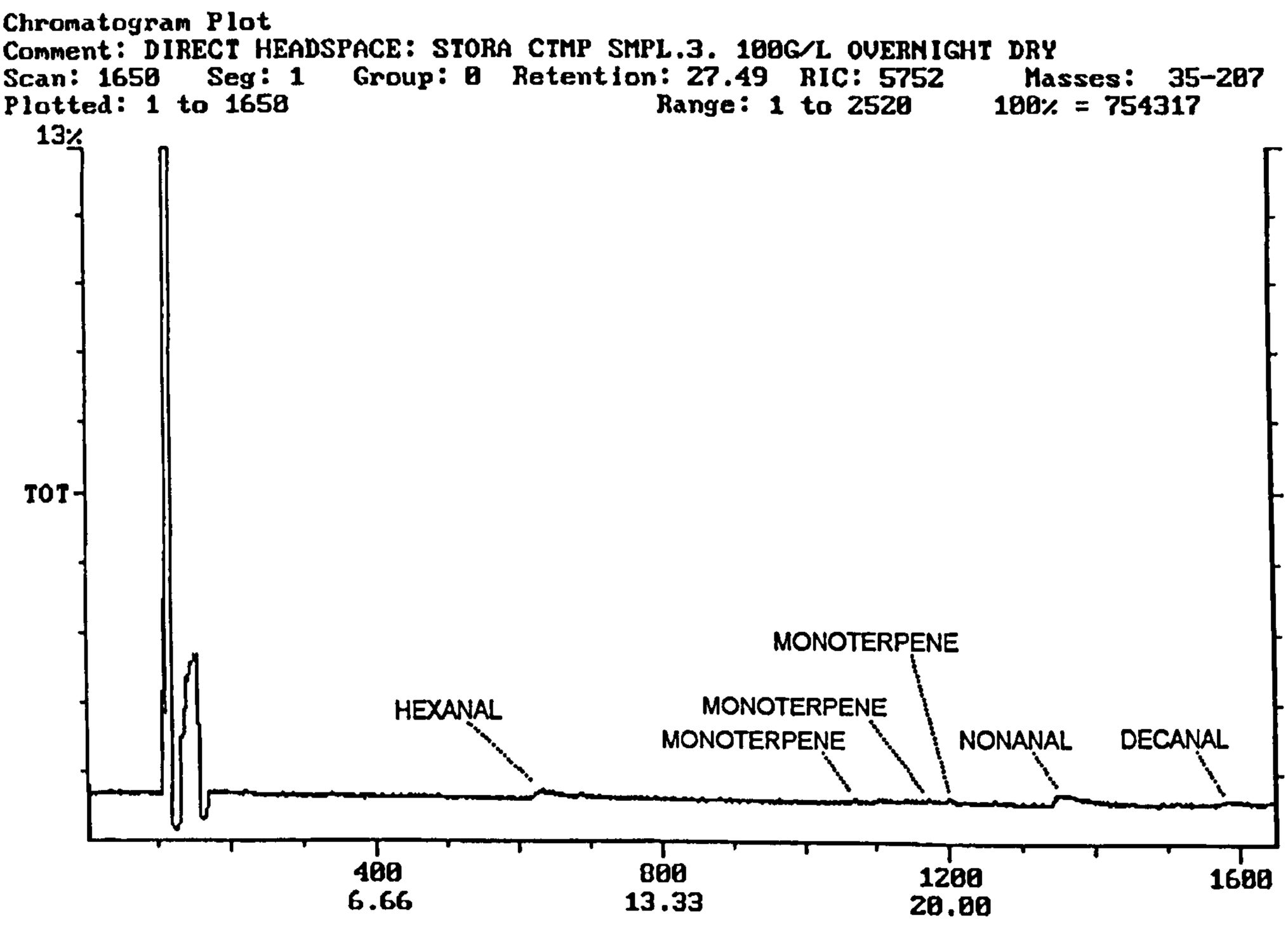


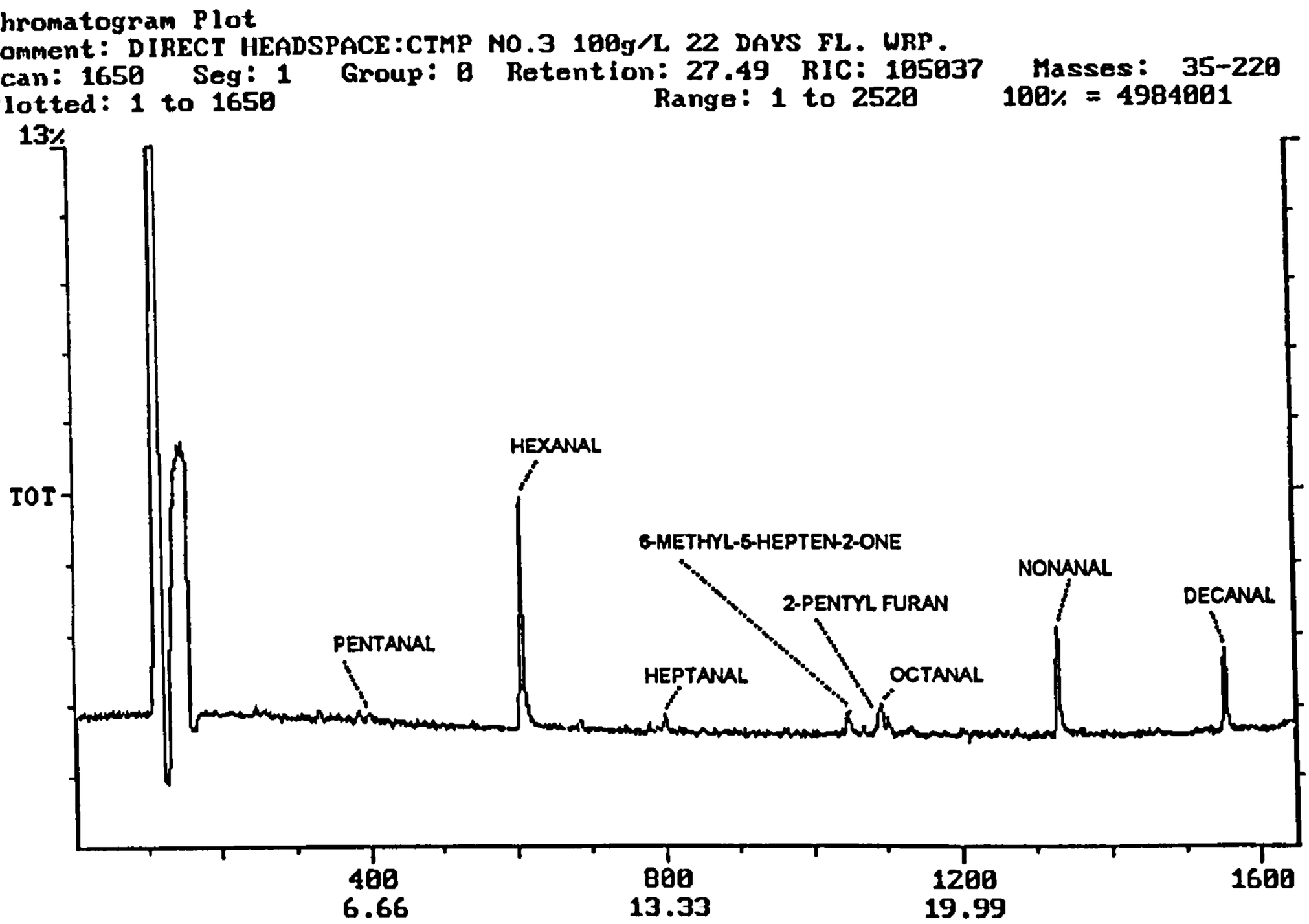
Figure 3.14 shows the chromatogram obtained at 22 days. The most significant features are the well resolved peaks for hexanal, nonanal and decanal. Additional compounds were heptanal, 6-methyl-5-hepten-2-one, 2-pentyl furan and octanal.

At 35 days, additional compounds were seen. These included 2-methyl propanal, 3-methyl butanal and pentanal. An increase in the peak size for hexanal was also evident.

3.3.1.2 The effect of UV-cured inks on the volatiles present in CTMP sheet

Sheets of paper were made from CTMP in a similar way to that described previously. However, only 20g of CTMP was pulverised with 1 litre of water. This produced thinner sheets that were more representative of the CTMP layers adopted in board materials.

Figure 3.14 Chromatogram acquired from the headspace vapours of a sample of laboratory prepared CTMP sheet stored for 22 days at room temperature.



The sheets produced were cut into two rectangular pieces, approximately 120 x 70 mm in dimensions. Tests were carried out in which these sheets were print proofed with UV-curable inks and subsequently cured. All samples of CTMP proofed with ink, were produced on the same day and were wrapped in aluminium foil during storage periods.

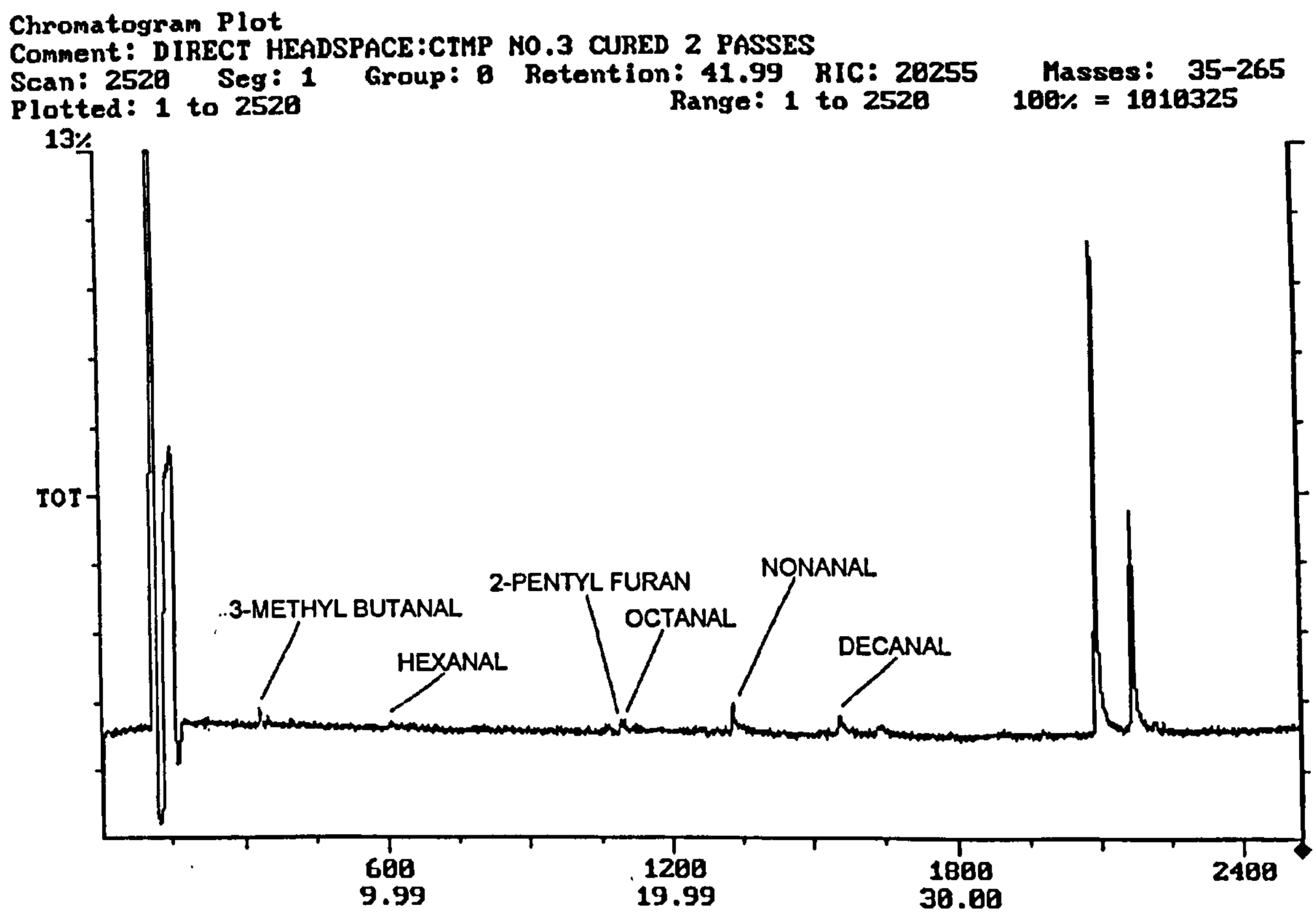
The UV-curable inks used in this series of tests were the Cadbury's 'Solid Blue' Unicure ink blend, the 'Opaque Orange' Unicure stock ink, the 'Cyan' Unicure stock ink and the 'Phthalo Green' Unicure stock ink. The inks were applied to the CTMP in the following way:

The desired quantity of ink was applied to a Duncan-Lynch proof printer and left to distribute on the rollers for 5 minutes. A hand-held double roll applicator was then inked on the assembly for 1 minute. The CTMP was subsequently proof printed with a thin film of ink by firm application of this roller to the sheet, in one swift pass. Prints were cured on a Wallace-Knight belt-curer, operating with a

belt speed of 0.5m s^{-1}. Samples were then analysed by static headspace GC/MS analysis on the same day, unless otherwise stated.

As a control, CTMP samples were irradiated with UV light without being proof printed with ink. Figure 3.15 shows the chromatogram acquired for a sample of CTMP sheet irradiated with two passes on the belt-curer. Peaks for nonanal and decanal can be seen, with smaller peaks for hexanal and 2-pentyl furan.

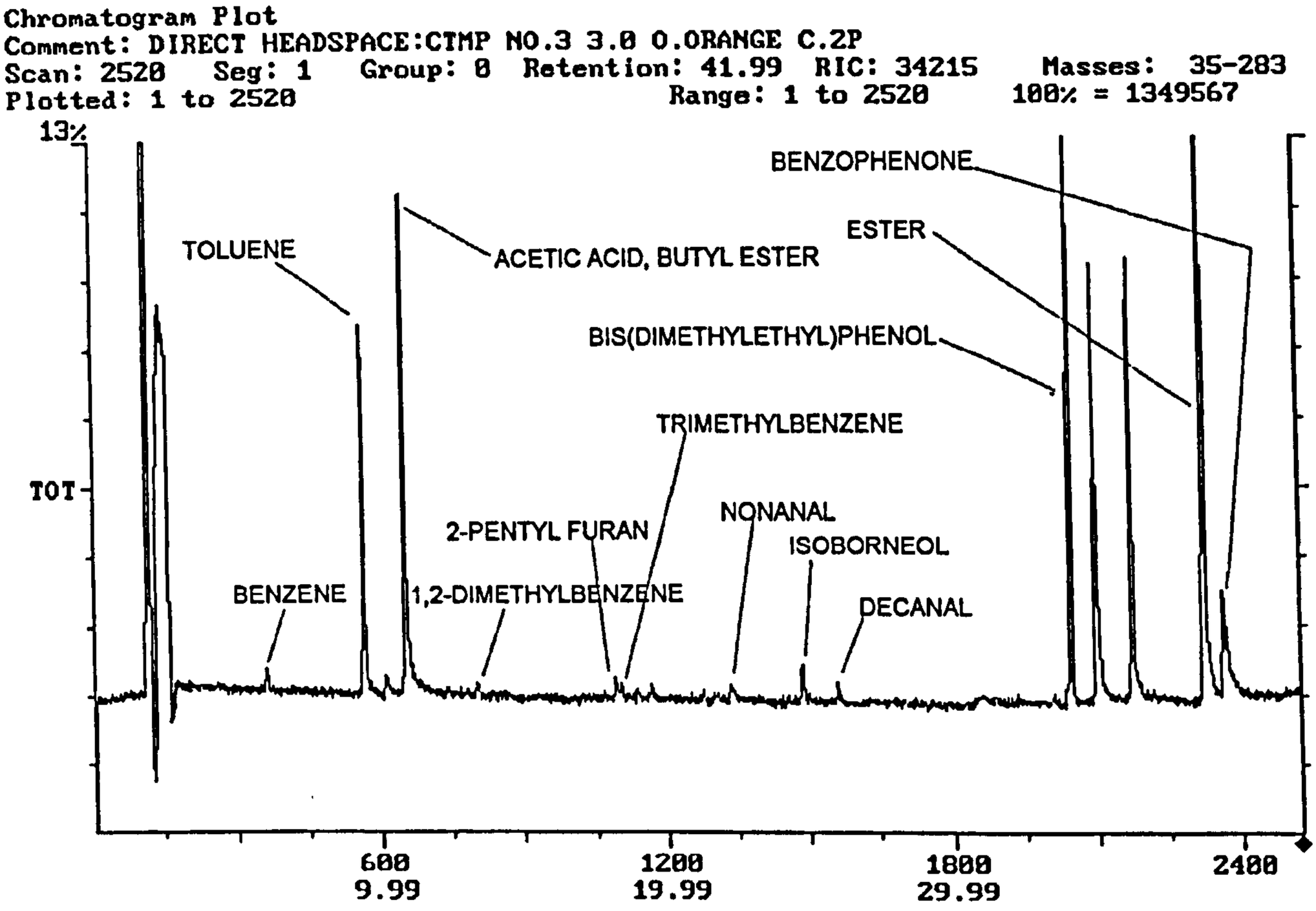
Figure 3.15 Chromatogram acquired from headspace vapours of a sample of laboratory prepared CTMP sheet irradiated by two passes on the belt-curer.



When this pattern is compared to that of a similar sample that has not been irradiated, there appears to be no significant difference, with the exception of 3-methyl butanal which is not present in the non-irradiated sample. Figure 3.16 shows the chromatogram acquired for CTMP, print proofed with the Opaque Orange ink of which 2.0cm^3 of the ink was applied to the proof printer. The ink was cured by 2 passes on the curer. Larger peaks for toluene, acetic acid-butyl ester, bis(dimethylethyl)phenol and a higher molecular weight ester are present. Smaller peaks for benzophenone, isoborneol, hexanal, nonanal, decanal, 2-

pentyl furan, 1,2-dimethylbenzene, benzene, some higher molecular weight esters and trimethylbenzene were also present.

Figure 3.16 Chromatogram acquired from headspace vapours of a sample of laboratory prepared CTMP sheet, print proofed with Opaque Orange ink and irradiated using two passes on the belt-curer.

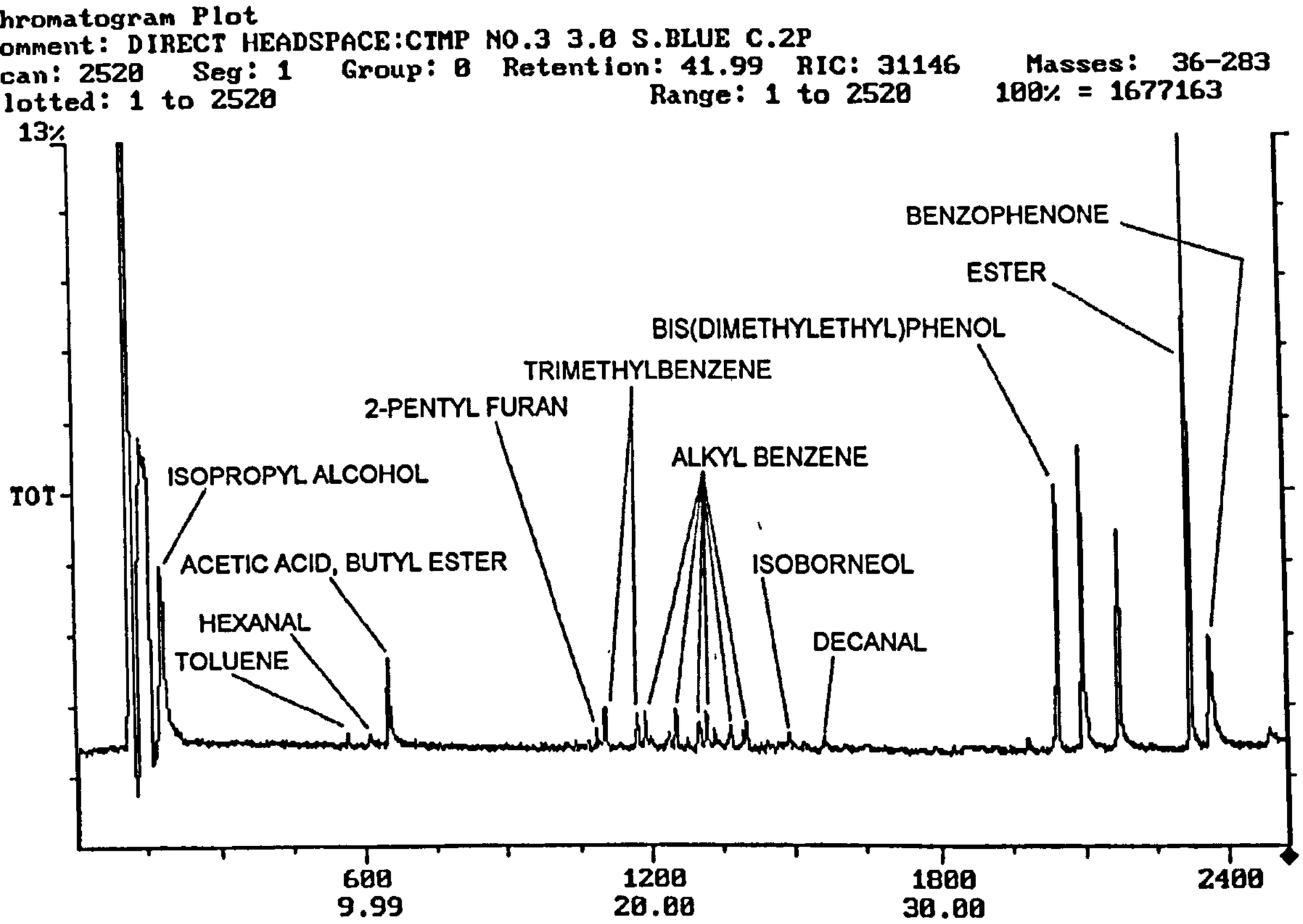


Samples of CTMP, print proofed with the Cyan ink, showed similar chromatograms. In addition, a peak was present for N,N-diethylaminobenzene and two peaks representing higher molecular weight esters were observed.

A similar picture was seen for samples print proofed with the Phthalo Green ink. A larger peak was present for isoborneol as were small peaks for either isobornyl acetate or bornyl acetate. A higher abundance of 1,2-dimethylbenzene was also apparent.

Figure 3.17 shows the chromatogram acquired for a sample of CTMP print proofed with the Solid Blue ink. In addition to the compounds already discussed, the presence of numerous alkylated benzenes was demonstrated.

Figure 3.17 Chromatogram acquired from headspace vapours of a sample of laboratory prepared CTMP sheet, print proofed with Solid Blue ink and irradiated using two passes on the belt-curer.



To assess the impact of multi-layer printing on the CTMP, a further series of tests was carried out. Samples were print proofed with one ink layer, that was cured using two passes on the curer. This layer was then over proofed with a different ink layer, that was cured in a similar way. Samples of CTMP were print proofed with the solid Blue ink, using the same amounts of ink as before. These samples were then over proofed with the Opaque Orange ink. These print proofed samples showed slightly larger peaks for hexanal, nonanal and 2-pentyl furan than those found in the samples printed with the individual inks.

To determine whether, or not, the increase in the presence of the above mentioned compounds was due to CTMP being exposed to four passes on the curer rather than two passes on the curer, samples of unproofed CTMP were irradiated by 4 passes on the curer. The peaks representing hexanal and 2-pentyl furan were smaller than those for the equivalent inked samples. However, the peaks for nonanal and decanal were very similar.

Samples of CTMP were subsequently print proofed with one ink and cured using 4 passes on the curer to ascertain whether there was a difference in

those samples where two inks were used, cured separately. For samples of CTMP print proofed with the Opaque Orange ink and cured using four passes, little difference was seen in the peak sizes for hexanal, nonanal, decanal and 2-pentyl-furan compared to the samples print proofed wet on dry.

To determine the affects of fount solution on the CTMP, during printing, $2cm^3$ of fount solution (5% fount concentrate, 8% isopropyl alcohol) were added to the ink during distribution on the proof printer. The amounts of hexanal, nonanal, decanal and 2-pentyl furan, in samples, were similar to those in samples where no fount solution had been added to the ink. No other significant differences were detected.

3.3.2 OXIDATION OF UNSATURATED FATTY ACIDS AND ITS SIGNIFICANCE WITHIN THE CARTON-BOARD PACKAGING SYSTEM

3.3.2.1 EXPERIMENTAL

Studies of the oxidation of unsaturated fatty acids within a CTMP matrix

In order to establish the precursors of the odorous volatiles forming in the above-mentioned CTMP samples, numerous tests were carried out. These were designed to simulate the natural occurrence of unsaturated species in board samples, and their subsequent oxidation.

Tests involving the addition of certain unsaturated fatty acids to 1 gram samples of machine-pressed, CTMP supplied by the Finnish board manufacturer, Metsa-Serla were carried out. Before use, this CTMP was vacuum-extracted at 120°C for 1.5 hours to ensure removal of any residual volatiles that may have been present through air-borne contamination, or, more importantly, through oxidation of any existing unsaturated species present in the pulp. Samples of the vacuum extracted CTMP were analysed by static headspace GC/MS to ensure complete volatile compound removal had been achieved.

The choice of the fatty acids used in the tests was determined by the nature of the volatile compounds found in Stora CTMP samples. The presence of pentanal, hexanal, heptanal, octanal, nonanal and decanal indicated the

presence of either oleic acid and/or linoleic acid, or their ester derivatives. The presence of linolenic acid was assumed to be unlikely due to its extreme susceptibility to oxidative attack. Linolenic acid autoxidises, approximately, 77 times more rapidly than does oleic acid and nearly 3 times more rapidly than linoleic acid. Linoleic acid undergoes autoxidation more rapidly than oleic acid (approximately 27 times faster (Gunstone,1984)).

Much literature exists regarding model experiments involving the formation of volatile compounds from fatty acids in foods (Selke *et al.*, 1980; Schieberle and Grosch, 1981; Grosch, 1987). This information is of both a qualitative and quantitative nature. However, little information that deals with the implications of the presence of fatty acids in carton packaging materials is available. The existing information regarding fatty acids in foods is arranged primarily according to the fatty acid precursor and whether or not the experiment was carried out at moderate temperatures (<80°C) or at elevated (>130°C) temperatures. The studies at moderate temperatures are of importance from the standpoint of packaging materials, since such studies represent the odorous compounds that can develop during storage when the lipids become autoxidised or photoxidised. It could be argued that studies at elevated temperatures may simulate the conditions that exist during pulping, refining and bleaching in packaging manufacture. It is important to consider this phenomenon in testing.

The vast majority of tests to date have been carried out using static headspace analysis of samples placed in standard 22 cm^3 glass vials, with silicone rubber septum caps. Unlike many other experiments on fatty acids, as reported in the literature, the headspace vial was also used as the reaction chamber.

A series of tests has been carried out under varying conditions, using samples of CTMP that had been impregnated with a small amount of fatty acid precursor, sealed in vials, and stored for different periods and temperatures. The volatiles forming as a result of fatty acid oxidation, during the storage period, accumulated in the particular vials and were analysed by static headspace sampling, coupled to capillary gas chromatography/mass spectrometry.

Much information has been published regarding the effect of time, temperature, humidity, oxygen availability, the presence of transition metal

catalysts, and photoxidation on unsaturated fatty acids with regard to food (Labuza, 1971; Pokorny, 1987; Kochhar, 1993). The following tests were carried out to determine the effect of these factors on packaging materials.

Determination of the effect of time and temperature on the presence of linoleic acid in CTMP

1 gram samples of CTMP were prepared in the manner described above. To the sample was added 10mg of linoleic acid, corresponding to 1%(w/w) of linoleic acid in CTMP. This corresponds to 11μl of the acid, at 20°C, which was evenly distributed over the surface of the sample by means of a syringe. The samples were then sealed in the glass vials and stored.

A total of twelve vials was stored at three different temperatures, four vials at 20°C, four vials at 40°C and four vials at 50°C. Vials at each temperature were sampled at 3, 7, 20, and 42 days by the static headspace procedure, using a headspace equilibration temperature of 105°C. In addition, a sample was analysed immediately after preparation and the data used as an initial point for the subsequent data series produced. The headspace conditions used were the same as those described in Section 3.5.5.

1 μl of internal standard (0.1%(v/v) benzene/methanol) was injected in to each vial, prior to analysis, to facilitate production of reproducible data and to remove errors associated with quantitative variation in sample introduction.

It could be argued that the volatiles found during the above analyses may result from non-oxidative thermal degradation, in addition to the oxidative mechanisms of interest, due to the use of a headspace temperature of 105°C. However, lower headspace temperatures may not be sufficient to vaporise some of the components that are present and, thus, low recoveries, or even no recovery of particular volatiles could result.

To determine the effect of a lower headspace temperature, the above test was repeated with a headspace sample equilibration temperature of 50°C. All other variables were kept the same. Vials at the three storage temperatures were sampled at 6, 15, 30 and 41 days. In addition, a sample was analysed immediately after preparation and the data used as an initial point for the subsequent data series produced.

A test was also carried out in which no headspace equilibration period was used. Vials at the three storage temperatures, sampled at 4, 21, 35 and 48 days, were purged with carrier gas and the headspace vapours passed into the GC without sample vial heating. This procedure was carried out to determine if headspace equilibration at 50°C had an effect on oxidation.

An additional test was carried out on the linoleic acid/CTMP system. To determine whether the formation of oxidation volatiles was a linear function of linoleic acid concentration, a test was undertaken in which 1 gram samples of CTMP were impregnated with 1.1μl of linoleic acid, via a syringe. This corresponded to a loading of 0.1%(w/w) linoleic acid in CTMP, at 20°C. Samples were sealed in vials and stored at 20°C, 40°C and 50°C for 3, 17, 32, 39 days. Sampling was carried out on the stored samples, and on an initial unstored sample, using a headspace equilibration temperature of 105°C

Determination of the effect of UV irradiation on the presence of linoleic acid in CTMP

1 gram samples of CTMP were prepared as before, except the impregnated samples were irradiated using four passes, at 0.5m/s, on a Wallace-Knight belt curer, fitted with a medium pressure mercury lamp, prior to being sealed in the headspace vials. A total of nine vials was stored at three different temperatures, three vials at 20°C, three vials at 40°C and three vials at 50°C. Vials at each temperature were then sampled at 5, 14, and 42 days, by the static headspace procedure at 105°C. As before, a sample was analysed immediately after preparation to provide an initial reference point.

A similar experiment was carried out using a headspace equilibration temperature of 50°C. Sample vials stored at 20°C, 40°C and 50°C were sampled at 3, 10, 25 and 41 days.

Determination of the effect of reduced oxygen partial pressure on the presence of linoleic acid in CTMP

Samples of impregnated CTMP were placed in vials and, prior to sealing were purged in a jet of oxygen-free nitrogen for 1 minute. Sample vials were stored at 20°C, 40°C and 50°C and sampled at 4, 14, 34, and 46 days by the headspace procedure at 105°C.

Determination of the effect of the relative humidity on the presence of linoleic acid in CTMP

Samples of impregnated CTMP in unsealed vials were placed, for 2 hours, in a desiccator, which contained a saturated solution of potassium acetate, that provided a constant relative humidity of 20%. Vials were then sealed and stored at 20°C, 40°C and 50°C for 6, 19, 29, and 42 days before sampling at an equilibration temperature of 105°C.

A series of samples was also placed in a desiccator for 2 hours, that contained a saturated solution of sodium hydrogen sulphate monohydrate, to provide a constant humidity of 52%. Vials were then sealed and stored at 20°C, 40°C and 50°C for 6, 19, 29 and 42 days, before sampling at an equilibration temperature of 105°C.

Determination of the effect of the presence of low amounts of copper on linoleic acid in CTMP

The profound effect of transition metal compounds on the rate of autoxidation of unsaturated fatty acids has been reported (Pokorny, 1987). Normally these compounds need only be present in very small amounts to have a significant effect.

A test was carried out in which a copper(II) ion solution, was added to samples CTMP to a loading of 0.05mg Cu/kg of CTMP. This translates to 0.00005mg of copper ions per 1 gram sample of CTMP. When the CTMP samples were dried out to room humidity, linoleic acid was added, as in previous tests. Samples were then placed in vials, sealed, and stored at 20°C, 40°C, and 50°C for 6, 21, 34, and 43 days. Headspace sampling was carried out at 105°C.

A test was also undertaken, using a copper (II) ion loading of 0.1mg Cu/kg of CTMP. Samples were stored at 20°C, 40°C, and 50°C for 4, 18, 32, and 48 days. Headspace sampling was carried out at 105°C.

A further test was carried out on impregnated samples of CTMP at a copper (II) ion loading of 0.5mg/kg of CTMP. Samples were stored at 20°C, 40°C, and 50°C for 4, 18, 31, and 44 days. The headspace equilibration temperature used was 50°C.

Determination of the effect of CTMP on the autoxidation of linoleic acid

In order to determine how the CTMP was influencing the oxidation process, under the conditions of storage and analyses, a test was carried out using linoleic acid in isolation. 11µl of linoleic acid were placed at the bottom of the test vials, corresponding to the same amount as 1%(w/w) of linoleic acid in 1 gram of CTMP at 20°C. The vials were, subsequently, sealed and stored at 20°C, 40°C, and 50°C for 4, 13, 28, and 40 days. Headspace sampling was carried out at 105°C.

Determination of the effect of time and temperature on the presence of methyl linoleate in CTMP

It is possible that the fatty acids remaining in carton-packaging, after manufacture, do not exist in the acid form but, rather in an esterified form. These esterified moieties can take the form of methyl, ethyl, or propyl linoleate, or longer chain ester derivatives, in the case of linoleic acid.

In order to determine the effect of the ester linkage on the autoxidation in CTMP, a test was carried out in which samples of CTMP were impregnated with 1%(w/w) of methyl linoleate, sealed, and stored at 20°C, 40°C, and 50°C for 6, 12, 31, and 48 days. Headspace sampling was carried out at 105°C.

An additional test was carried out using CTMP samples that were impregnated with 1%(w/w) methyl linoleate. These samples were sealed and stored at 20°C, 40°C, and 50°C for 6, 12, 31, and 48 days. However, headspace sampling was carried out at 50°C.

Determination of the significance of the formation of *trans,trans* 2,4-decadienal as a precursor in the formation of volatile oxidation products from linoleic acid/alkyl linoleate in CTMP

The formation of 2,4-decadienal from linoleic acid 9-hydroperoxide, during oxidation, has been well studied (Grosch *et al.*, 1981; Schieberle and Grosch, 1981; Grosch, 1987). This intermediate readily decomposes, to form a host of odorous volatiles. To determine the extent and the nature of this volatile compound formation, samples of CTMP were impregnated with 1%(w/w) *trans,trans* 2,4-decadienal, sealed, and stored at 20°C, 40°C and 50°C for 4, 12, 29, and 47 days. Headspace sampling was carried out at 105°C.

Determination of the effect of singlet oxygen on the oxidation of linoleic acid in CTMP

The presence of singlet oxygen and its effect of the oxidation of unsaturated species has been reported in studies of the photoxidaton of fatty acids (Labuza, 1971; Chan, 1977). To determine the effect of singlet oxygen on linoleic acid impregnated CTMP, 50µl of a 0.2 grams solution of tetrasulpho-zincphthalocyanine in 100 cm^3 of water was added to each 1 gram sample of CTMP. Each was allowed to dry prior to 11µl linoleic acid being added. The samples were then irradiated using four passes on the Wallace-Knight UV curing unit. The belt speed was set to 0.5m/s. The samples were sealed and stored at 20°C, 40°C, and 50°C for 3, 17, 33, and 48 days. A headspace equilibration temperature of 105°C was used for sampling.

Determination of the effect of UV-cured ink on the presence of linoleic acid in CTMP

To discover whether, or not, the UV-curable inks had an effect on the oxidation of linoleic acid in CTMP pulp, a test was carried out in which the impregnated CTMP was proofed with a uniform layer of Coates Unicure Phthalo Green UV-curable ink. This ink was applied with the use of a roller, inked from a Duncan-Lynch proof printer loaded with 2.0cm^3 of the ink. The printed samples were

promptly irradiated by using four passes on the Wallace-Knight curer, set with a belt speed of 0.5m/s. Samples were then sealed and stored at 20°C, 40°C and 50°C for 4, 17, 31, and 45 days and analysed using a headspace temperature of 105°C. The test was repeated using an equilibration temperature of 50°C, during which samples were sealed and stored at 20°C, 40°C and 50°C for 7, 21, 32 and 49 days.

Determination of the effect of time and temperature on the presence of oleic acid in CTMP

Much attention has been given to the consequences of the presence of linoleic acid or its ester derivatives on the formation of volatiles in CTMP pulp. Oleic acid undergoes much slower rates of oxidation compared to linoleic acid. However, the odorous volatiles produced may build up to become significant. A test was carried out in which 1%(w/w) oleic acid was added to 1 gram samples of CTMP. The samples were then sealed and stored at 20°C, 40°C, and 50°C for 13, 27, 57, and 85 days. Headspace sampling was carried out at 105°C. As in previous tests, a sample was analysed immediately after preparation and used as an initial reference point.

Determination of the effect of time and temperature on the presence of a combination of oleic and linoleic acid in CTMP

In the natural carton system, it is likely that more than one fatty acid species will be present. There will be a combination of different acids. The mutual pro-oxidative and anti-oxidative effects of mixtures of two fatty acids of different reactivity has been reported (Labuza, 1971). To determine the effect of one fatty acid on the autoxidation of another, a test was carried out in which samples of CTMP were impregnated with 0.5%(w/w) oleic acid and 0.5%(w/w) linoleic acid. These samples were sealed and stored at 20°C, 40°C, and 50°C for 4, 18, 32, and 48 days.

Studies of the oxidation of unsaturated fatty acids within a carton-board matrix

A number of experiments has been conducted to investigate the oxidation behaviour of linoleic acid in CTMP. However, the complete carton-board containing a diverse number of components could provide a different environment for oxidation to take place. To investigate how linoleic acid oxidises within the carton-board matrix, a series of tests was carried in which 1 gram samples of Stora Koppargloss (705μm thickness grade) were purified, as before, by evacuating in an oven for 1.5 hours at 120°C. To the purified samples were added 10mg of linoleic acid, corresponding to 1%(w/w) of linoleic acid/Koppargloss. A number of tests was carried out in which linoleic acid/Koppargloss samples were conditioned under varying conditions.

Determination of the effect of time and temperature on the presence of linoleic acid in Koppargloss carton-board

Samples of linoleic acid/Koppargloss were sealed in the glass vials and stored. A total of twelve vials was stored at three different temperatures, four vials at 20°C, four vials at 40°C and four vials at 50°C. Vials at each temperature were then sampled at 7, 20, 34, and 55 days by the static headspace procedure. In addition, a sample was analysed immediately after preparation and the data used as an initial point for the subsequent data series produced.

Determination of the effect of singlet oxygen on the oxidation of linoleic acid in Koppargloss carton-board

To determine the effect of singlet oxygen on the linoleic acid impregnated Koppargloss, 50μl of a 0.2 grams solution of tetrasulphozincphthalocyanine in 100 cm^3 of water was added to each 1 gram sample of Koppargloss. Each was allowed to dry prior to 10 mg linoleic acid being added. The samples were then irradiated using four passes on the Wallace-Knight UV curing unit. The belt speed was set to 0.5m/s. The samples were sealed and stored at 20°C,

40°C, and 50°C for 6, 20, 34, and 55 days and sampled using an headspace equilibration temperature of 105°C.

Determination of the effect of UV-cured ink on the presence of linoleic acid in Koppargloss Carton-board

To discover the effect of UV-curable inks on the oxidation of linoleic acid in Koppargloss carton-board, a number of tests was carried out in which Koppargloss, impregnated with linoleic acid, was print proofed with a uniform layer of UV-curable ink. This ink was applied with the use of a roller, inked from a Duncan-Lynch proof printer loaded with 2.0cm^3 of the ink, as previously described. The printed samples were promptly irradiated by four passes on the Wallace-Knight curer, set with a belt speed of 0.5m/s.

In the first test, impregnated Koppargloss samples were proofed with Coates Unicure Phthalo Green UV-curable ink. Samples were sealed and stored at 20°C, 40°C and 50°C for 6, 22, 40, and 50 days. A headspace temperature of 105°C was used. The test was repeated using a headspace equilibration temperature of 50°C, during which samples were sealed and stored at 20°C,40°C and 50°C for 16, 31, and 45 days.

Another test was carried out in which impregnated samples of Koppargloss were print proofed with the Coates Unicure Phthalo Green UV-curable ink mixed with fount solution/isopropyl alcohol (IPA).

This was achieved by applying 2.0 cm^3 of the ink to the proof printer and then adding 1.0 cm^3 of fount/IPA solution to the rollers. The fount/IPA consisted of 5%(v/v) Dupont Fountsol F-5 and 8%(v/v) IPA in deionised water.

As before, four samples were prepared for storage at each temperature in addition to the unstored sample used as the initial reference point. A fresh 1 cm^3 aliquot of fount/IPA was applied to the rollers after every three samples were proofed, to replenish IPA lost to evaporation.

Samples were sealed and stored at 20°C, 40°C and 50°C for 8, 21, 35, and 45 days. A headspace temperature of 105°C was used.

To determine the effect of different photoinitiators on the oxidation of linoleic acid, two inks were evaluated by print proofing them on to samples of linoleic

acid/Koppargloss and irradiating, as before. Both inks were black Coates Unicure inks. One contained a benzophenone initiator (sample no. UCL882). The other contained isopropyl thioxanthone and was free from benzophenone (sample no. UB903).

Samples of impregnated Koppargloss proofed with the benzophenone containing ink were sealed and stored at 20°C, 40°C and 50°C for 5, 16, 31 and 44 days. Samples proofed with the thioxanthone containing ink were sealed and stored at 20°C, 40°C and 50°C for 4,15, 30 and 43 days. A headspace equilibration temperature of 105°C was used.

Two additional inks were tested, a cyan coloured benzophenone-containing ink and a cyan ink containing no benzophenone initiator. Both these inks were supplied by Swale Process, Urmston, Manchester.

The samples were prepared in the same way as the samples proofed with the Coates inks. Samples print proofed with the benzophenone ink were sealed and stored at 20°C, 40°C and 50°C for 5, 15, 28 and 46 days. Samples print proofed with the benzophenone free ink were sealed and stored at 20°C, 40°C and 50°C for 4, 11, 28 and 46 days. A headspace equilibration temperature of 105°C was used.

Determination of the extent of photoxidation in linoleic acid/Koppargloss systems proof printed with Phthalo Green ink and irradiated

Photoxidation of linoleic acid is brought about by the addition of excited singlet state oxygen to the unsaturated bonds, via an 'ene' reaction. To determine the effect of singlet oxygen, if any, on the formation of volatile oxidation products, a known singlet oxygen quencher was introduced into the linoleic acid/Koppargloss system prior to print proofing with the ink. Any volatile compound formation occurring should then be attributed to autoxidation and not photoxidation.

The singlet oxygen quencher used was the tertiary amine, 1,4-diaazabicyclo[1,1,1]octane (DABCO). 100µl of a solution of 0.05 grams of DABCO in 100 cm^3 methanol was applied as an even film to the Koppargloss samples. The methanol was allowed to evaporate. The samples were then impregnated with linoleic acid and print proofed with Phthalo Green ink, as

described in previous tests, and irradiated with UV light. Samples were sealed and stored at 20°C, 40°C and 50°C for 7, 18, 31 and 42 days. A sample was analysed immediately after preparation to act as an initial point in the subsequent data series produced. A headspace equilibration temperature of 105°C was used.

The test described above was repeated using 1,3-diphenylisobenzofuran (DPBF) instead of DABCO. DPBF is a singlet oxygen scavenger like DABCO. It has an intense yellow colour which is bleached colourless in the presence of singlet oxygen (Fowler and Devonshire, 1991). It is possible, therefore to gain a visual determination of the involvement of singlet oxygen.

Samples of Koppargloss carton-board were soaked in a solution containing 0.1 grams of 1,3-diphenylisobenzofuran in 100 cm^3 of methanol. These samples were then dried thoroughly in air. The samples of carton-board with a bright yellow colouration were impregnated with 10 mg of linoleic acid as in previous tests and printed with the green ink in the same manner as before. The intensity of the yellow of the underside of the carton-board samples was assessed after curing, just prior to headspace analysis and just after headspace analysis.

Determination of the effect of time and temperature on the presence of trilinolein in Koppargloss carton-board

A number of experiments has been carried out to determine the extent of volatile compound formation resulting from the oxidation of linoleic acid within CTMP and Koppargloss carton-board. A large proportion of the fatty acid component of softwoods is in the form of triglycerides. Linoleic acid maybe formed readily from the thermal decomposition of trilinolein, which is its triglyceride derivative. This is likely to occur during the pulping processes involved in carton-board manufacture. It is possible, however, that some of the fatty acid component of carton-board may be in the form of trilinolein.

To determine the significance of this, relative to the presence of free linoleic acid, a test was carried out in which 1 gram samples of purified Koppargloss where impregnated with 10 mg of trilinolein (10µl at 20°C) by means of a glass syringe. Samples were then stored at 20°C, 40°C and 50°C for 4, 14, 29 and

42 days. A sample was analysed immediately after preparation and was used for the initial point in subsequent data series. A headspace equilibration temperature of 105°C was used. The test was repeated using a equilibration temperature of 50°C in which samples were stored at 20°C, 40°C and 50°C for 6, 16, 28 and 44 days.

3.3.2.2 RESULTS AND DISCUSSION

Oxidation of unsaturated fatty acids within a CTMP matrix

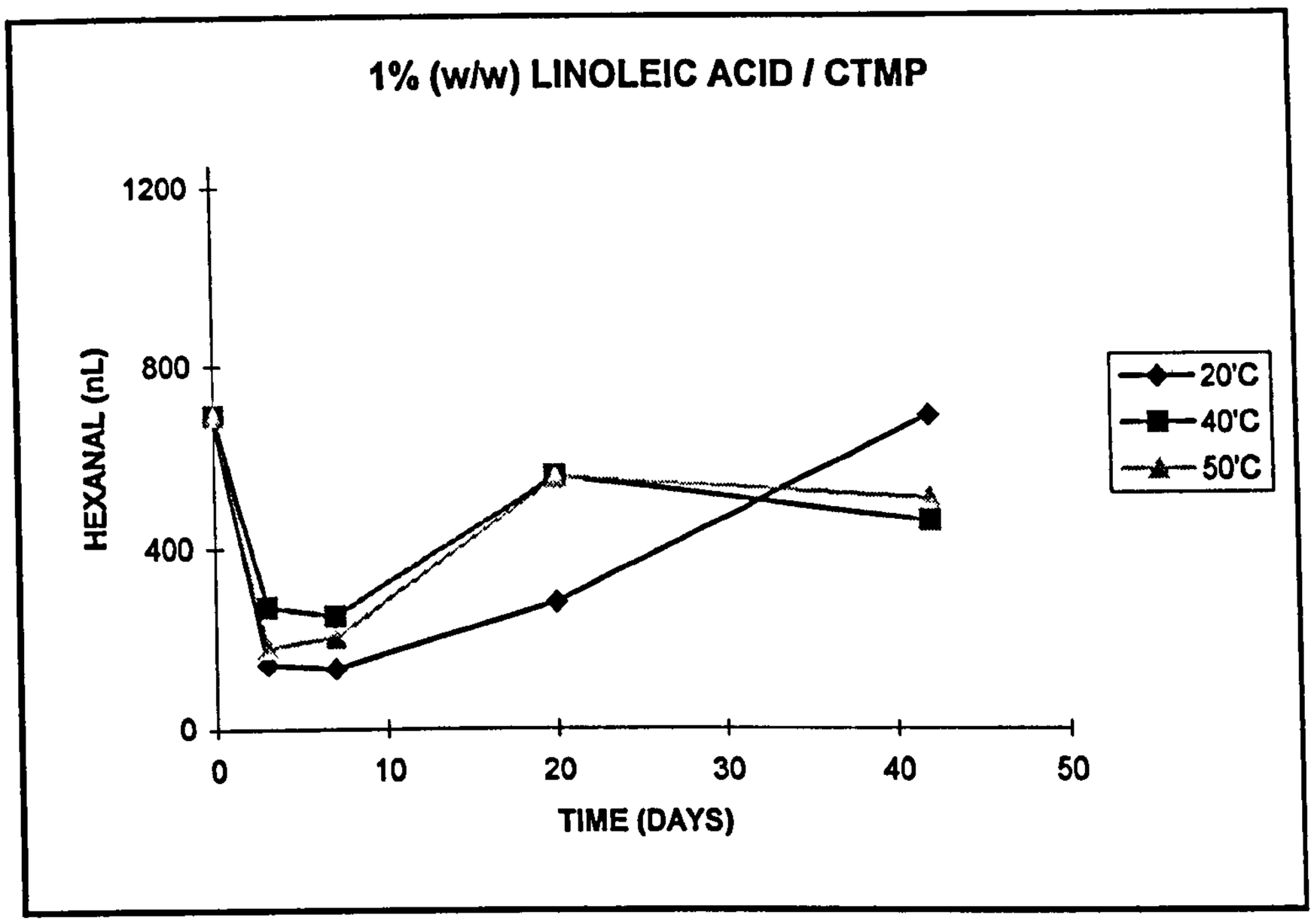
A number of the volatile products was identified throughout the tests. A large proportion of these volatiles was identified by mass spectrometry and quantitation was achieved. This quantitation was carried out using identified compounds in a series of calibration standards, made up in increasing concentrations by the addition of measured amounts of analyte compounds to 1 gram samples of CTMP, sealed in nitrogen-purged vials.

Determination of time and temperature on the presence of linoleic acid in CTMP

The most obvious observation was the difference in the number of volatiles recovered using the two headspace equilibration temperatures of 105°C and 50°C. This may be due to a number of reasons, including the reduced vaporisation of less volatile components and the less thermal degradation of oxidation precursors and intermediates, such as hydroperoxides, at 50°C.

Hexanal is the most dominant volatile compound throughout the tests at both headspace temperatures. From samples headspaced at 105°C, it can be seen that the concentration of hexanal is far in excess of other volatiles present, even for the initial sample at 0 days storage. The formation of hexanal in this particular case could be due to thermal degradation rather than to gradual oxidative decomposition. Figure 3.18 shows the amount of hexanal recovered (in nL) during the test, at storage temperatures of 20°C, 40°C, and 50°C.

Figure 3.18 Amounts of hexanal recovered (in nL) from samples of linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 42 days (Headspace sampling temperature: 105°C).



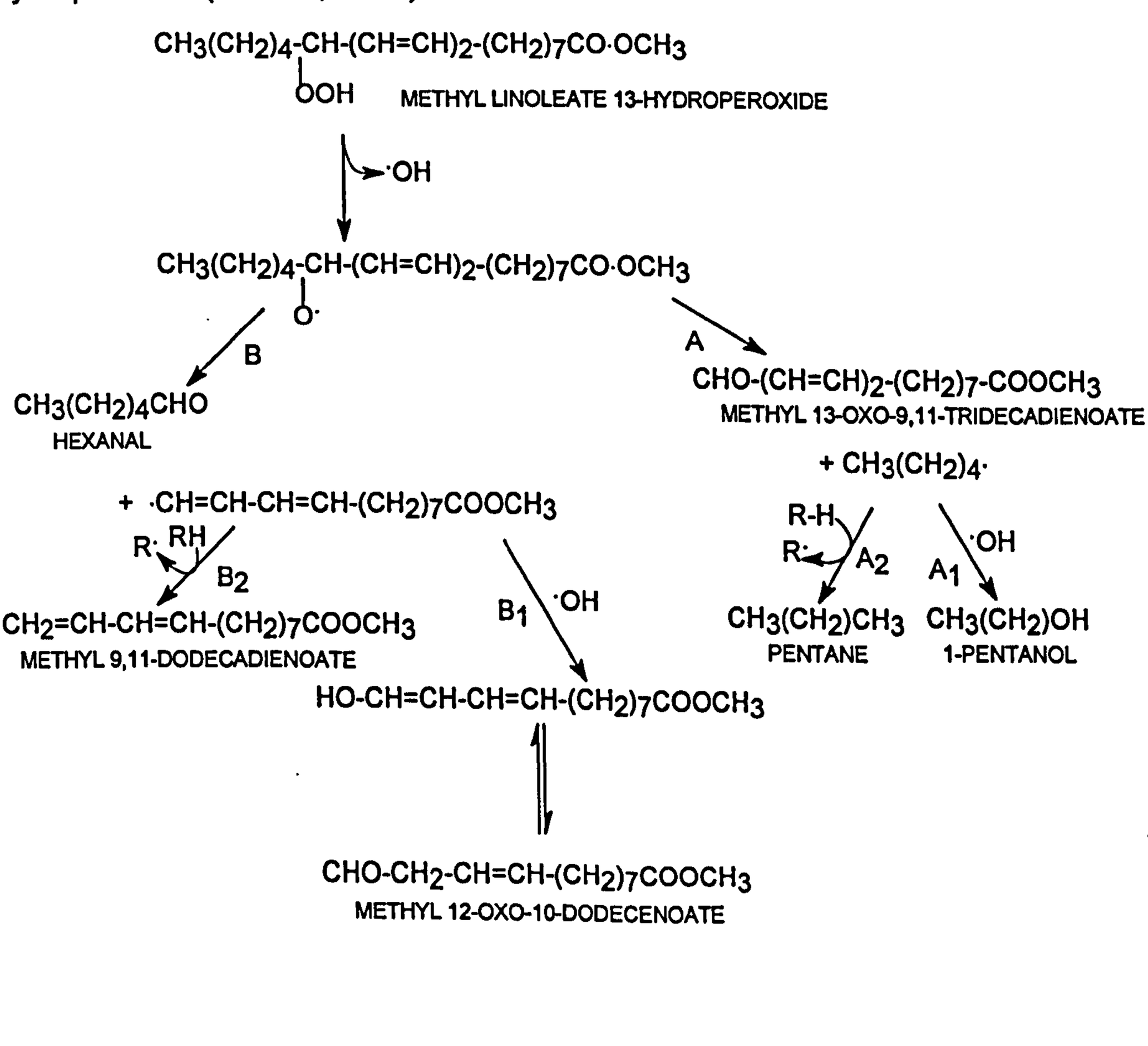
Over a 6 week period, the amount of hexanal produced, initially, is most rapid at 40°C and least rapid at 20°C. Surprisingly, the initial formation at 50°C is slightly less than at 40°C. The amount of hexanal present peaks at a similar amount for samples stored at 40°C and 50°C, at around 3 weeks. At 6 weeks the amount of hexanal present is greatest for the sample stored at ambient temperature. This may indicate that hexanal undergoes decomposition once it has been formed. This decomposition is enhanced at higher temperatures. Hexanal may undergo further oxidation to form the hexanoic acid. It is possible that this carboxylic acid could have become strongly bound to the cellulose/lignin in the CTMP, thus, preventing sampling by headspace analysis. It has been reported that the major components in the autoxidation of hexanal, octanal, and 2-octenal are the corresponding fatty acids (Schieberle and Grosch, 1981).

It is widely accepted that lipid hydroperoxides are the primary oxidation products of unsaturated fatty acids (Labuza, 1971; Gunstone, 1984; Grosch, 1987; Kochhar, 1993). During autoxidation, hydroperoxide formation occurs via the abstraction of hydrogen, by a peroxy radical, from the α-methylene group of

a fat molecule. In the case of linoleic acid, this occurs at the doubly reactive allylic C-11, with formation of a pentadienyl radical. This intermediate radical reacts with oxygen to produce a mixture of conjugated 9- and 13-diene monohydroperoxides. Monohydroperoxides produced by autoxidation are unstable and break down readily in to a wide variety of volatile products and non-volatile products.

The reaction pathway for the formation of volatile compounds, from unsaturated lipids, has been proposed by various workers (Grosch, 1987 and Kochhar, 1993). It involves the homolytic cleavage of the -OOH group, to produce an alkoxy radical and a hydroxyl radical. The alkoxy radical undergoes ß-scission of the C-C bond, with the formation of an aldehyde and an alkyl or vinyl radical. In the case of linoleic acid, or methyl linoleate, the breakdown of the 13-hydroperoxide isomer, by this mechanism, results in the formation of hexanal, among other volatiles. The reaction pathway for the formation of hexanal by this route is illustrated in Figure 3.19.

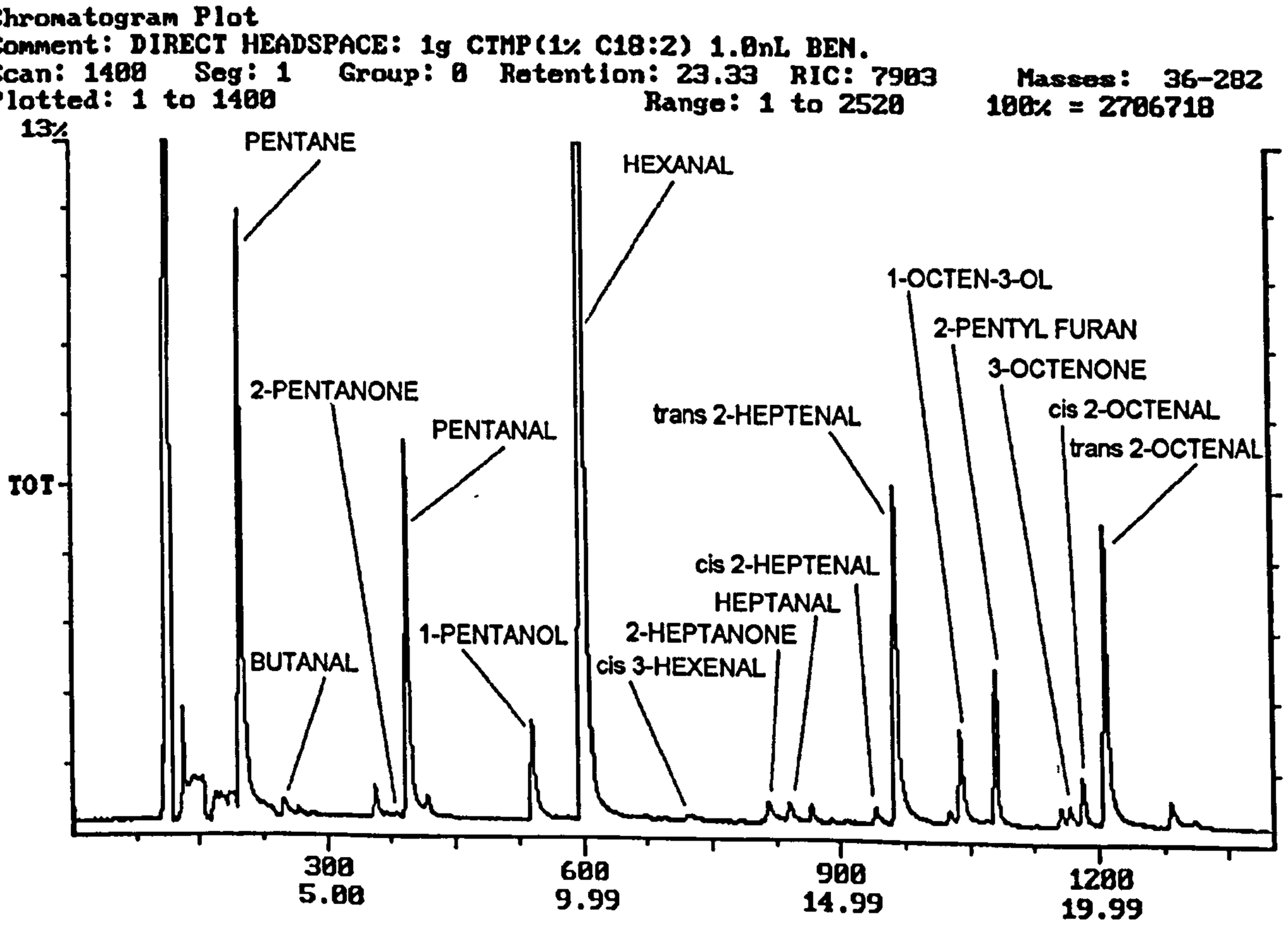
Figure 3.19 Reaction mechanism for the decomposition of methyl linoleate 13-hydroperoxide (Grosch, 1987).



Pentane and 1-pentanol can be formed through this reaction mechanism. These two compounds were detected throughout the test at all storage temperatures. However, the amounts of pentanol found were significantly greater than those found for pentane. The amounts found increased with storage temperature. This indicates that H-abstraction from the acid/CTMP system was not favoured over combination of the pentyl radicals with hydroxyl radicals. It could also indicate that there was an abundance of hydroxyl radicals in the CTMP/fatty acid system, possibly due to the cleavage of the -OOH groups during hydroperoxide decomposition.

Numerous other compounds were also found during the thermal decomposition study, and oxidative decomposition, of linoleic acid/CTMP samples headspaced at 105°C. The chromatogram (Figure 3.20) shows the volatiles formed from 1%(w/w) linoleic acid in CTMP resulting from thermal decomposition during the 60 minute headspace equilibration period at 105°C. The sample had not been stored. Thus, no significant hydroperoxide formation could have occurred through oxidation at lower temperatures.

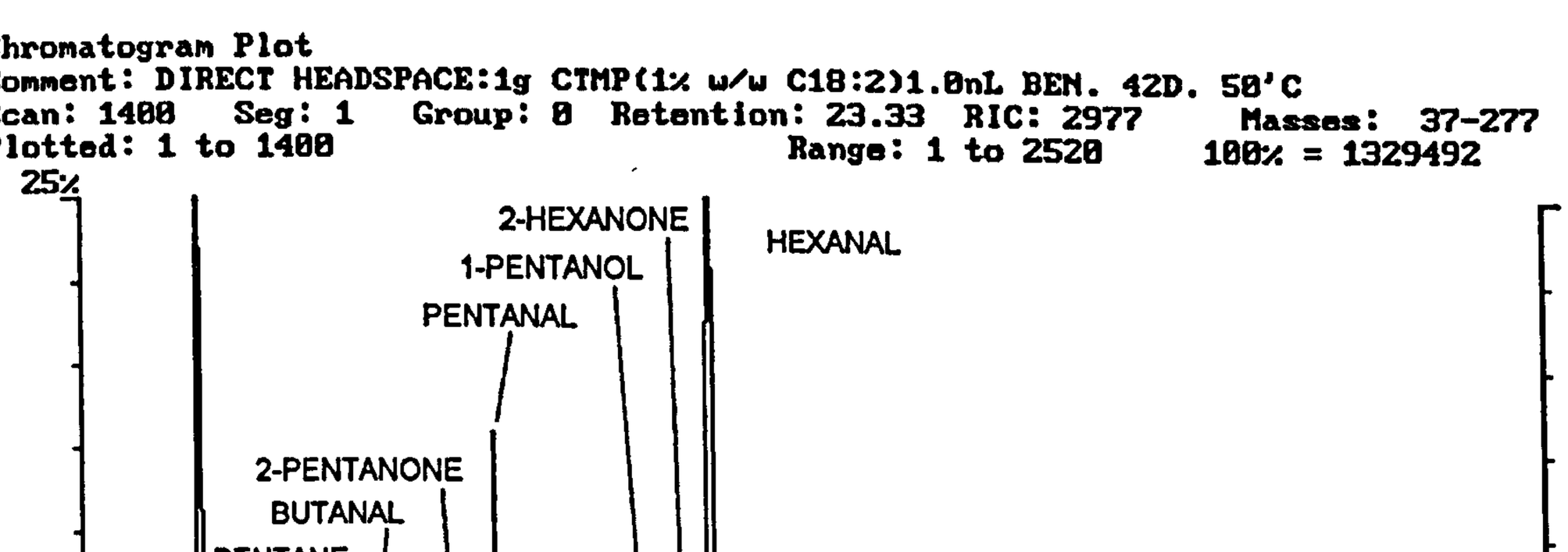
Figure 3.20 Chromatogram acquired from the headspace vapours of a one gram sample of CTMP/1%(w/w) linoleic acid that had undergone thermal degradation, at 105°C, for 60 minutes.



In addition to hexanal, pentane and 1-pentanol, the formation of other volatiles is evident. These include butanal, 2-pentanone, pentanal, 3-hexenal, 2-heptanone, heptanal, 2-heptenal, 1-octen-3-ol, 2-pentyl furan, 3-octen-2-one, and 2-octenal.

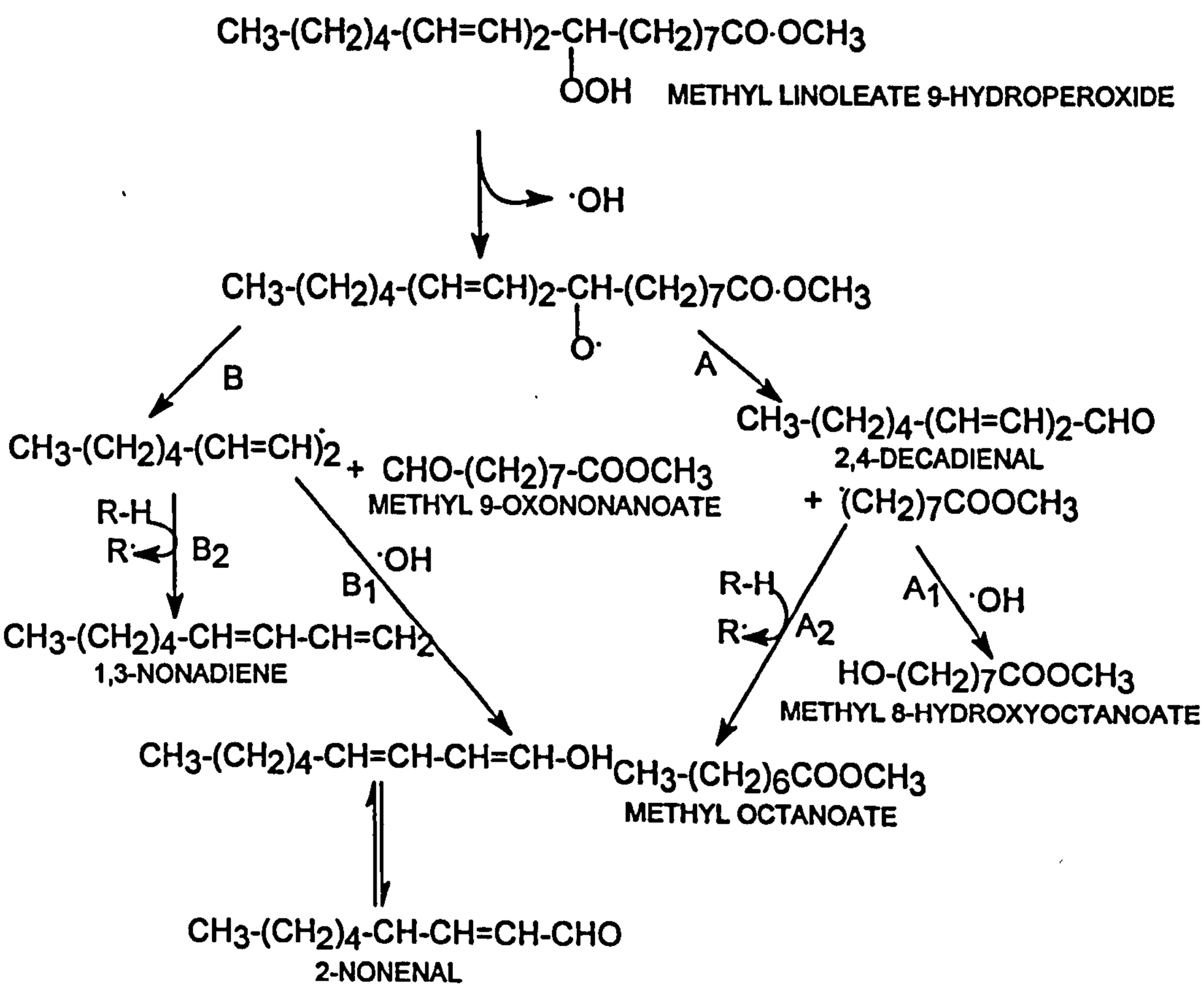
Figure 3.21 shows the chromatogram for the volatile oxidation products recovered from a sample that had been stored at 50°C for 42 days. The volatiles present in the headspace sample are very similar to those recovered from the sample represented in the previous chromatogram. This indicates that thermal and oxidative decomposition, under the conditions that existed, proceeded by similar mechanisms. Volatiles recovered included butanal, 2-pentanone, pentanal, 2-heptanone, heptanal, 2-heptenal, 1-octen-3-ol, 2-pentyl furan, 3-octen-2-one, and 2-octenal, among others, as with the previous sample.

Figure 3.21 Chromatogram acquired from the headspace vapours of a one gram sample of CTMP/1%(w/w) linoleic acid that had been stored, at 50°C, for 42 days.



These compounds did not form by decomposition of linoleic acid 13-hydroperoxide (Figure 3.19), but rather, by different mechanisms. The formation of a major precursor of a number of these compounds can be explained in the decomposition of linoleic acid 9-hydroperoxide. The mechanism for the decomposition of methyl linoleate 9-hydroperoxide is illustrated in Figure 3.22.

Figure 3.22 Reaction mechanism for the decomposition of methyl linoleate 9-hydroperoxide (Grosch, 1987).

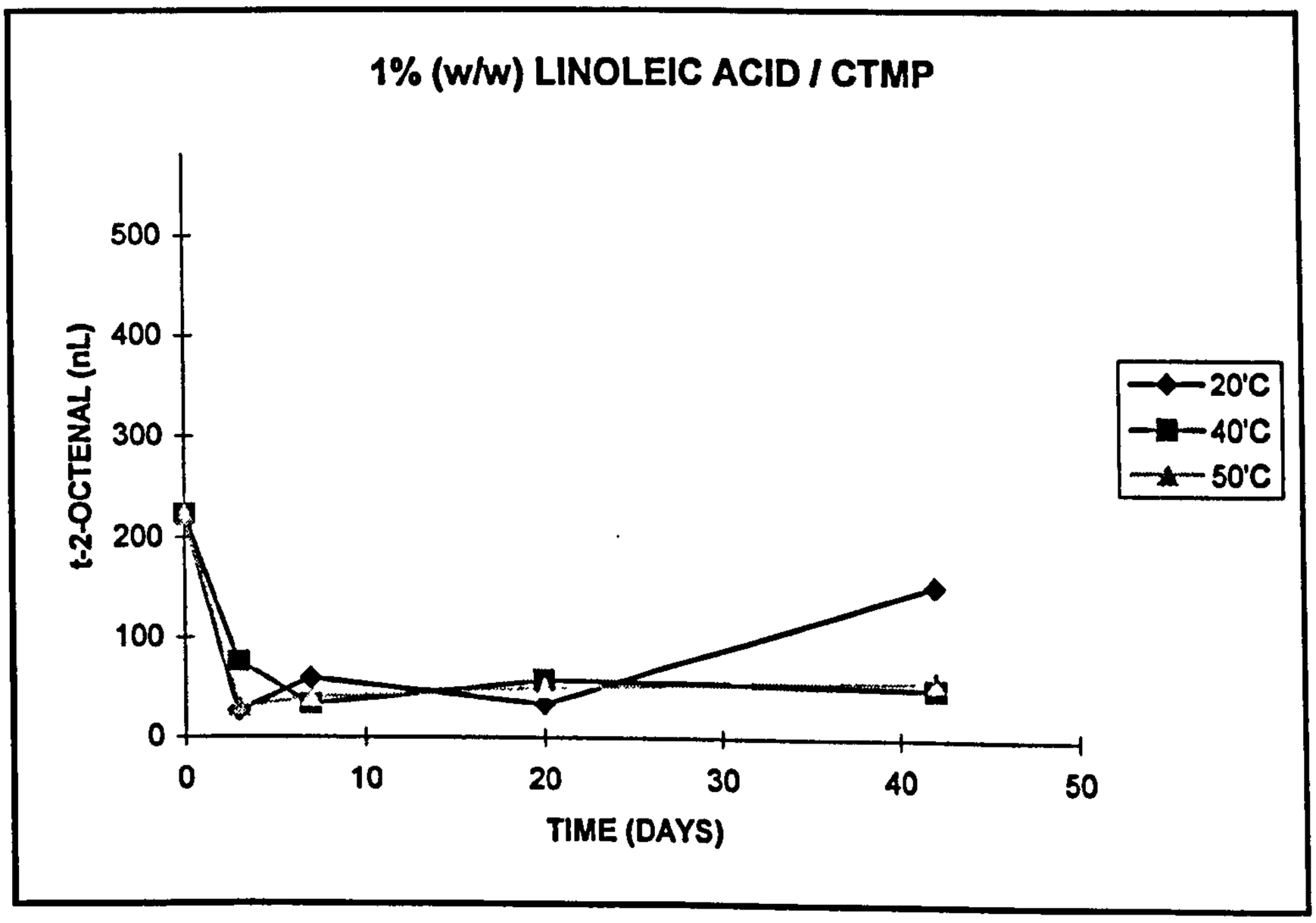


Scission of the alkoxy radical by route A leads to the formation of 2,4-decadienal. 2,4-decadienal autoxidises at room temperature to produce a mixture of volatiles. These include butenal, hexanal, 2-heptenal, 2-octenal, benzaldehyde, glyoxal, *trans*-2-buten-1,4-dial, furan, ethanol, acrolein, pentane, amongst others (Matthews *et al.*, 1971). The two major volatiles formed are hexanal and *trans*-2-octenal. 2,4-decadienal, in particular *trans*, *trans* 2,4-decadienal was not detected in the headspace volatiles of the linoleic

acid/CTMP samples, at either a sampling temperature of 50°C, or 105°C. This is due to the rapid breakdown of the compound, if formed.

trans 2-octenal is formed from the decomposition of 2,4-decadienal (Matthews *et al.*, 1971; Schieberle and Grosch, 1981). If 2,4-decadienal decomposition is the major route to the formation of *trans* 2-octenal then formation of 2,4-decadienal, as an intermediate, can be determined from the presence of *trans* 2-octenal. Figure 3.23 shows the amounts of *trans* 2-octenal detected for samples of linoleic acid/CTMP throughout the test using a headspace equilibration temperature of 105°C.

Figure 3.23 Amounts of *trans* 2-octenal recovered (in nL) from samples of linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 42 days (Headspace sampling temperature: 105°C).

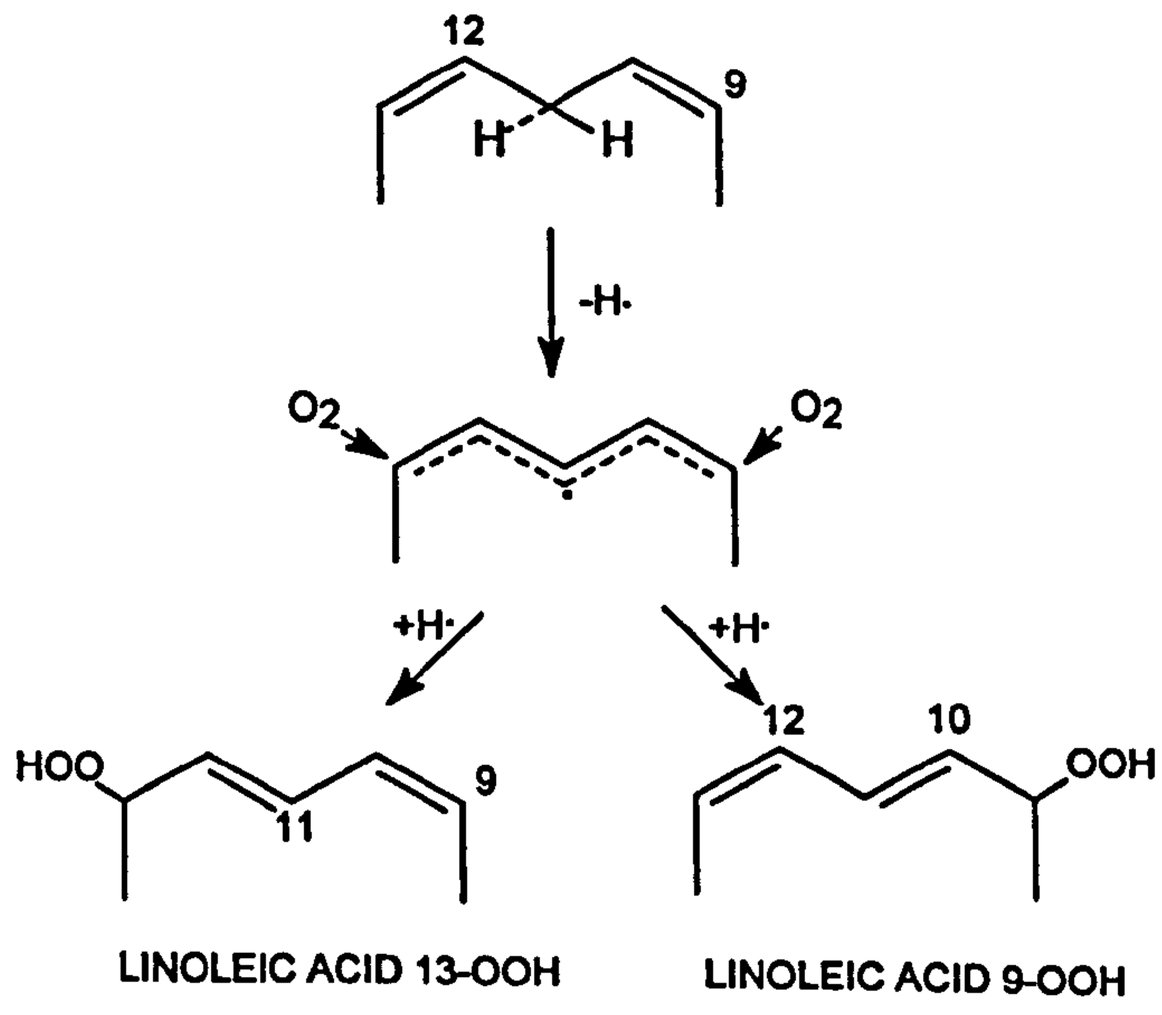


Consideration needs to be made for the possibility of *trans* 2-octenal decomposing within the test system. If this is the case, then formation of *trans 2-octenal* is not a good indicator of the formation of 2,4-decadienal. Figure 3.23 shows that, at longer storage times, the amount of *trans*-2-octenal increased more for the sample stored at room temperature, than for those stored at higher temperatures. This could indicate either its breakdown when

exposed to higher temperatures for longer periods or its subsequent oxidation to a fatty acid.

The nature of the monohydroperoxide is of great significance to the formation of odorous volatiles from unsaturated fatty acids. As mentioned before, autoxidation of linoleic acid usually involves hydrogen abstraction at the doubly degenerate allylic C-11, with the formation of a pentadienyl radical. This intermediate reacts to produce a mixture of monohydroperoxides. The major isomers are the 9- and 13-hydroperoxides. The reaction scheme for the formation of these two major isomers is illustrated in Figure 3.24.

Figure 3.24 Reaction scheme for the formation of two major hydroperoxides from linoleic acid autoxidation.



It has been reported that volatile compounds predicted to be products of the decomposition of linoleic acid 9-hydroperoxide are 2,4-decadienal, methyl octanoate and methyl 9-oxo-nonanoate. However, in tests, small amounts of hexanal have also been recovered (Grosch, 1987). The same author, has reported that the composition of the volatile products from the decomposition of methyl linoleate 13-hydroperoxide, yielded, in addition to the expected hexanal, even larger amounts of 2,4-decadienal, methyl octanoate and methyl 9-oxononanoate.

It is accepted that isomerisation of hydroperoxides takes place before decomposition (Ellis *et al.*, 1968; Gunstone, 1984; Grosch, 1987). This explains the presence of volatile compounds from the decomposition of 13-

hydroperoxide in a sample of the 9-isomer, and vice-versa for the products of the decomposition of the 9-hydroperoxide in the 13-isomer. It could be argued that hexanal present from the decomposition of 9-hydroperoxide may result from the decomposition of 2,4-decadienal. However, this is unlikely since the experiments, in which hexanal formation occurred, were carried out in the absence of oxygen (Grosch, 1987).

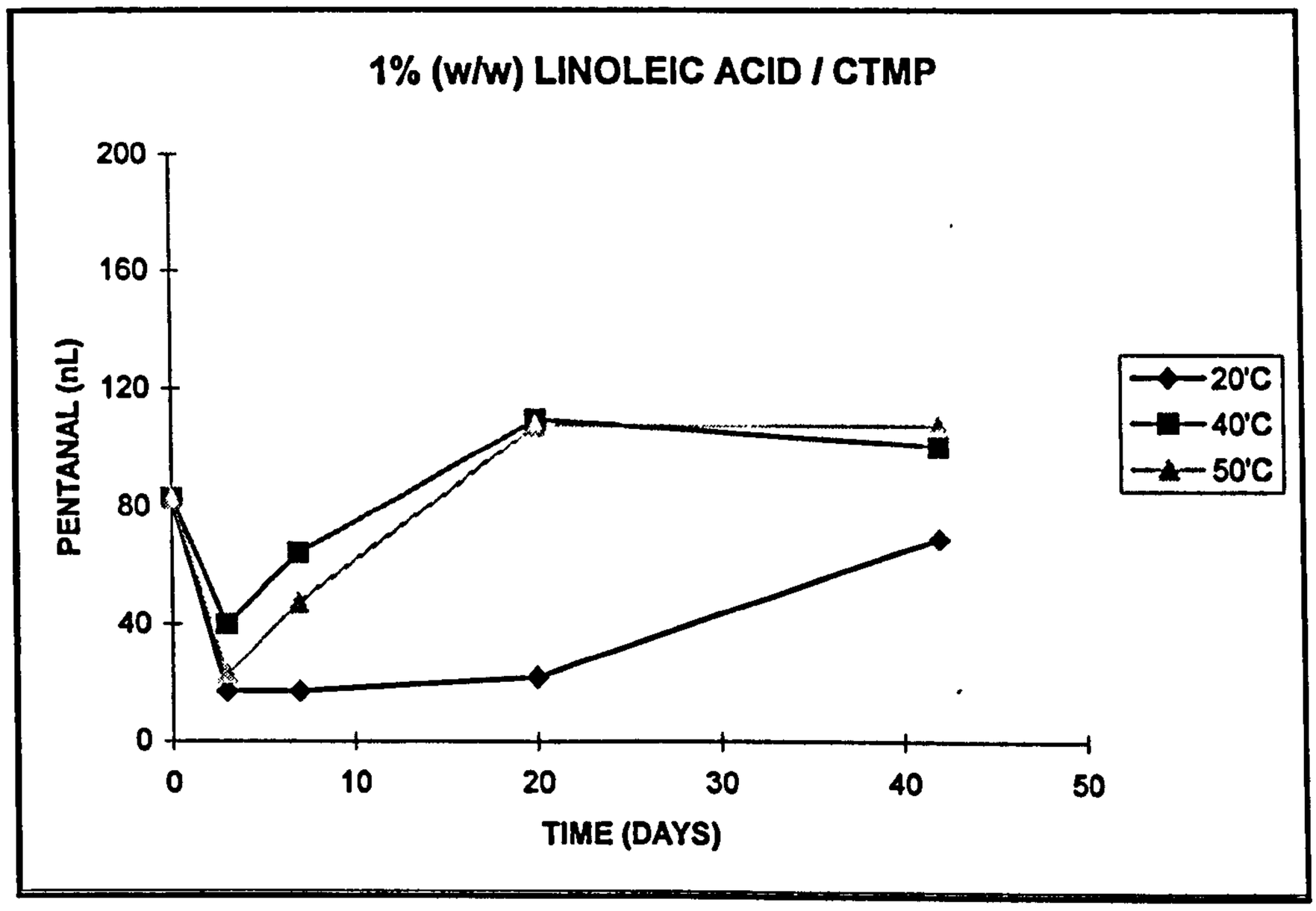
Isomerisation favours the conversion of the 13-hydroperoxide to the 9-isomer. This was indicated by the relatively large amount of 2,4-decadienal produced from the 13-isomer, as opposed to the small amounts of hexanal formed from the 9-isomer. It is thought that a mechanism exists that favours the formation of 2,4-decadienal, when further oxidation of this 2,4-decadienal is prevented. One theory states that route A rather than B is favoured for the ß-scission of monohydroperoxides, since route A allows the direct formation of stabilised oxodienes from 9- and 13-hydroperoxides (Grosch, 1987). These products are resonance stabilised. Thus, by this mechanism, the production of 2,4-decadienal is favoured over that of hexanal.

Schieberle and Grosch (1981) and Grosch (1987) have shown that similar compositions of aldehydes are produced from the decomposition of 9- and 13-hydroperoxides, in the presence of oxygen. The major product was hexanal. 2,4-Decadienal was absent. The predominance of hexanal is widely accepted to be due to the greater oxidative susceptibility of 2,4-decadienal (Schieberle and Grosch, 1981).

As mentioned earlier, 2,4-decadienal was not detected in the headspace vapours of samples of linoleic acid/CTMP. It is likely that this unsaturated aldehyde underwent more rapid decomposition than did the hydroperoxide precursor and, thus, was broken down as it was formed. It could be argued that, due to its lower volatility, 2,4-decadienal may not be vaporised by headspace analysis at lower temperatures. However, if this was so, trace amounts of the volatile compound would still be recovered. Throughout the test, no traces of 2,4-decadienal were found.

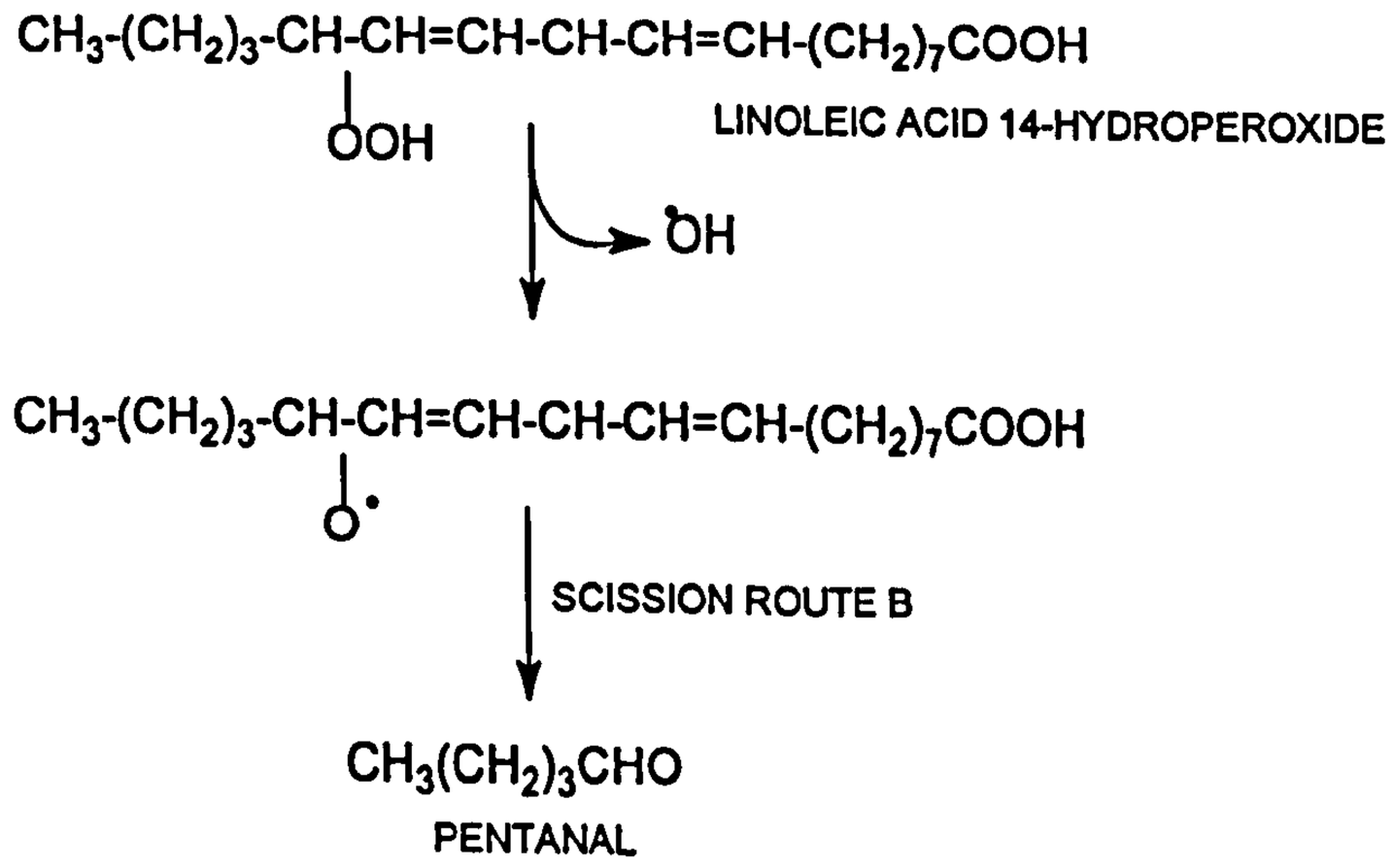
Another major volatile compound found during the test was pentanal. Figure 3.25 shows the amounts of pentanal detected from samples of linoleic acid/CTMP, headspaced at 105°C.

Figure 3.25 Amounts of pentanal recovered (in nL) from samples of linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 42 days (Headspace sampling temperature: 105°C).



It has been suggested that the decomposition of linoleic acid 14-hydroperoxide leads to the formation of pentanal (Grosch, 1987). The reaction pathway, illustrated in Figure 3.26, is the likely mechanism for the formation of pentanal by this route.

Figure 3.26 The reaction pathway for the formation of pentanal from linoleic acid 14-hydroperoxide.



The formation of the 14-isomer involves hydrogen abstraction from a methylene group with only one neighbouring double bond. The intermediate radical is much less stable. This is why the 14-isomer has been reported in low concentrations by previous workers (Gunstone, 1984; Belitz and Grosch, 1987). It has been reported that 14-hydroperoxide, along with the 8-isomer, has been found in a 1:1 mixture, making up 2-3% of total monohydroperoxides, for linoleic acid autoxidised at temperatures between 0°C and 60°C (Grosch, 1987). 8-hydroperoxide decomposition is said to produce 2,5-undecadienal. However, due to rapid autoxidation, no reports of its detection have been made.

It has been suggested by Matthews et al (1971), that pentanal may also be formed by dissociation of 2,4-decadienal, following allylic hydrogen abstraction.

Another possible pathway for the formation of pentanal could lie in a reaction pathway that involves the addition of oxygen to the pentyl radical produced from the decomposition of linoleic acid 13-hydroperoxide, via scission route A. The result would be a primary hydroperoxide that could lose a hydroxyl radical to form an alkoxy radical. Subsequently, this could form an aldehyde.

1-octen-3-ol was present in samples of linoleic acid/CTMP throughout the test, in which a headspace sampling temperature of 105°C was used. It is accepted that unsaturated alcohols may form as the result of the hydrogen abstraction by an alkoxy radical, resulting from the decomposition of a hydroperoxide. 1-octen-3-ol may also form via the decomposition of linoleic acid 10-hydroperoxide. This 10-isomer, and the 12-isomer, have been reported in small amounts in pyrolysed methyl linoleate (Grosch, 1987). The reaction pathway for the formation of 1-octen-3-ol, via methyl linoleate 10-hydroperoxide, is illustrated in Figure 3.27 (Grosch, 1987).

Ho et al. (1978) have discussed methyl linoleate 10-hydroperoxide, produced by the action of singlet oxygen, as a possible precursor for 2-pentyl furan. The reaction pathway for such a formation is shown in Figure 3.28.

Figure 3.27 Reaction scheme proposed for the homolytic breakdown of methyl linoleate 10-hydroperoxide with the formation of 1-octen-3-ol and 10-oxo-*trans*-8-decenoate.

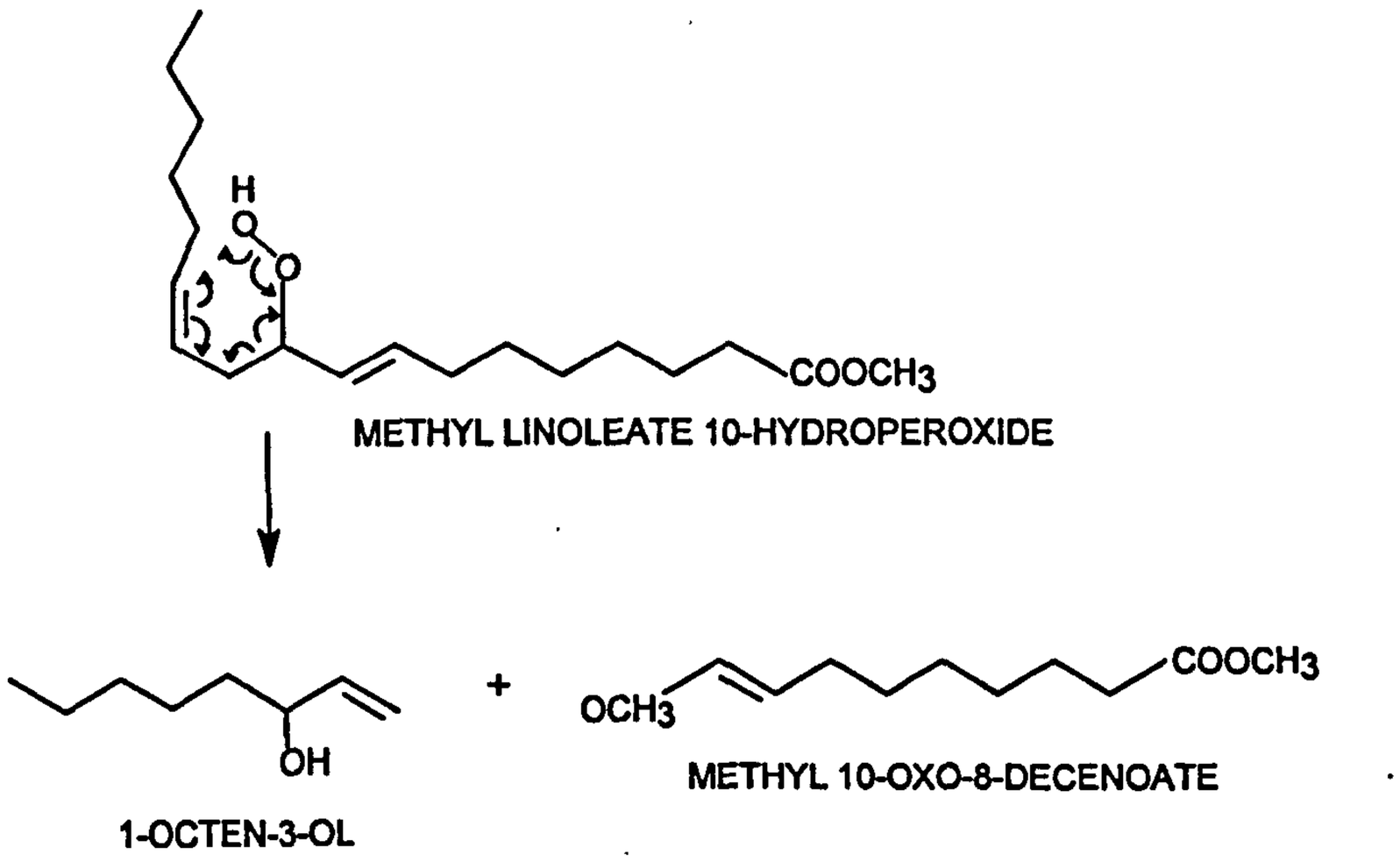
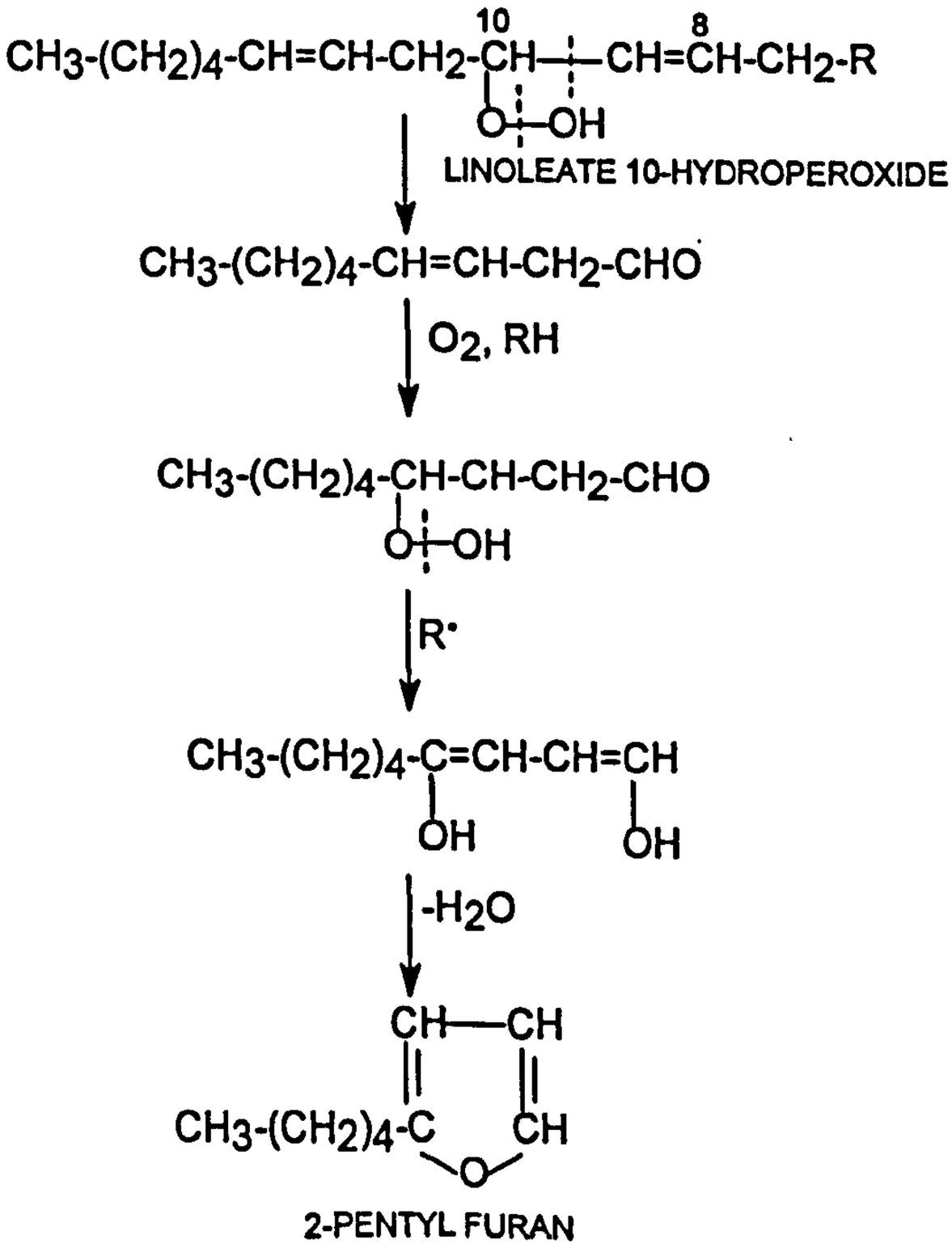


Figure 3.28 Reaction scheme for the formation of 2-pentyl furan from the decomposition of methyl linoleate 10-hydroperoxide.



This route to the formation of 2-pentyl furan is disputed by some workers. Frankel (1983) has suggested that 2-pentyl furan may form from linoleic acid 9-hydroperoxide via a cyclic peroxide and pentylfuraldehyde intermediate. This mechanism also involves the action of singlet oxygen. However, no pentylfuraldehyde was found during the test and no known singlet oxygen generators were thought to be present. Therefore, the mechanism illustrated above (Figure 3.28) appears to be the most feasible.

As discussed earlier, thermal decomposition and oxidative decomposition appears to follow similar reaction pathways, i.e. through the formation of hydroperoxides. However, the nature of the primary initiation step, illustrated below, might differ.

$$RH + O_2 \longrightarrow \text{free radicals or } ROOH$$

This initial reaction has a very high activation energy (~140-260 kJ mol^{-1}) (Labuza, 1971). Therefore, if a peroxide is to form, something must assist the formation. Some workers suggest the role of singlet oxygen in this primary reaction. Hydroperoxide formation requires a change in total electron spin, since both the substrate and product are in singlet states, while oxygen is in the triplet state. Conservation of spin is violated, making the reaction improbable. However, if singlet oxygen is involved, then the reaction is possible for the formation of hydroperoxides (Labuza, 1971). Singlet oxygen can be formed through photochemical reactions in the presence of a sensitiser. The question is, however, where and what is the photosensitiser in the linoleic acid/CTMP system?.

Some workers suggest that trace metals, if present, may be responsible for primary initiation (Labuza, 1971). Such a reaction is shown below:

$$RH + M^{n+} \longrightarrow R^{\cdot} + H^{+} + M^{(n-1)+}$$

Metals that are oxidised by a one electron transfer are most active. This fits very well in to singlet oxygen theory, since most sensitisers are bound metal complexes.

It has also been proposed that an increased temperature may result in the direct attack of oxygen on the substrate, since the high activation energy could

be overcome (Labuza, 1971). During thermal decomposition, it is likely that this mechanism could be significant.

Using external calibration standards, a number of the more abundant volatiles formed during oxidation of linoleic acid within CTMP, recovered using static headspace analysis, were quantified. Quantitation employed the same procedure as described previously for the quantitation of volatiles in printed carton-board samples. Table 3.3 shows the amounts of the volatiles recovered from the sample of linoleic acid/CTMP stored for 42 days at 50°C, represented by the chromatogram in Figure 3.21.

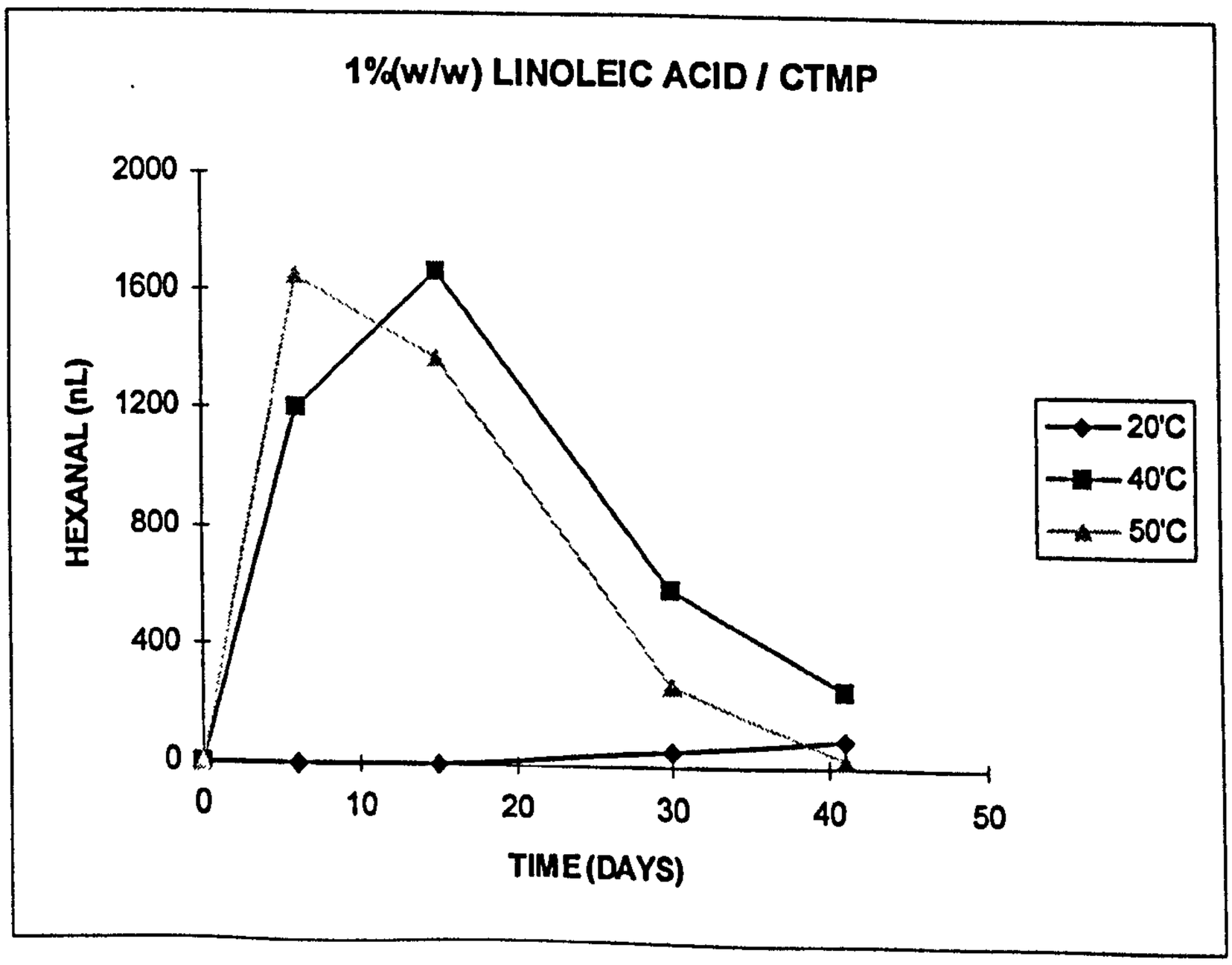
Table 3.3 Amounts of volatile compounds (in nL) recovered from a one gram sample of 1%(w/w) linoleic acid/CTMP stored for 42 days at 50°C using static headspace GC/MS analysis .

NAME OF COMPOUND	RETENTION TIME (in minutes)	QUANTITATION ION (in m/z)	CALCULATED AMOUNT (in nL)
Pentanal	6.58	58	107
Hexanal	10.03	44	506
Heptanal	14.04	70	4
Octanal			
Nonanal	22.14	57	1
trans 2-heptenal	16.18	83	34
trans 2-octenal	20.21	70	57
2-Pentanone	6.36	86	1
2-Hexanone	9.71	58	1
2-Heptanone	13.61	58	14
6-Undecanone	28.23	43	31
1-Butanol	5.89	56	10
1-Pentanol	9.03	42	117
1-Octen-3-ol	17.40	57	24
1-Butoxy-2-propanol			
Methyl octanoate	22.96	74	1
Pentyl pentanoate	25.34	85	1
2-Pentyl furan	18.06	138	27
Pentane	3.09	41	6
Heptane	7.34	71	1
Nonane			
Decane	18.84	57	1
1-Hexene	4.44	56	3
1-Heptene	6.98	56	7
1-Undecene	22.28	55	3
1-Dodecene			
Toluene	9.31	91	7
Ethylbenzene	12.94	106	4
1,2-Dimethylbenzene			
Styrene			
a-Methyl styrene			
Propylbenzene	16.68	91	4
1,2,3-Trimethylbenzene			
1,3,5-Trimethylbenzene	17.04	105	7

The compounds quantified in the above table were those found in significant amounts in carton-board samples and those identified from the oxidation of linoleic acid within CTMP. Table 3.3 shows that the hexanal is clearly the most abundant volatile compound found in this sample. Other volatile compounds present in significant amounts include pentanal, 1-pentanol, *trans* 2-heptenal, *trans* 2-octenal, 6-undecanone, 2-pentyl furan, and 1-octen-3-ol. Acyclic compounds associated with the carton-board surface binder were present in small amounts, in addition to the *n*-alkanes and *n*-alkenes.

Use of a headspace equilibration temperature of 105°C results in partial thermal degradation of linoleic acid. It may also increase the rate of primary initiation in the system. To determine the effect of this elevated temperature, samples of CTMP impregnated with 10 mg of linoleic acid, corresponding to 1%(w/w) linoleic acid/CTMP, as in previous tests, were stored for up to 42 days at 20°C, 40°C and 50°C and sampled using an equilibration temperature of 50°C. Figure 3.29 shows the amounts of hexanal detected for samples of linoleic acid/CTMP using a headspace equilibration temperature of 50°C.

Figure 3.29 Amounts of hexanal recovered (in nL) from samples of linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 42 days (Headspace sampling temperature: 50°C).



Despite the lower headspace equilibration temperature, the amounts of hexanal recovered from samples stored at 40°C and 50°C, compared to those recovered at the higher headspace temperature, are significantly greater. Hexanal, therefore, has a considerable vapour pressure even at this low temperature. This finding also suggests that hexanal may be decomposed at higher temperatures to non-volatile products, and that formation of non-volatile products is favoured over hexanal production initially.

At the storage temperatures of 40°C and 50°C, hexanal formation is very rapid within the first week of storage. After this time, the amount of hexanal begins to decline at 50°C. At 40°C, it rises further for another week after which it starts to decline.

The amounts of hexanal recovered from samples stored at ambient temperature, are less than those at the higher headspace temperature. This suggests that hydroperoxide formation at this temperature is much slower and that little decomposition of hydroperoxides occurs during the headspace equilibration period at 50°C, compared to that at 105°C.

Figure 3.29 also shows that no hexanal was recovered from the sample that had not been stored. Therefore, no thermal decomposition of linoleic acid occurred during the equilibration period at 50°C, to produce volatile products.

Figure 3.30 shows the chromatogram acquired from a sample of linoleic acid/CTMP stored for 15 days at 40°C. The major volatiles, in addition to hexanal, are hexane, pentanal, 1-pentanol and 2-pentyl furan. Only trace amounts of *trans* 2-octenal were found, indicating that this compound is not formed when an equilibration period of 50°C is used. Alternatively, this compound may not be sufficiently volatile at this temperature to be recovered in significant amounts.

If this compound is not formed, it is likely that decomposition of linoleic acid 9-hydroperoxide to 2,4-decadienal does not occur and any of the 9-isomer formed isomerises to the 13-hydroperoxide and decomposes to form hexanal, and other products.

Figure 3.30 Chromatogram acquired from the headspace vapours of a one gram sample of CTMP/1%(w/w) linoleic acid that had been stored, at 40°C, for 15 days.

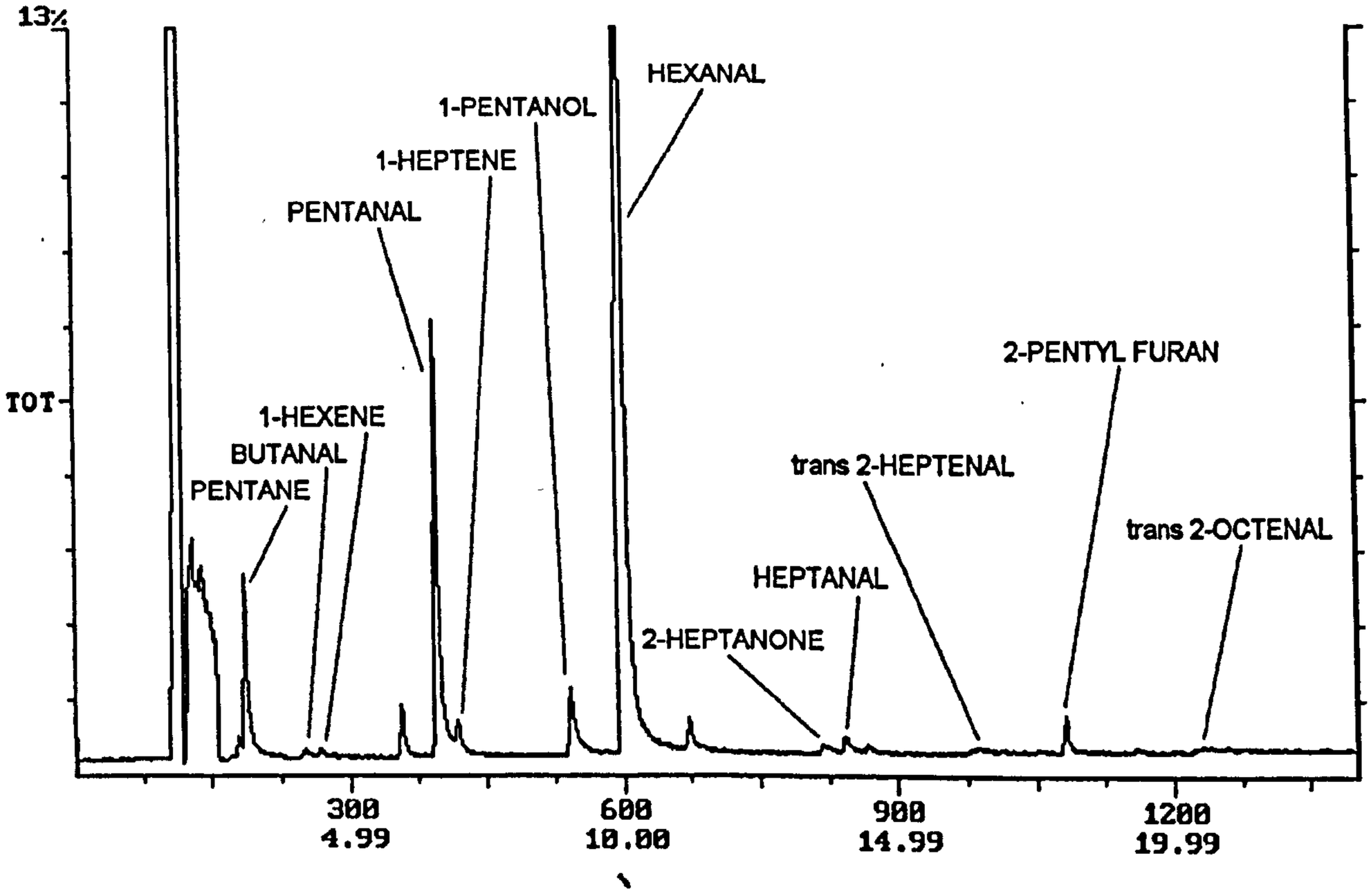
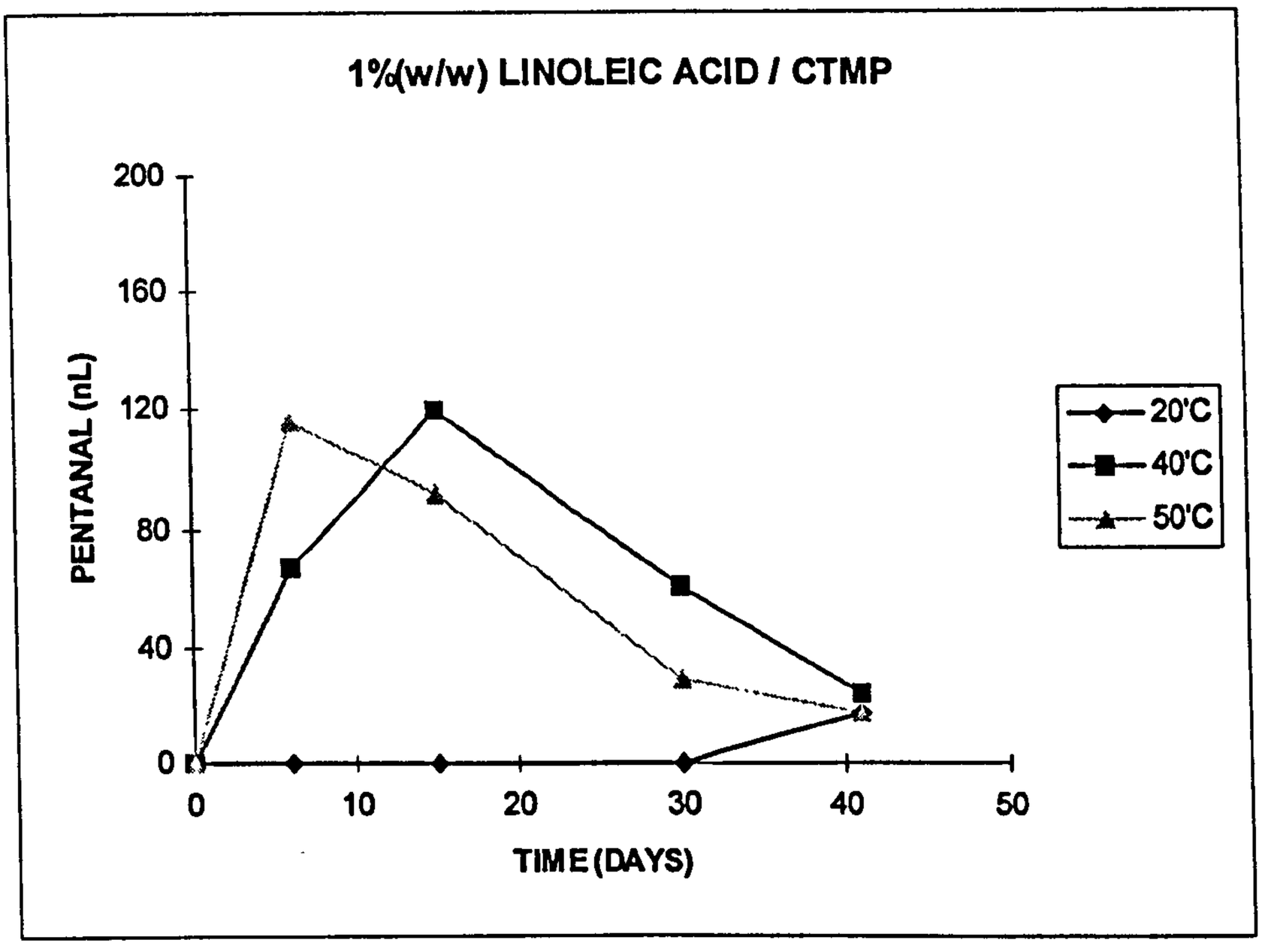


Figure 3.31 shows the amounts of pentanal recovered from samples of linoleic acid/CTMP using an equilibration temperature of 50°C. The levels of pentanal recovered from samples stored at 40°C and 50°C peak at similar amounts to those samples acquired using an equilibration temperature of 105°C. However, the pentanal levels decline more rapidly for the samples acquired using the lower equilibration temperature.

It is unlikely that pentanal is formed from 2,4-decadienal, due to the absence of *trans* 2-octenal. However, it possible that it may form via the addition of oxygen to the pentyl radical resulting from scission route A, during the decomposition of linoleic acid 13-OOH. The primary hydroperoxide formed may then lose a hydroxyl radical to yield an alkoxy radical, which can then form pentanal. The lifetime of the initial pentyl radical may be extended sufficiently, at the lower equilibration temperature, for an increased number of molecular collisions to occur with atmospheric oxygen.

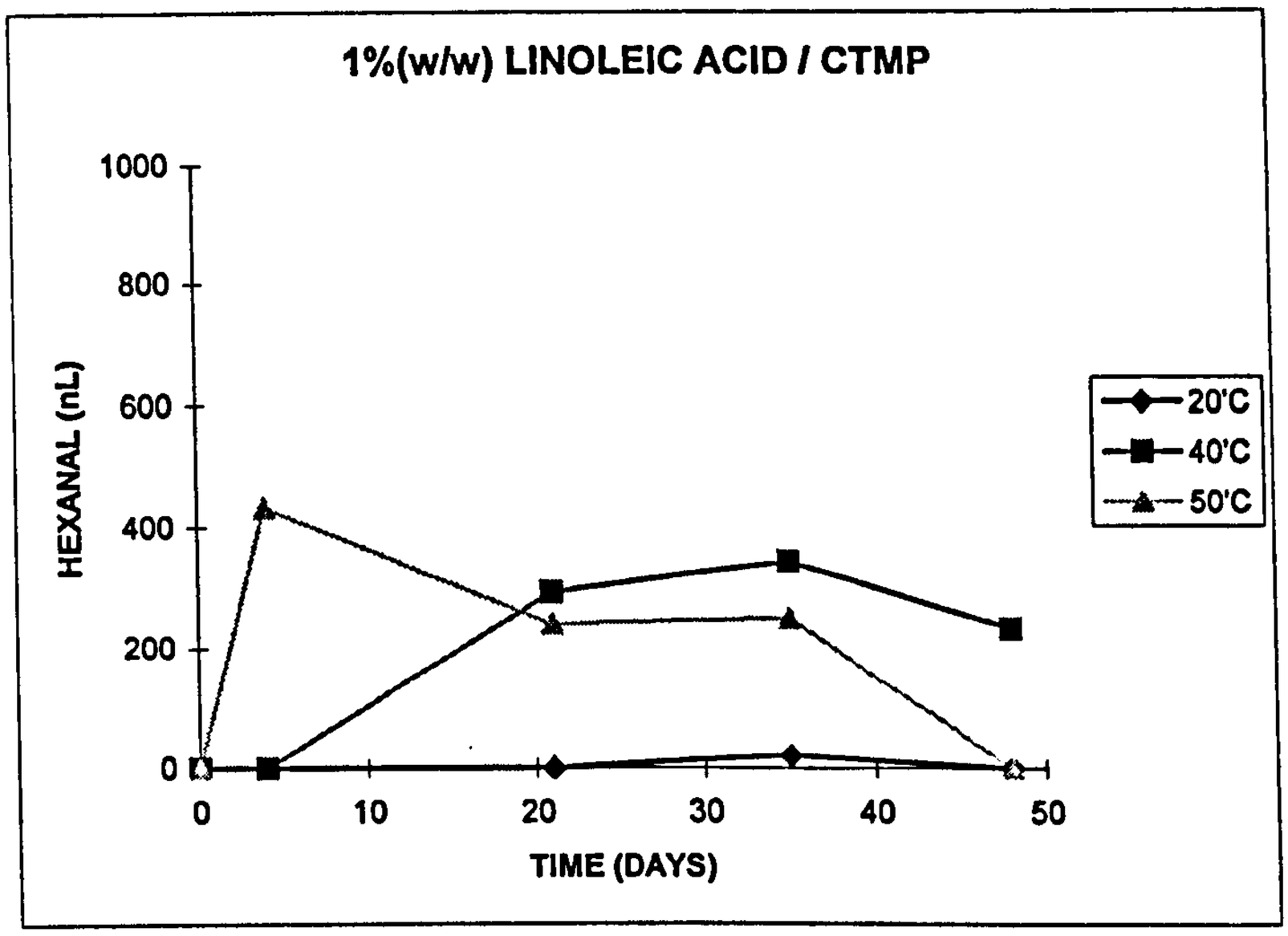
Figure 3.31 Amounts of pentanal recovered (in nL) from samples of linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 42 days (Headspace sampling temperature: 50°C).



It is evident that a headspace equilibration temperature of 50°C did not result in the thermal decomposition of linoleic acid within the CTMP system, where a low concentration of hydroperoxides existed, i.e. in the sample that was unstored and analysed shortly after preparation. However, it was less than certain how much of an effect a headspace equilibration temperature of 50°C had on the decomposition of hydroperoxides formed during storage. To determine this effect, the test involving linoleic acid/CTMP was repeated. However, when samples were analysed, no headspace equilibration period was employed. Figure 3.32 shows the amounts of hexanal recovered from samples of linoleic acid/CTMP where no headspace equilibration period was used.

The amounts of hexanal recovered are considerably less than those recovered for samples acquired using an equilibration temperature of 50°C. Greater amounts of hexanal in equilibrated samples may be due to an accelerated decomposition of hydroperoxides, to form volatile products, during equilibration. Alternatively, the low recovery of hexanal, and other volatiles, using no equilibration period may be due to these compounds having low vapour pressures at the temperatures at which these vials were stored.

Figure 3.32 Amounts of hexanal recovered (in nL) from samples of linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 48 days (No headspace equilibration period).



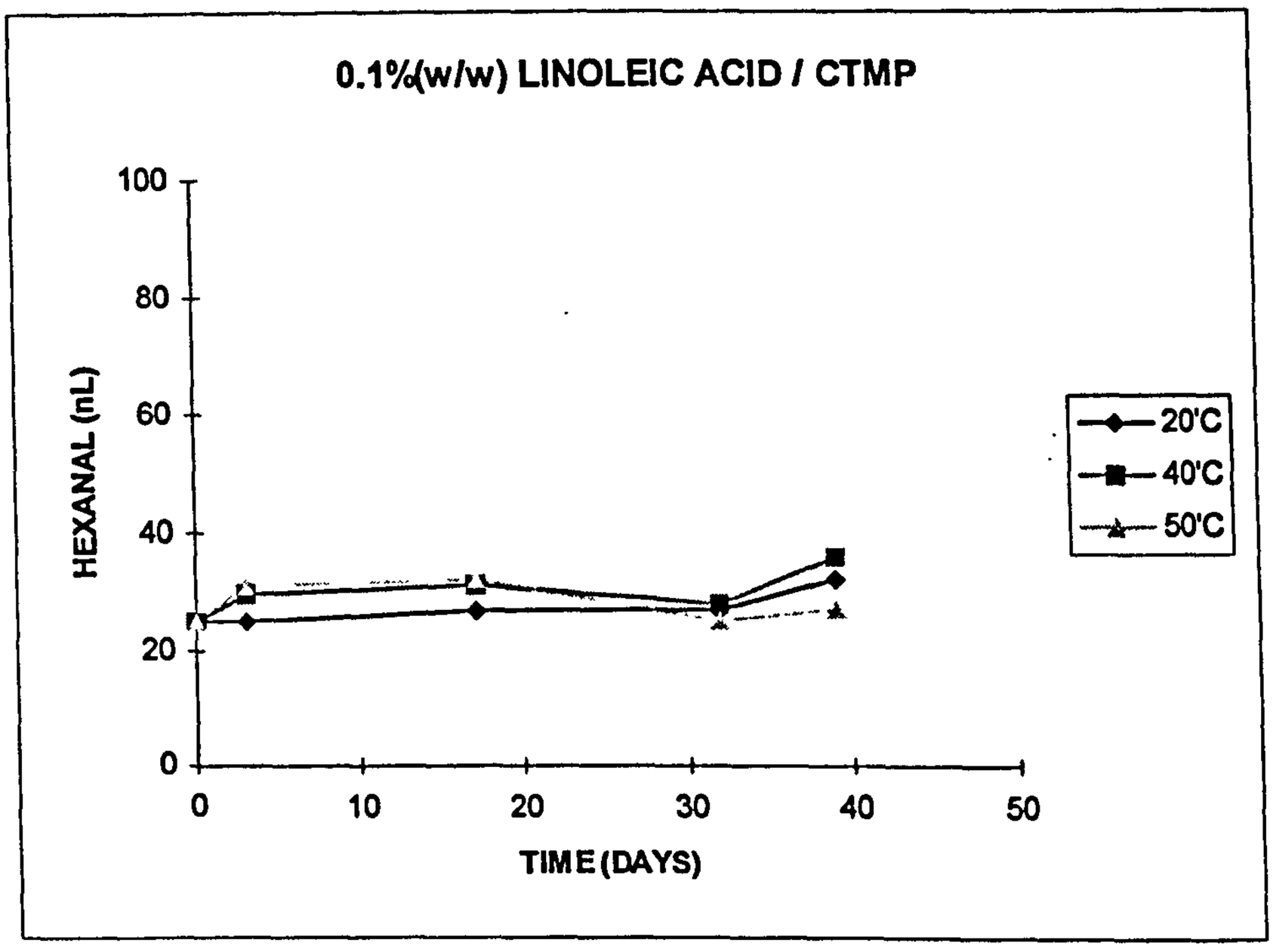
If either of these hypothesis were applicable, it does not explain why a significantly lesser amount of hexanal was recovered from samples stored at 50°C. Volatiles formed in these vials should have similar vapour pressures to samples equilibrated at 50°C. These lower recoveries can only be explained by a delay between samples being removed from the storage oven before sampling. During this period, samples may cool to temperatures approaching ambient temperature. Thus, the vapour pressure of the volatiles present may drop significantly, making it difficult to determine the effect of headspace equilibration on the decomposition of hydroperoxides.

To determine whether volatile compound formation is a linear function of linoleic acid concentration in CTMP, a test was carried out in which 1 gram samples of CTMP were impregnated with 1.1μl of linoleic acid, corresponding to 0.1%(w/w) linoleic acid in CTMP. The samples were sealed and stored in a similar way to previous samples. If volatile compound formation was to be a linear function of linoleic acid concentration, then it should be expected that the

amounts of volatiles recovered from these samples will be approximately ten times less than for the corresponding 1%(w/w) linoleic acid in CTMP samples.

Figure 3.33 shows the amounts of hexanal recovered from samples during the test.

Figure 3.33 Amounts of hexanal recovered (in nL) from samples of 0.1% (w/w) linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 39 days (Headspace sampling temperature: 105°C).



The amounts of hexanal recovered are vastly lower than for the corresponding samples impregnated with 1.0%(w/w) linoleic acid/CTMP. Even if the amounts of hexanal represented on the graph were multiplied by a factor of 10, the amounts of hexanal are still significantly less.

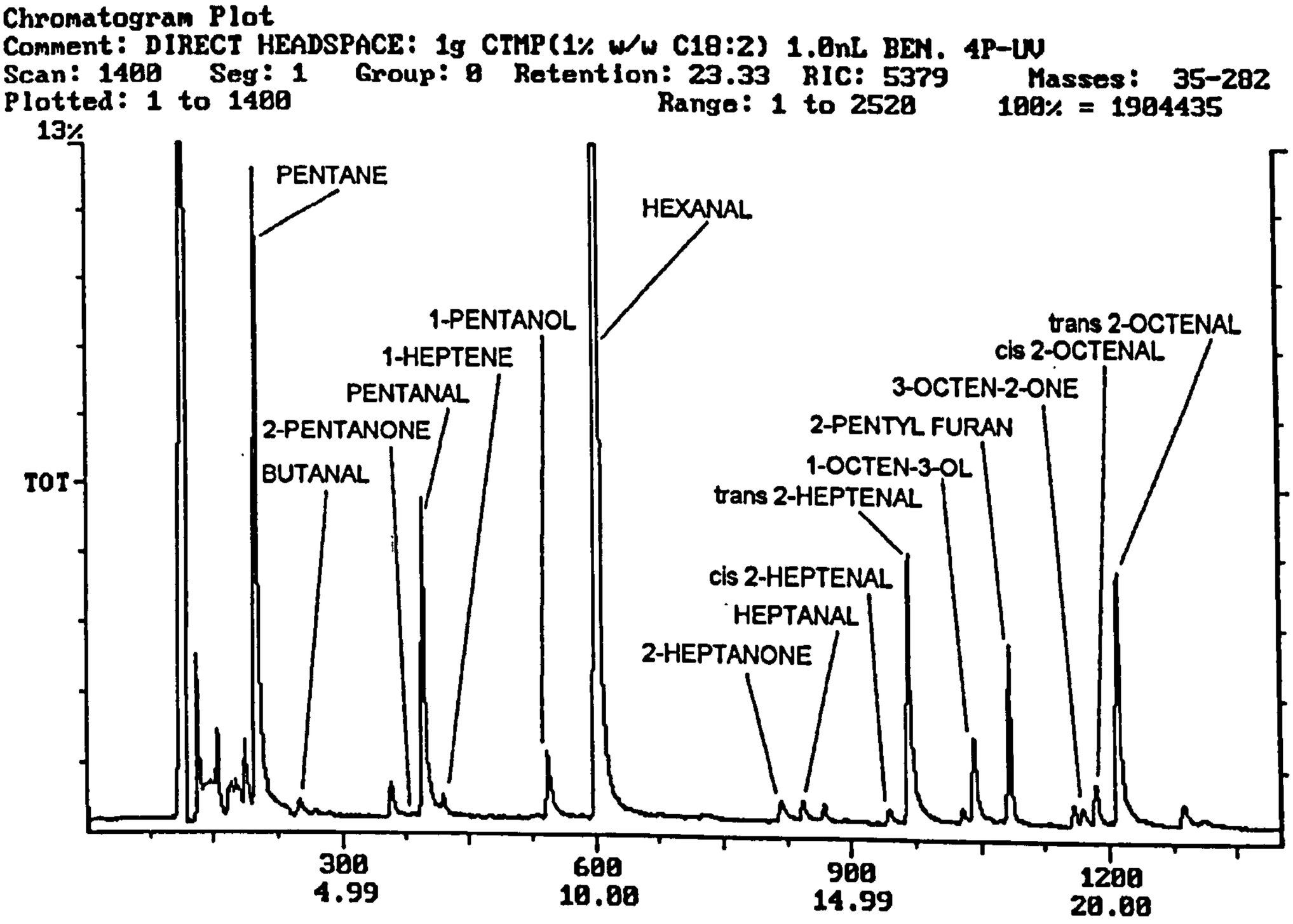
These findings demonstrate that formation of oxidation volatiles is not a linear function of linoleic acid concentration. The main reason for this probably lies in the availability of free radicals within the system. Due to the lower abundance of linoleic acid, hydrogen abstraction from linoleic acid molecules, to produce pentadienyl radicals (see Figure 3.24), by neighbouring linoleic acid peroxy radicals will occur at a much slower rate due to the lower density of the fatty acid. This will result in a slower rate of initiation. A slower rate of initiation will result in a smaller concentration of hydroperoxides being formed. A lower

concentration and density of hydroperoxides will reduce the bimolecular decomposition of hydroperoxides and, thus, significantly reduce the formation extent of volatile products.

Determination of the effect of UV irradiation on the presence of linoleic acid in CTMP

The chromatogram in Figure 3.34 shows the headspace volatiles, recovered at an equilibration temperature of 105°C, from a sample of linoleic acid/CTMP that had been UV irradiated using four passes on a belt curer. The sample had not been stored. Thus, the volatiles resulted from thermal decomposition.

Figure 3.34 Chromatogram acquired from the headspace vapours of a UV irradiated, one gram sample of CTMP/1%(w/w) linoleic acid that had undergone thermal degradation, at 105°C, for 60 minutes.

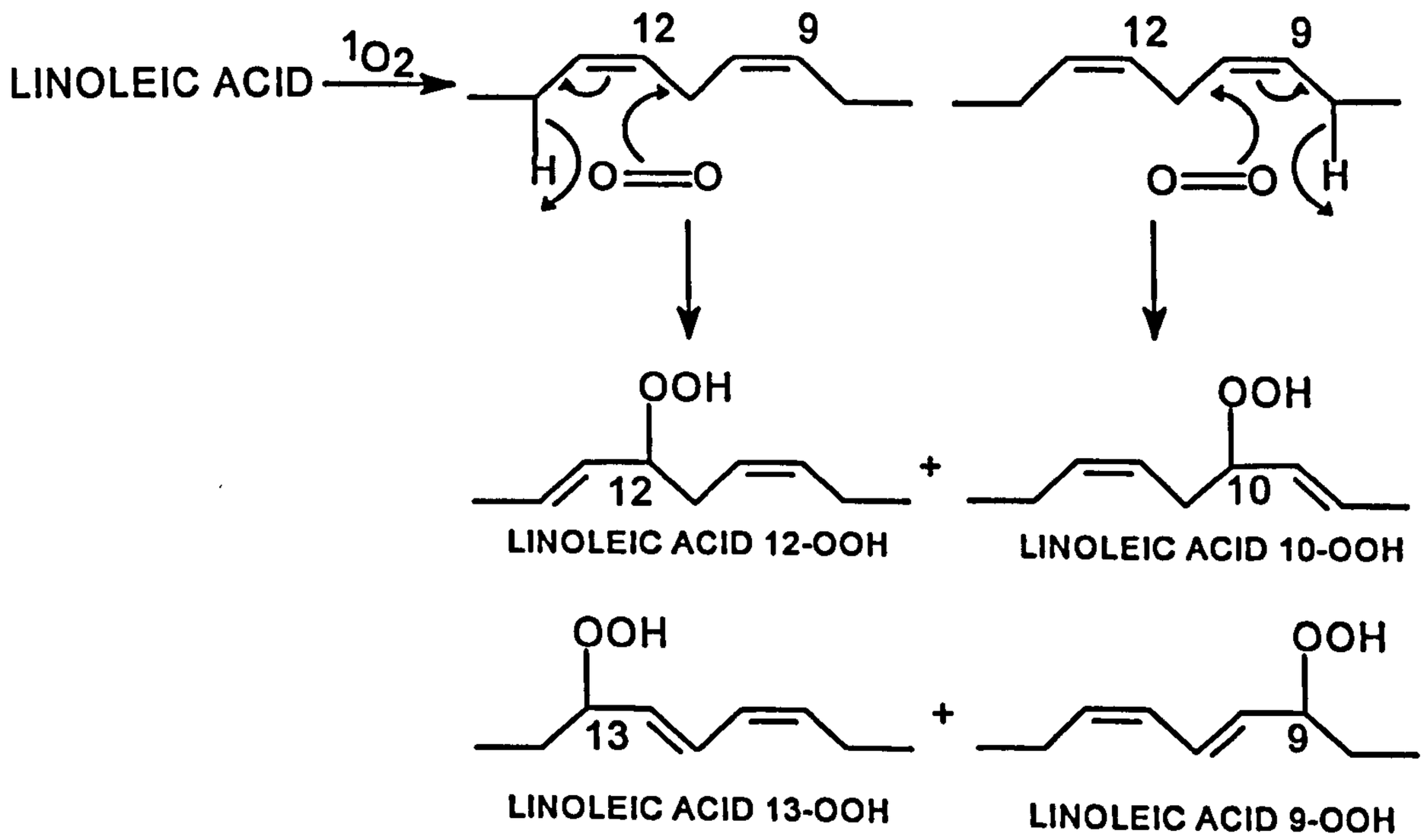


The chromatogram is not so different from that acquired from non-irradiated samples (see Figure 3.20). This suggests that irradiation does not affect the reaction pathways involved in the oxidation to any great extent. However, the

amounts of volatiles recovered were less for irradiated samples than for the non-irradiated samples. This is surprising, since photoxidation, resulting from UV radiation, is widely accepted to be significantly faster than autoxidation. It has been reported that photoxidation of methyl linoleate proceeds 1600 times faster than autoxidation. There is no induction period (Gunstone, 1984).

Photoxidation proceeds by a different mechanism from free-radical autoxidation. The singlet oxygen produced reacts with a double bond by concerted addition and, thus, attaches itself at either end of the bond in a *trans* configuration. This mechanism is illustrated in Figure 3.35.

Figure 3.35 Reaction scheme for the formation of isomeric hydroperoxides of linoleic acid by photoxidation.



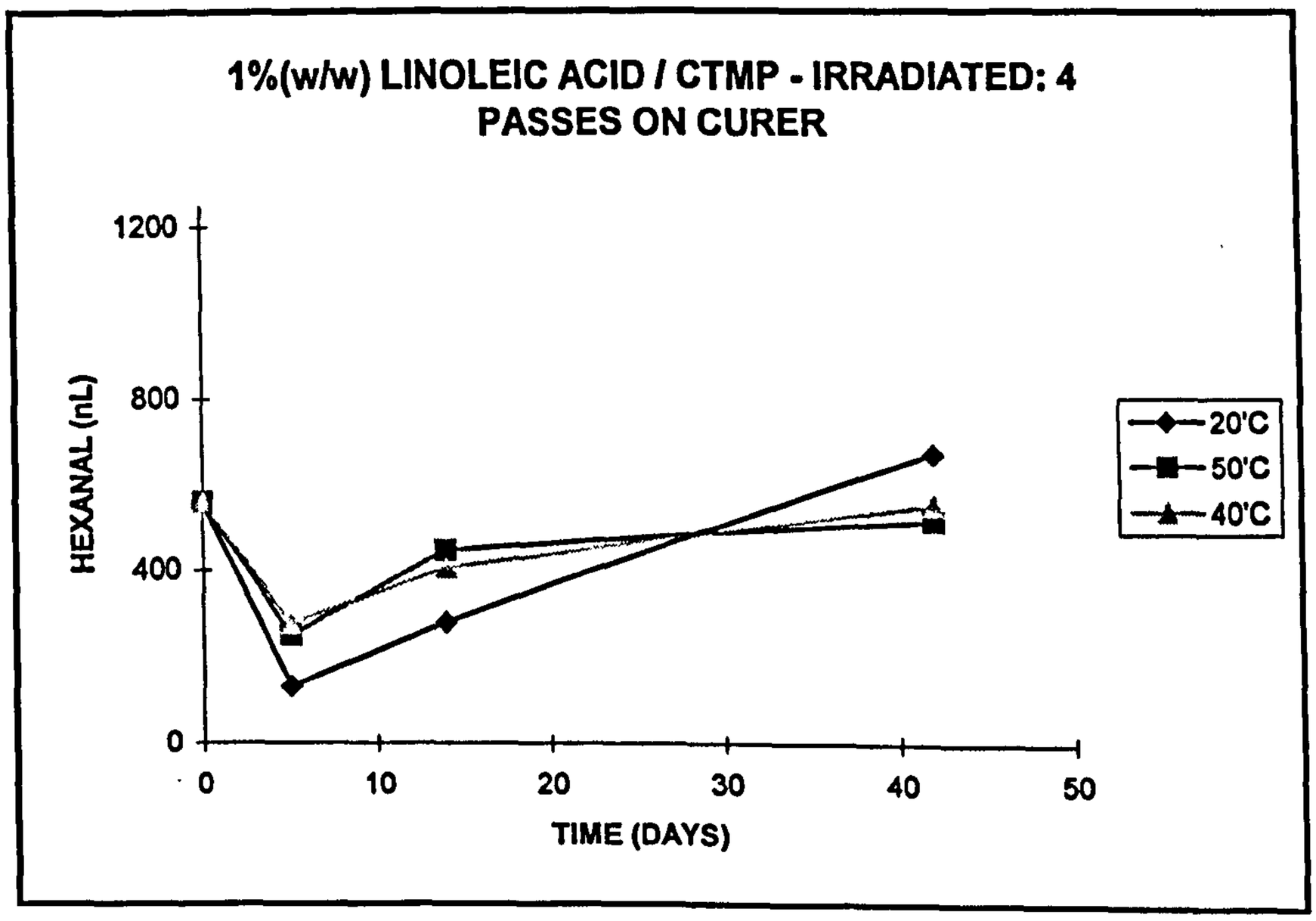
The linoleic acid unit yields a mixture of 9-, 10-, 12-, and 13-hydroperoxides. The relative proportions of these isomers are approximately 31%: 18%: 18%: 33%, respectively (Belitz and Grosch, 1987). It is thought that the internal isomers are present in smaller proportions due to the hydroperoxides having a homoallylic structure that allows 1,3-cyclisation to hydroperoxy cyclic peroxides (Kochhar, 1993).

The results of the tests carried out, in which the amounts of volatile were no greater, suggest that singlet oxygen is not involved and photoxidation does not occur. This may be due to the lack of photosensitiser in the linoleic acid/CTMP system. The explanation for the apparent smaller amounts of volatiles,

compared to those in the non-irradiated samples, suggests that some other competing reaction mechanisms exist. These reactions may involve the addition of radical species to a chain to produce non-volatile, high molecular weight species (oligomers) that are not detected by headspace analysis.

Hexanal was still found to be the most abundant volatile compound detected in all the samples, during the test. Figure 3.36 shows the amounts of hexanal that were recovered from samples of irradiated linoleic acid/CTMP, headspaced at 105°C.

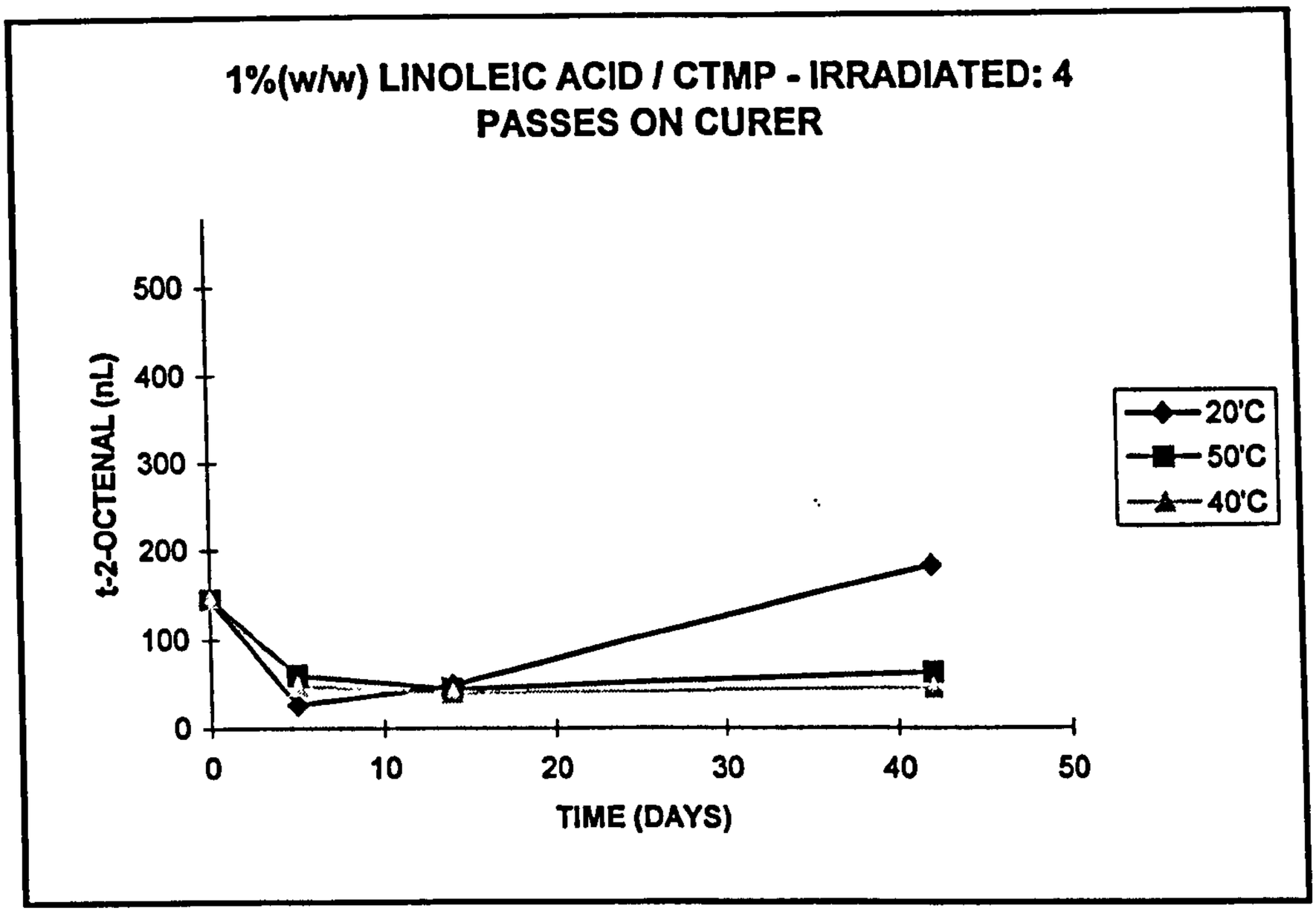
Figure 3.36 Amounts of hexanal recovered (in nL) from samples of irradiated linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 42 days (Headspace sampling temperature: 105°C).



As in the non-irradiated samples, the rate of hexanal formation declines after 2 weeks, at the two higher storage temperatures, but continued to increase at room temperature. This may be due to the breakdown of hexanal at higher temperatures, to the further oxidation of hexanal to the corresponding fatty acid, and/or the involvement of competing reactions, such as oligomerisations that are favoured at higher temperatures.

The amounts of *trans* 2-octenal recovered from irradiated samples showed little difference to those recovered from non-irradiated samples. Figure 3.37 shows the amounts of *trans* 2-octenal recovered during the test.

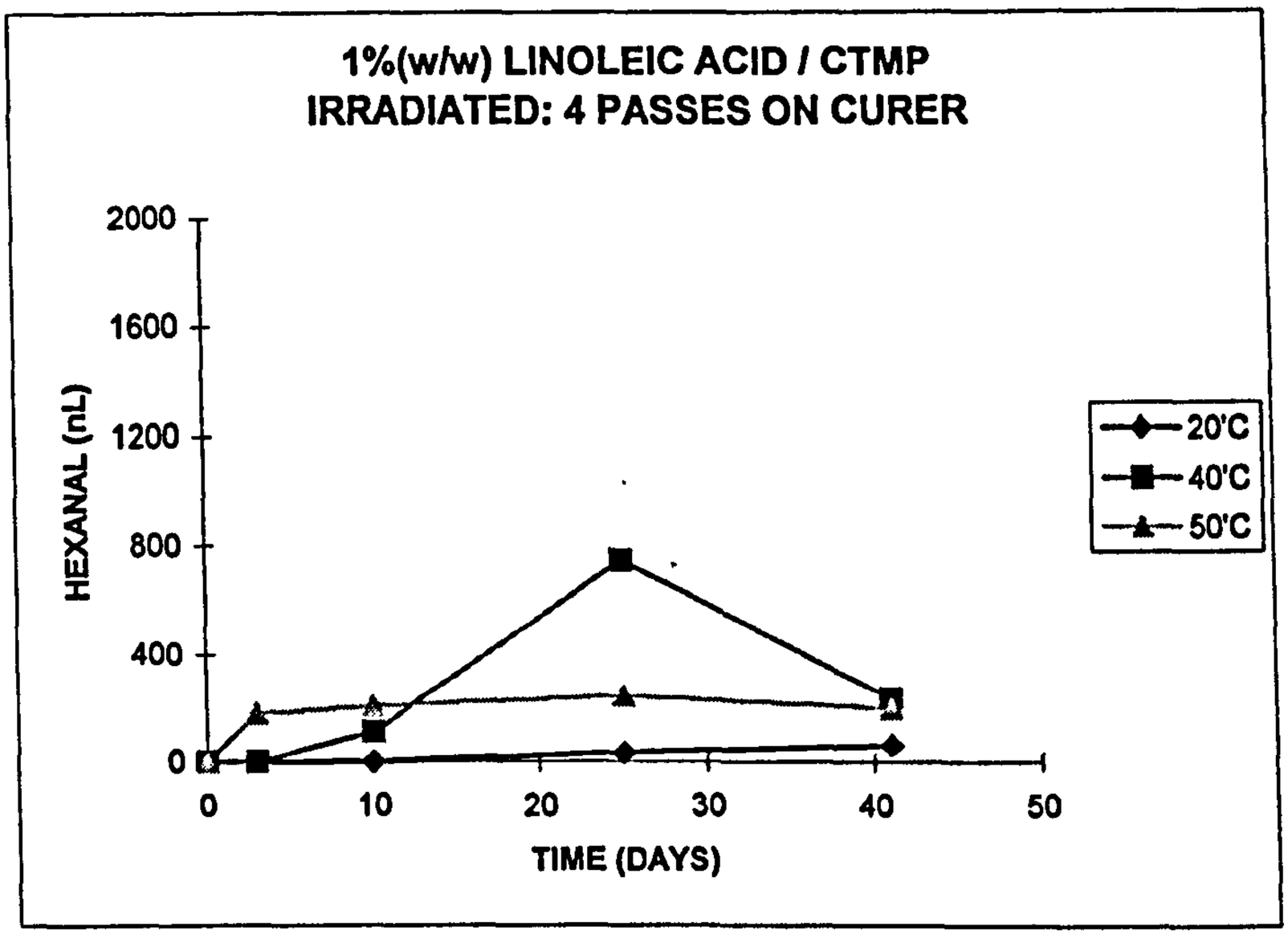
Figure 3.37 Amounts of *trans* 2-octenal recovered (in nL) from samples of irradiated linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 42 days (Headspace sampling temperature: 105°C).



If octenal is a good indicator to the brief existence of 2,4-decadienal, then UV irradiation of samples has little effect on 2,4-decadienal formation relative to the non-irradiated samples. It has been reported that the relative formation of 2,4-decadienal decreases in photoxidised methyl linoleate (Tressl et al., 1981). This, again, suggests that the sample system contains no effective photosensitisers. Tressl *et al.* (1981) states, also, that the formation of *trans* 2-heptenal should increase with photoxidation. No significant differences were detected in the levels of *trans* 2-heptenal between non-irradiated and irradiated samples of linoleic acid/CTMP.

The test was repeated, using samples linoleic acid/CTMP that had been irradiated with UV light. However, a headspace equilibration period of 50°C was used. Figure 3.38 shows the amounts of hexanal recovered during the test.

Figure 3.38 Amounts of hexanal recovered (in nL) from samples of irradiated linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 41 days (Headspace sampling temperature: 50°C).



The amounts of hexanal recovered, compared to samples that had not been irradiated with UV light, were significantly less. This suggests that the irradiation process had an effect on the oxidation process that was more pronounced at the lower headspace equilibration temperature. The amounts of hexanal recovered, compared to those sampled acquired using an equilibration period of 105°C, were also less.

It is likely that irradiation with UV light, even in the absence of a suitable photosensitiser, results in the formation of different products from oxidation. These products are likely to be non-volatile compounds of higher molecular weight (e.g. oligomers).

Determination of the effect of reduced oxygen partial pressure on the presence of linoleic acid in CTMP

The chromatogram in Figure 3.39 shows the headspace volatiles, collected at 105°C for a sample of linoleic acid/CTMP that had undergone thermal degradation at a significantly reduced oxygen partial pressure. It is obvious that the lower levels of oxygen have retarded considerably, the formation of volatiles. The only two volatiles recovered were pentane and hexanal, in small amounts.

Figure 3.39 Chromatogram acquired from the headspace vapours of a one gram sample of CTMP/1%(w/w) linoleic acid that had under gone thermal degradation, at low oxygen partial pressure, at 105°C, for 60 minutes.

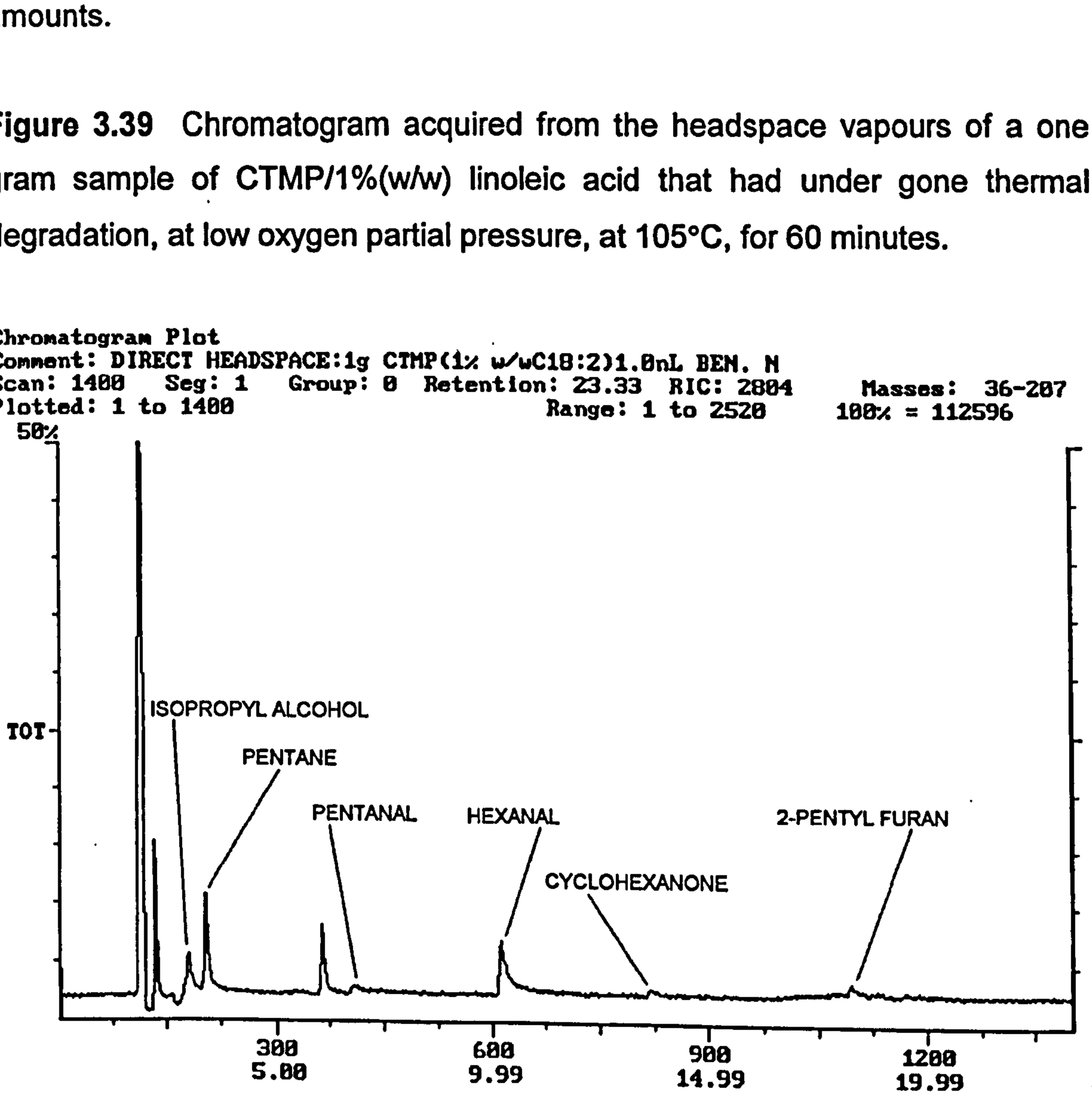
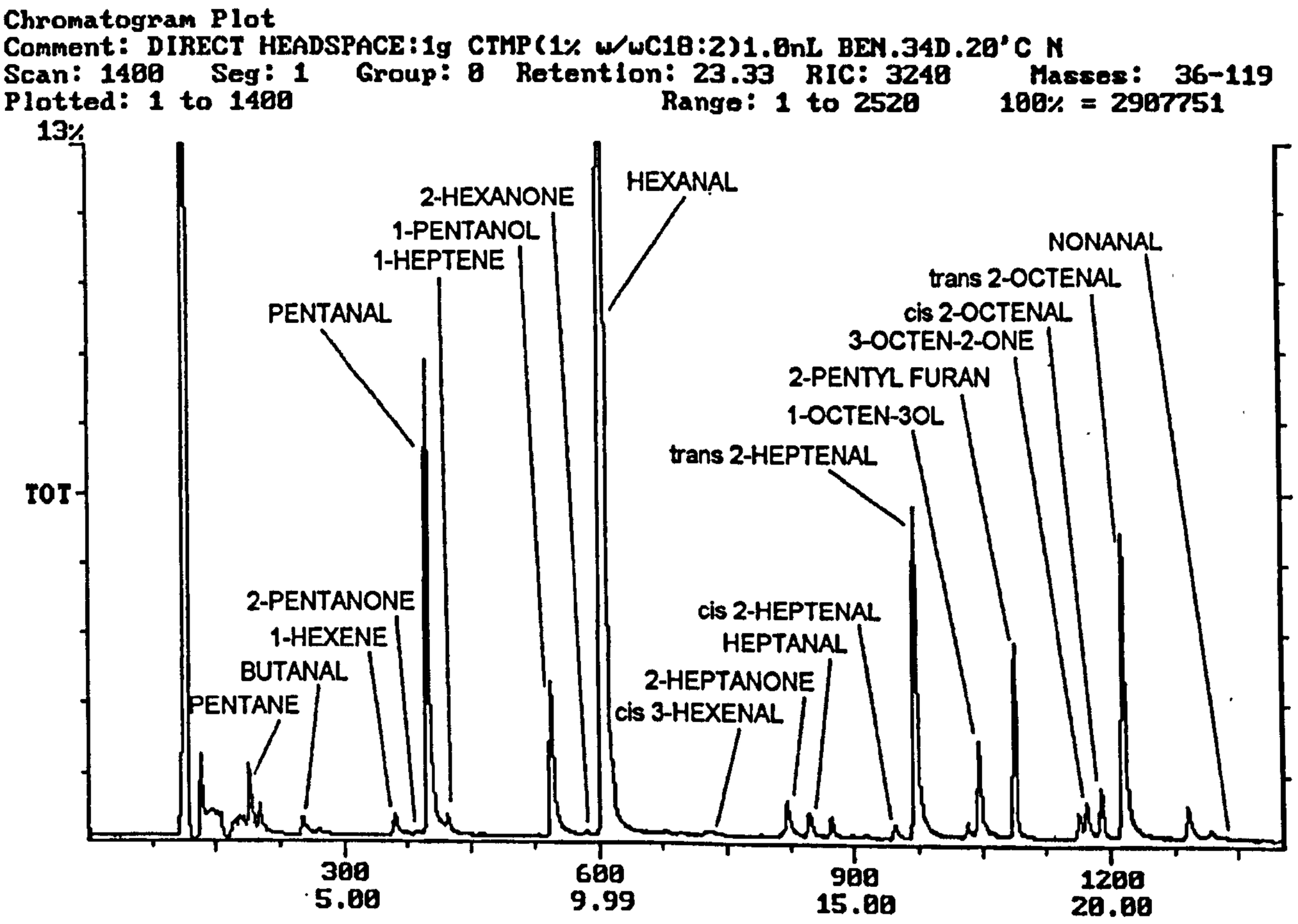


Figure 3.40 shows the chromatogram for the headspace volatiles recovered from a sample of linoleic acid/CTMP stored, under reduced oxygen partial pressure, for 34 days at 20°C. It shows relatively extensive formation of volatiles, the nature of which are comparable with those recovered from samples that were oxidised at atmospheric oxygen partial pressures.

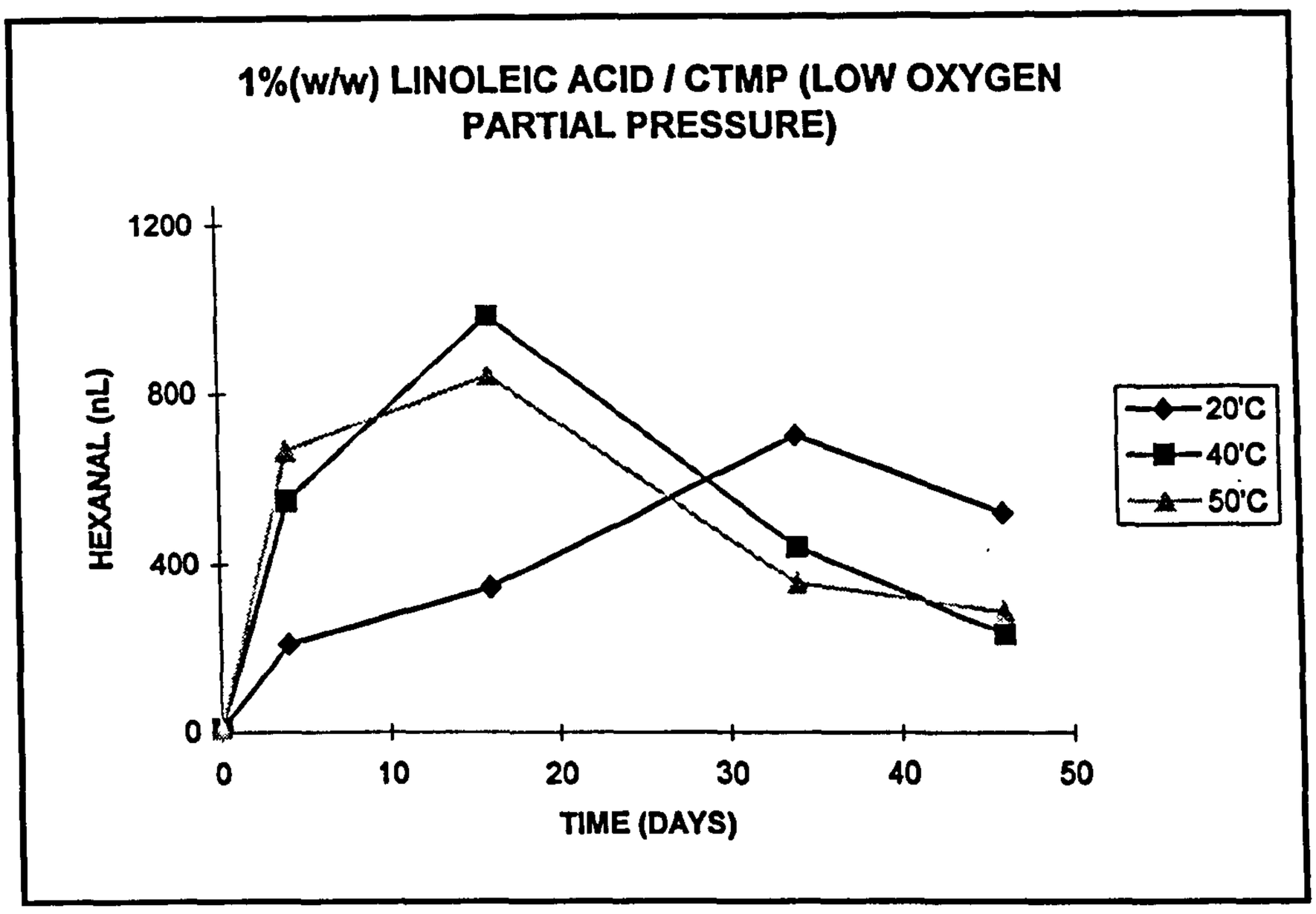
Figure 3.40 Chromatogram acquired from the headspace vapours of a one gram sample of CTMP/1%(w/w) linoleic acid that had been stored, at 20°C, for 34 days, at low oxygen partial pressure.



The amounts of hexanal, initially small, increased rapidly at the higher storage temperatures, in particular at 40°C, before declining after 2-3 weeks. At ambient temperature, the hexanal levels increased for up to approximately 5 weeks before starting to decline. Figure 3.41 shows the amounts of hexanal recovered during the test.

The amounts of hexanal found in samples, stored for approximately two weeks at 40°C and 50°C, were greater than those found from samples that had been stored at normal oxygen partial pressures. This could suggest that, under normal conditions, hexanal, and other volatiles, may be broken down readily to other compounds or oxidised. However, at reduced oxygen pressures this breakdown is retarded. Therefore, the build up of hexanal in the system is not counteracted by competing breakdown reactions.

Figure 3.41 Amounts of hexanal recovered (in nL) from samples of linoleic acid/CTMP stored, under reduced oxygen partial pressure, at 20°C, 40°C and 50°C for up to 46 days (Headspace sampling temperature: 105°C).



Another reason for the increased hexanal levels may lie in the nature of the termination reactions involved in the free radical processes. There are three major termination reaction options:

(i) $ROO^{\bullet} + ROO^{\bullet} \longrightarrow$

(ii) $ROO^{\bullet} + R^{\bullet} \longrightarrow$ NON-RADICAL PRODUCTS

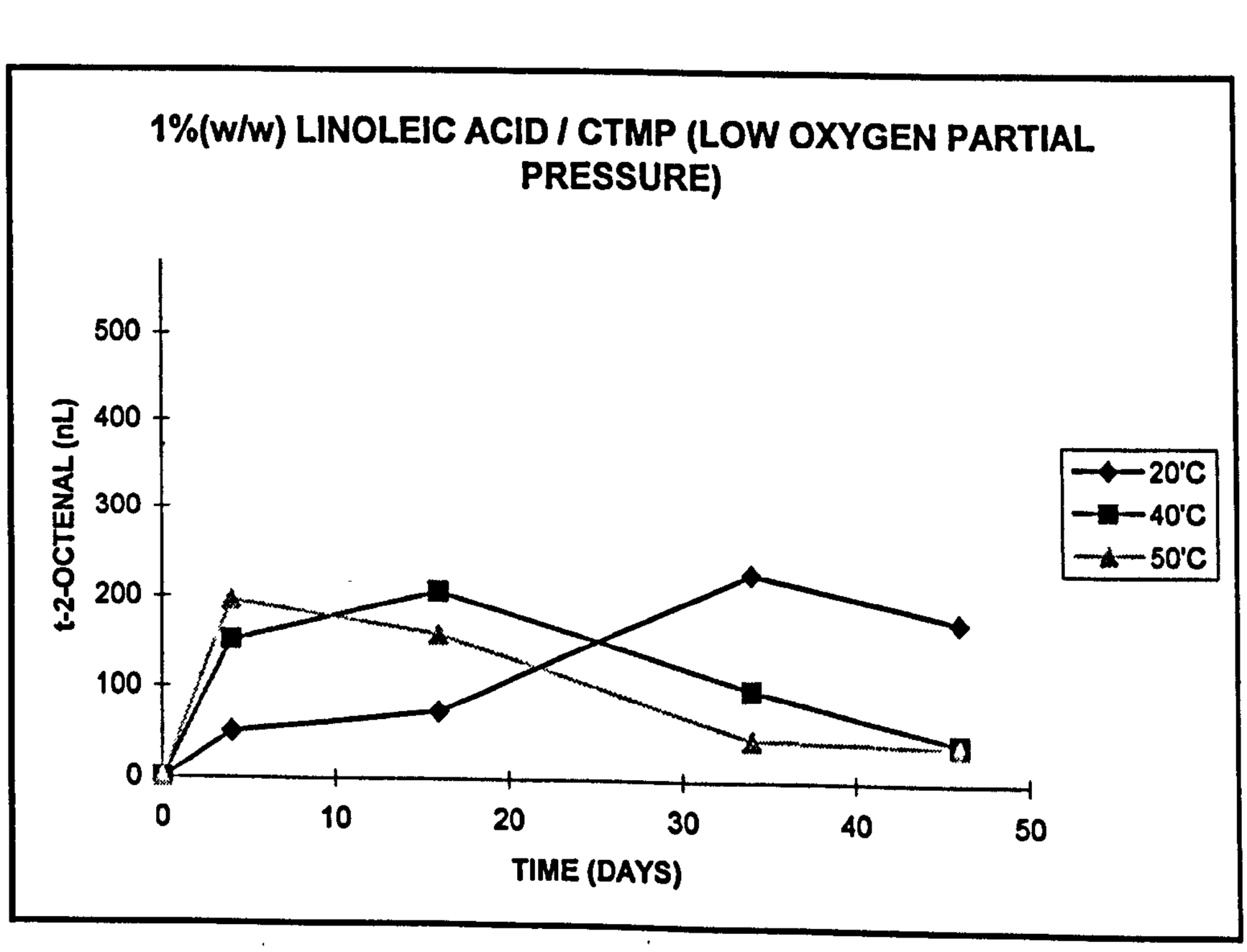
(iii) $R^{\bullet} + R^{\bullet} \longrightarrow$

At high oxygen partial pressures, termination reaction (i) predominates. However, as the oxygen partial pressure falls, reaction (ii) becomes more significant and reaction (i) starts to decline. If the oxygen pressure continues to fall then reaction (i) becomes insignificant, reaction (ii) passes a peak and begins to decline and reaction (iii) becomes important (Labuza, 1971). During the test carried out on linoleic acid/CTMP, it is possible that reaction (i),

normally predominant at atmospheric oxygen pressure, is less extensive and reactions (ii) and (iii) are more significant. Thus, combination of peroxy radicals is less frequent, leaving more peroxy radicals available, to form hydroperoxides, and/or decompose in to volatile oxidation products.

The levels of *trans* 2-octenal recovered from samples, followed a similar pattern to that of hexanal. However, the amounts of *trans*-2-octenal found at ambient temperature, after 6 weeks storage, were significantly greater than those found at higher temperatures. This may indicate that this unsaturated compound is readily broken down or oxidised at higher temperatures. However, at the lower oxygen pressure and temperature, this oxidation is less rapid. Figure 3.42 shows the amounts of *trans* 2-octenal, recovered from samples of linoleic acid/CTMP, stored under a reduced oxygen partial pressure.

Figure 3.42 Amounts of *trans* 2-octenal recovered (in nL) from samples of linoleic acid/CTMP stored, under reduced oxygen partial pressure, at 20°C, 40°C and 50°C for up to 46 days (Headspace sampling temperature: 105°C).



Generally, the amounts of *trans*-2-octenal are greater than those found from samples stored under a normal oxygen pressure. If, as before, it is assumed that the presence of octenal is a good indicator of the involvement of 2,4-

decadienal, then it can be assumed that the lower oxygen partial pressure favours the formation of 2,4-decadienal, via linoleic acid 9-hydroperoxide. This is in agreement with the findings of Grosch *et al.* (1981), who suggested that hexanal formation, via isomerisation of linoleic acid 9-hydroperoxide to the 13-isomer, is favoured in the presence of oxygen. Thus, as the oxygen pressure decreases, formation of 2,4-decadienal is favoured over that of hexanal.

The amounts of pentanal recovered from samples during the test showed a similar pattern to those given by hexanal and *trans* 2-octenal.

Another point concerns the availability of oxygen at low partial pressures. It has been reported that at higher temperatures, oxygen is less soluble in the substrate that is undergoing oxidation. Thus, at higher temperatures, the rate of oxidation becomes more limited with respect to the oxygen pressure, especially for linoleic acid (Labuza, 1971). If the amounts of hexanal recovered from samples can be considered to be an indicator of the overall oxidation of the samples in the test, this theory could explain one of the reasons why smaller amounts of hexanal were recovered at 50°C, than at 40°C, for some samples, over the storage period. It may, also, suggest why the levels of hexanal decline, at both 40°C and 50°C, after 2-3 weeks; possibly as a result of the falling oxygen pressure, as oxygen is taken up in peroxide formation, and the remaining oxygen being subject to reduced solubility at the higher temperatures.

Determination of the effect of the relative humidity on the presence of linoleic acid in CTMP

The process of fatty acid oxidation can be divided into different stages. Firstly, primary initiation occurs. As discussed before, this involves the formation of the first few hydroperoxides by addition of oxygen to the fatty acid chains that have undergone a hydrogen abstraction reaction.

The second stage includes the development of the chain reaction. At this stage, sufficient peroxides are present for the initiation to proceed by a process that requires less energy; the monomolecular decomposition in to free radicals:

$$ROOH \longrightarrow RO^{\cdot} + {}^{\cdot}OH$$

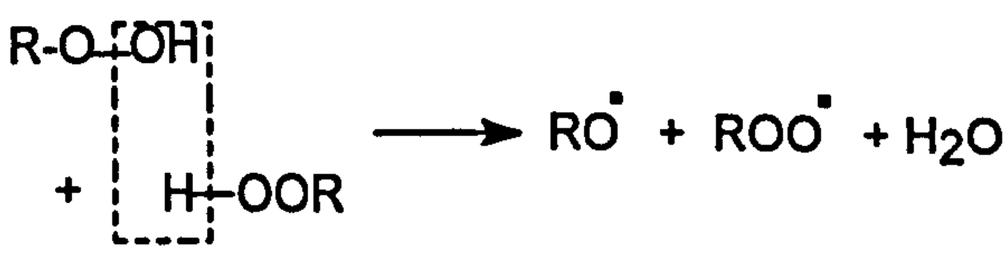
$$ROOH \longrightarrow R^{\bullet} + HO_2^{\bullet}$$

During monomolecular decomposition, the concentration of substrate is considered to be constant, due to the low extent of oxidation. The rate and time course of oxidation are proportional to the square route of the extent of oxidation. The monomolecular decomposition stage is considered to be relevant up to an oxidation extent of approximately 0.02 moles of hydroperoxide/mole of substrate (Labuza, 1971), after which bimolecular decomposition becomes significant.

During bimolecular decomposition, the rate of oxidation is directly proportional to the peroxide concentration. The bimolecular decomposition reaction can be written as below:

$$2ROOH \longrightarrow ROO^{\bullet} + RO^{\bullet} + H_2O$$

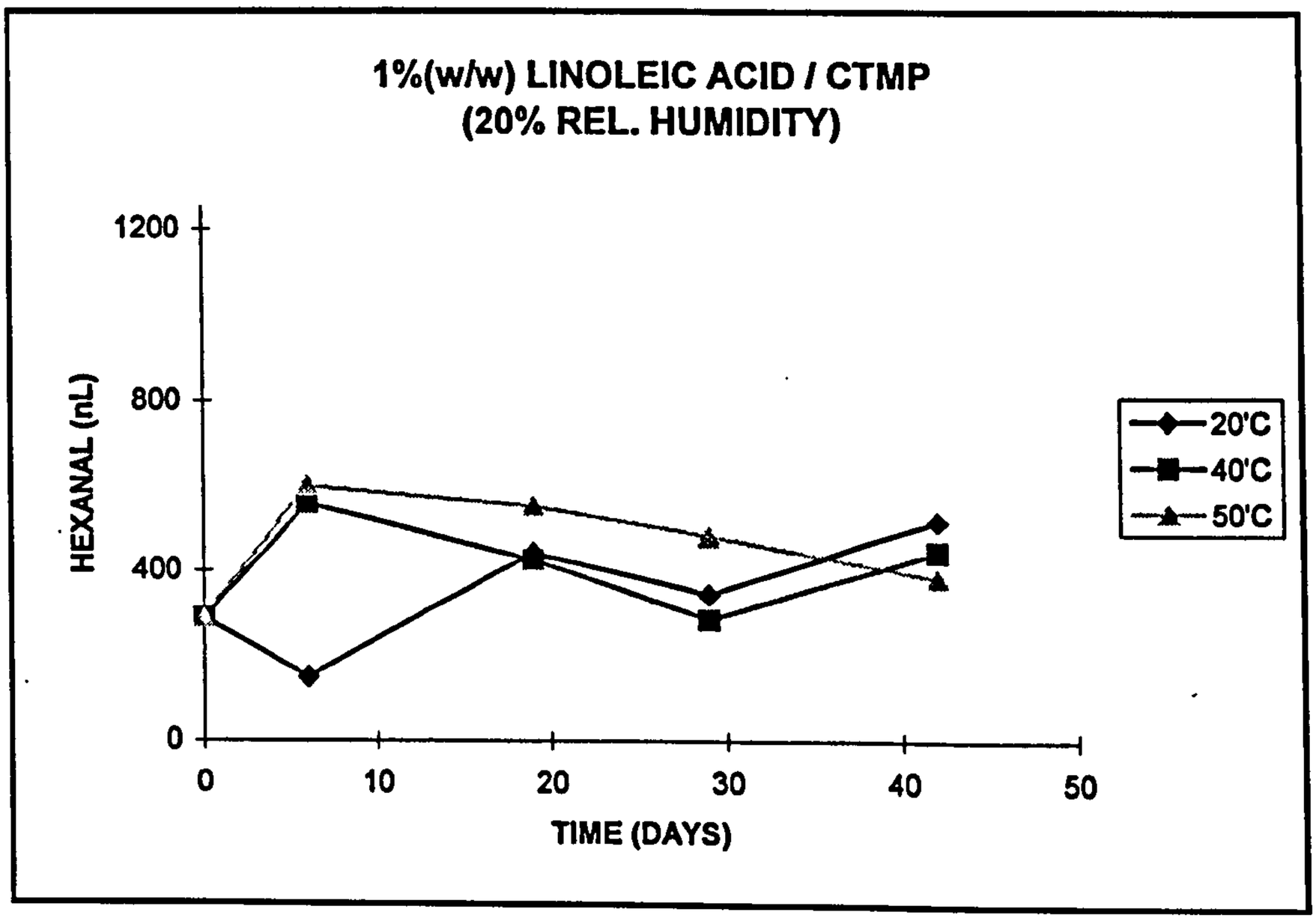
During the bimolecular reaction, peroxides have been observed to form hydrogen-bonded complexes with each other (Labuza, 1971). Once peroxide composition is high enough, diffusion limitations are overcome and peroxides are in close enough vicinity to be able to associate. It has been reported that hydrogen bonded dimer decomposition is energetically favourable (Labuza, 1971):



Due to the nature of the above reaction, the solvent polarity effects the rate of decomposition. Thus, as the solvent polarity increases the reaction should proceed at a slower rate, due to hydrogen bonding between the peroxide and the solvent. Therefore, the water content of the sample system has important consequences for the rate of oxidation. As the humidity rises, a drop in the amounts of volatile compounds recovered should be detected. Figure 3.43 shows the amounts of hexanal that were recovered from samples of linoleic

acid/CTMP stored at a 20% relative humidity. Figure 3.44 shows the amounts of hexanal recovered from samples that were stored at a 52% relative humidity.

Figure 3.43 Amounts of hexanal recovered (in nL) from samples of linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 42 days at 20% relative humidity (Headspace sampling temperature: 105°C).

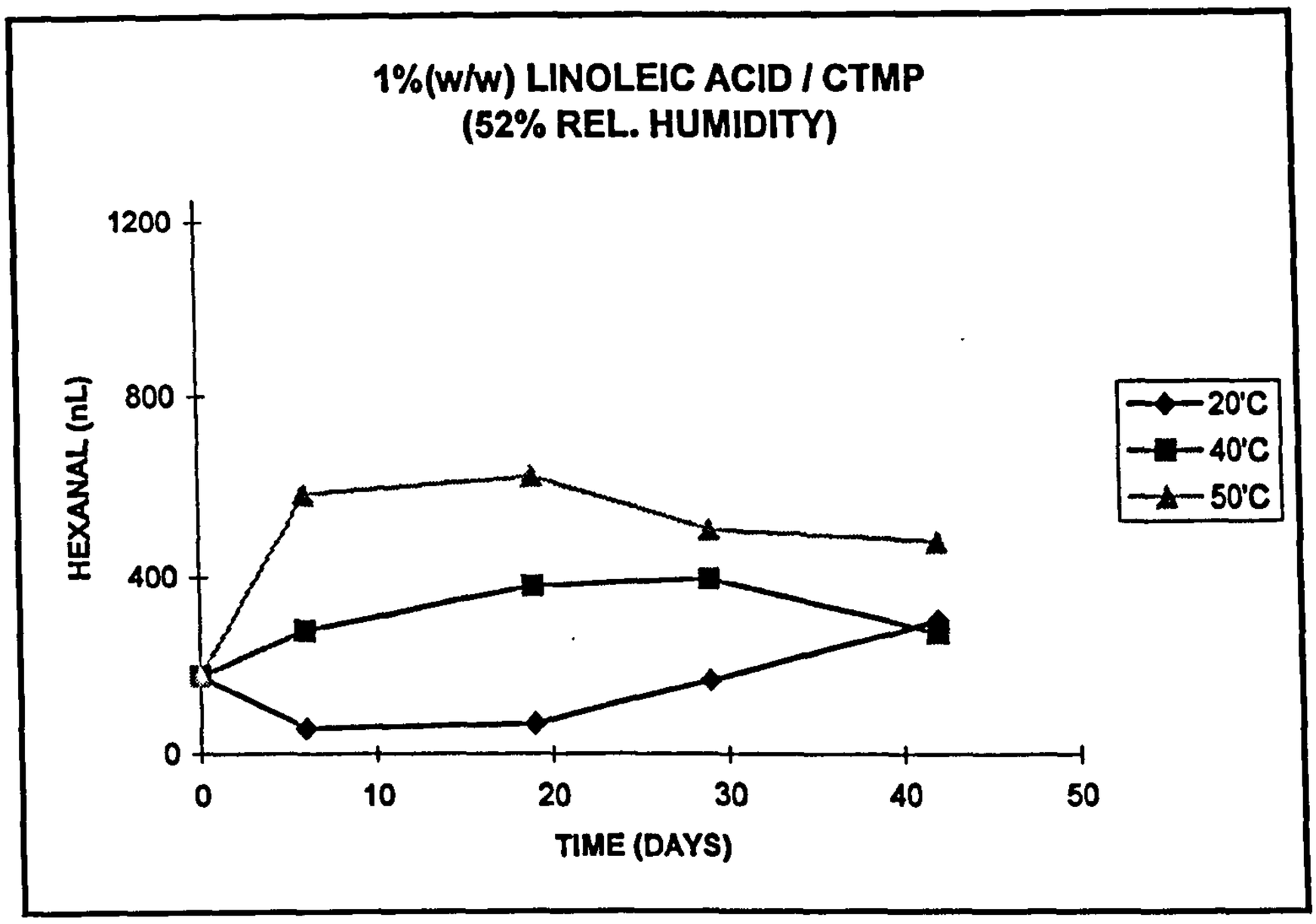


In general, the amounts of hexanal recovered from samples stored at 52% relative humidity are initially slightly less than those recovered from samples stored at 20% relative humidity. However, at longer storage periods, the amounts of hexanal recovered were more equal. This may be due to the humidity, at which the samples were initially stored at 20% relative humidity, rising due to the generation of water. This water may be formed in both the monomolecular and bimolecular decomposition stages illustrated above.

It has also been reported that solvent polarity can effect the termination reaction (Labuza, 1971):

$$ROO^{\bullet} + ROO^{\bullet} \longrightarrow \text{TERMINATION PRODUCTS}$$

Figure 3.44 Amounts of hexanal recovered (in nL) from samples of linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 42 days at 52% relative humidity (Headspace sampling temperature: 105°C).



As mentioned before, this reaction is significant at higher oxygen partial pressures. Therefore, at high oxygen pressures and high relative humidity, the above termination reaction may be moderately suppressed.

Consequently, this may affect samples at short storage periods, when the oxygen pressure is still relatively high. This may explain why the amounts of hexanal recovered from samples stored under ambient temperature, at the 20% relative humidity, are slightly greater than the amounts recovered from equivalent samples, stored at the 52% relative humidity.

Considering the levels of *trans* 2-octenal recovered from samples, the results were not too dissimilar at the two different humidities for samples stored at 40°C and 50°C. However, at ambient temperature, the amounts detected after longer storage periods were greater at the lower humidity. This may indicate that the 13-hydroperoxide isomer is favoured at lower humidity and that lower amounts of 2,4-decadienal are formed via the 9-isomer.

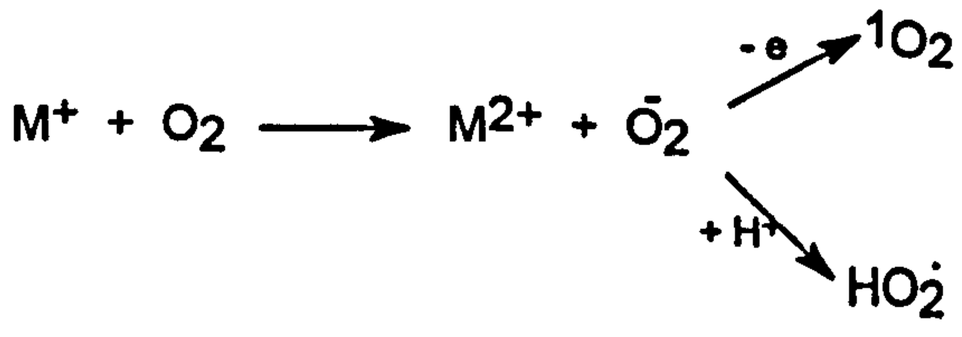
The amounts of pentanal recovered during the tests were comparable at both relative humidities and no significant differences were found.

Determination of the effect of the presence of small amounts of copper on linoleic acid in CTMP

Transition metal ions, that possess two or more valency states, with a suitable oxidation-reduction potential between them, both decrease the induction period and increase the rate of oxidation. These metal ions include copper ions, cobalt ions, iron ions, nickel ions, and manganese ions, as well as others of minor importance. The major action of these metal ions is in the reduction of the activation energy of the initiation step to 60-100 kJ mol^{-1}. Some metal ions are so active that their effect is pronounced even at concentrations as low as 0.01 ppm, i.e. of the order of their natural occurrence in fats and oils. All materials of tree and plant origin contain traces of heavy metal ions, which partially enter the lipophilic phase. These heavy metal ions are usually bound as salts of both phospholipids and free fatty acids.

Trace amounts of heavy metal ions can produce free radicals by direct reaction with intact lipids (RH):

The metal ion in its lowest valency state can react readily with oxygen, forming an oxygen radical ion:



This oxygen anion can either lose an electron to give singlet oxygen, or reacts with a proton to form a peroxy radical, able to initiate a chain reaction. The metal ion loses an electron to return to the higher valency state.

In the complete absence of oxygen, the above reaction is retarded by the metal ion in its lower valency state so that the H-abstraction reaction from the substrate can be considered to be reversible.

Heavy metal ions also produce free radicals, via the decomposition of lipid hydroperoxides, as illustrated:

$$ROOH + M^{3+} \longrightarrow ROO^{\cdot} + H^{\cdot} + M^{2+}$$

$$ROOH + M^{2+} \longrightarrow RO^{\cdot} + {}^{\cdot}OH + M^{3+}$$

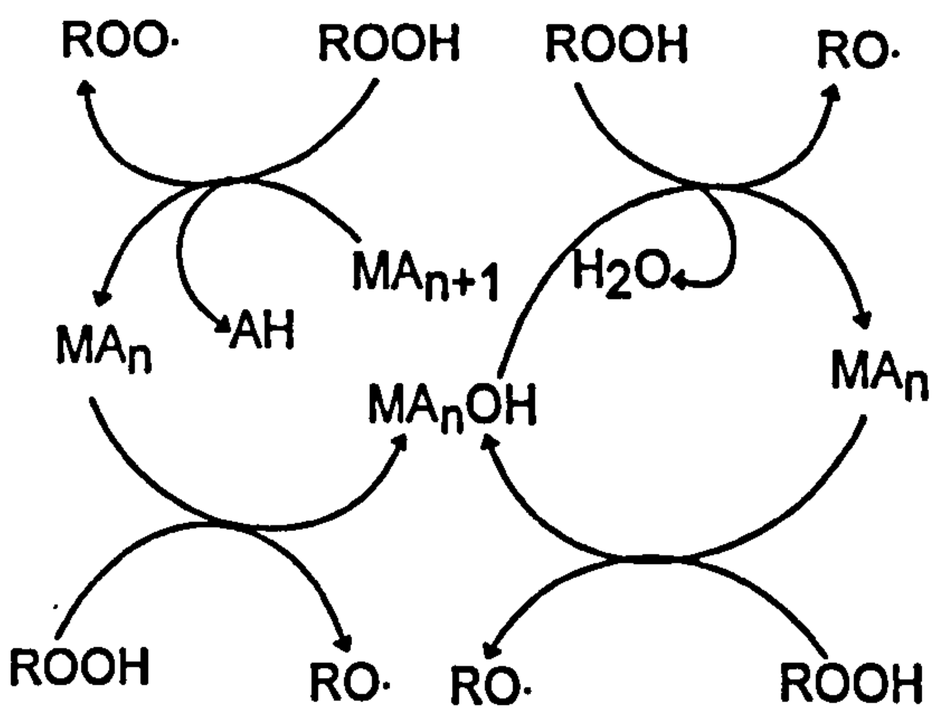
Metals in both valency states can take part. It is these two simultaneous reactions that are the most important source of free radicals in nearly all cases of lipid oxidation.

Heavy metals also take part in further oxidation reactions of secondary reaction products, for example, aldehydes and alcohols, produced by the decomposition of hydroperoxides. Primary hydroxyl groups of alcohols are oxidised to aldehydes (Pokorny, 1987). Secondary alcohols are oxidised to ketones in a similar way. Aldehydes are oxidised, in turn, to carboxylic acids.

Heavy metal ions can react with the phenolic groups of antioxidants, for example, tocopherols, oxidising them to quinones, and so deactivating, at least partially, the antioxidants present.

In a low polarity medium such as fats and oils, metal catalysis may proceed via a non-ionic mechanism (Pokorny, 1987) shown in Figure 3.45.

Figure 3.45 Non-ionic mechanism suggested for the metal ion catalysed oxidation of fats and oils.



In the early stages of lipid oxidation, when the rate of hydroperoxide decomposition is negligible compared with the rate of formation, but has already become the main source of chain initiation (and the heavy metal ion

content [M^{n+}] is very small), then the initiation rate r_i is proportional to the content of heavy metal ions:

$$r_i = k\,[ROOH]\,[M^{n+}]$$

At very low concentrations of the metal ion catalyst, the catalyst soon becomes deactivated and partly precipitates as inactive hydrated oxides. This deactivation takes place during the induction period, so that the metal ion is converted to an inactive form by the time the maximum rate of autoxidation is reached.

At very low partial pressures of oxygen, the rate-decreasing effect of the low oxygen pressure is enhanced by the presence of metal salts, especially copper salts. Several explanations have been put forward. At a low oxygen partial pressure, oxygen-dependent reactions are catalysed to a lesser extent than at atmospheric pressure. Less peroxide is formed, and fewer oxidation chains are started. The oxygen-independent reactions are started at reduced oxygen pressure at the same rate as at atmospheric oxygen pressure. Therefore, chain terminations are not restricted by the lack of oxygen, and predominate. In addition, copper ions compete for the oxygen present, and may, also, compete with oxygen for the $R^{\cdot}$ radicals (Pokorny, 1987).

At very high concentrations of metal ions, especially copper (II) ions, the effect on hydroperoxide decomposition and on termination reactions is so great that the hydroperoxide content becomes very small. Hence, the overall rate of oxidation decreases. The pro-oxidant activity of heavy metals can, thus, revert to antioxidant activity.

The relative activities of metal ions depend on several factors, of which the concentration and the temperature are important. For example, iron ions at a certain concentration may be more active than copper ions, at a similar concentration, under one set of conditions and copper ions may be more active under a different set of conditions.

In general, the nature of the anion has little effect on the catalytic activity of the metal cation. However, anions possessing metal-chelating activity, such as citric acid anions, suppress the pro-oxidative activity of metals ions.

It has been reported that copper (II) salts increase the pro-oxidative effect of iron (III) salts, at low metal concentrations, while iron salts increase the activity

of copper salts (Pokorny, 1987). The synergistic effect was not observed using high amounts of metal ions.

The pro-oxidant activity of heavy metal ions is affected by the polarity of the reaction medium, both because of the effect on the metal ion-hydroperoxide co-ordination complex and on the micelle formation. Metal ions usually lose activity in the presence of water due to lipid-insoluble hydrates being formed.

In lipids, transition metal ions do not exist in the free form, but are always surrounded by solvent molecules (often water), molecules of oxidation products or, frequently, by other metal ions, so that metal ions can form polymeric micelles, especially at high concentrations. Metal ions affect oxidation even when surrounded by ligands, because electrons can be transported through the outer layer of ligands to the oxidising molecule. In addition, the oxidising molecule can replace a weakly bound ligand molecule before the reaction. If the metal ion is surrounded by strongly bound ligands, such as EDTA (ethylenediamine tetraacetic acid), no ligand displacement occurs and, thus, no oxidation takes place.

As mentioned above, copper ions can be an effective catalyst for autoxidation of unsaturated lipids at a loading of 0.01mg/kg of substrate. To determine, the effect of a small amount of copper ions on the linoleic acid/CTMP system, copper ions at concentrations of 0.05mg/kg and 0.1mg/kg were added to series of samples that were stored and then analysed as described previously.

Figure 3.46 shows the amounts of hexanal recovered from one gram samples of linoleic acid/CTMP with 0.00005mg of copper ions added, and Figure 3.47 shows the amounts of hexanal recovered from one gram samples with 0.0001mg of copper ions added.

Despite fluctuation in the amounts of hexanal recovered from samples loaded with 0.05 mg copper /kg of substrate, the general trend shows that hexanal amounts gradually increase with storage time for samples stored at 20°C. For samples stored at 40°C, hexanal amounts generally increase to a maximum before declining after 4-5 weeks. Figures 3.46 and 3.47 show that there is no significant difference in the amounts of hexanal recovered at the two different copper concentrations. One observation, however, concerns samples stored at 40°C and 50°C. At the higher copper ion concentration, hexanal build up occurs more slowly.

Figure 3.46 Amounts of hexanal recovered (in nL) from samples of linoleic acid/CTMP (+ 0.05mg Copper/kg substrate) stored at 20°C, 40°C and 50°C for up to 43 days (Headspace sampling temperature: 105°C).

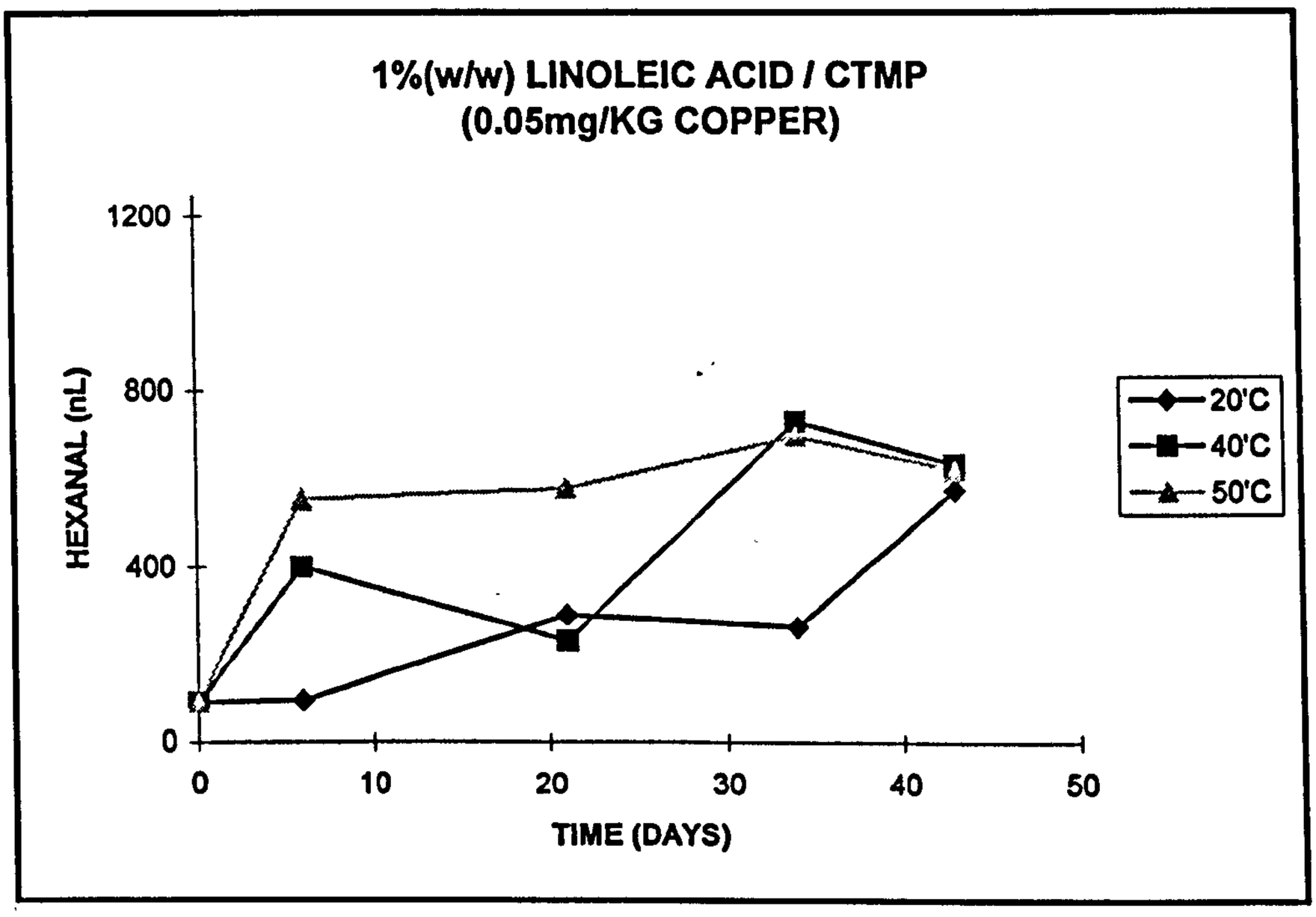
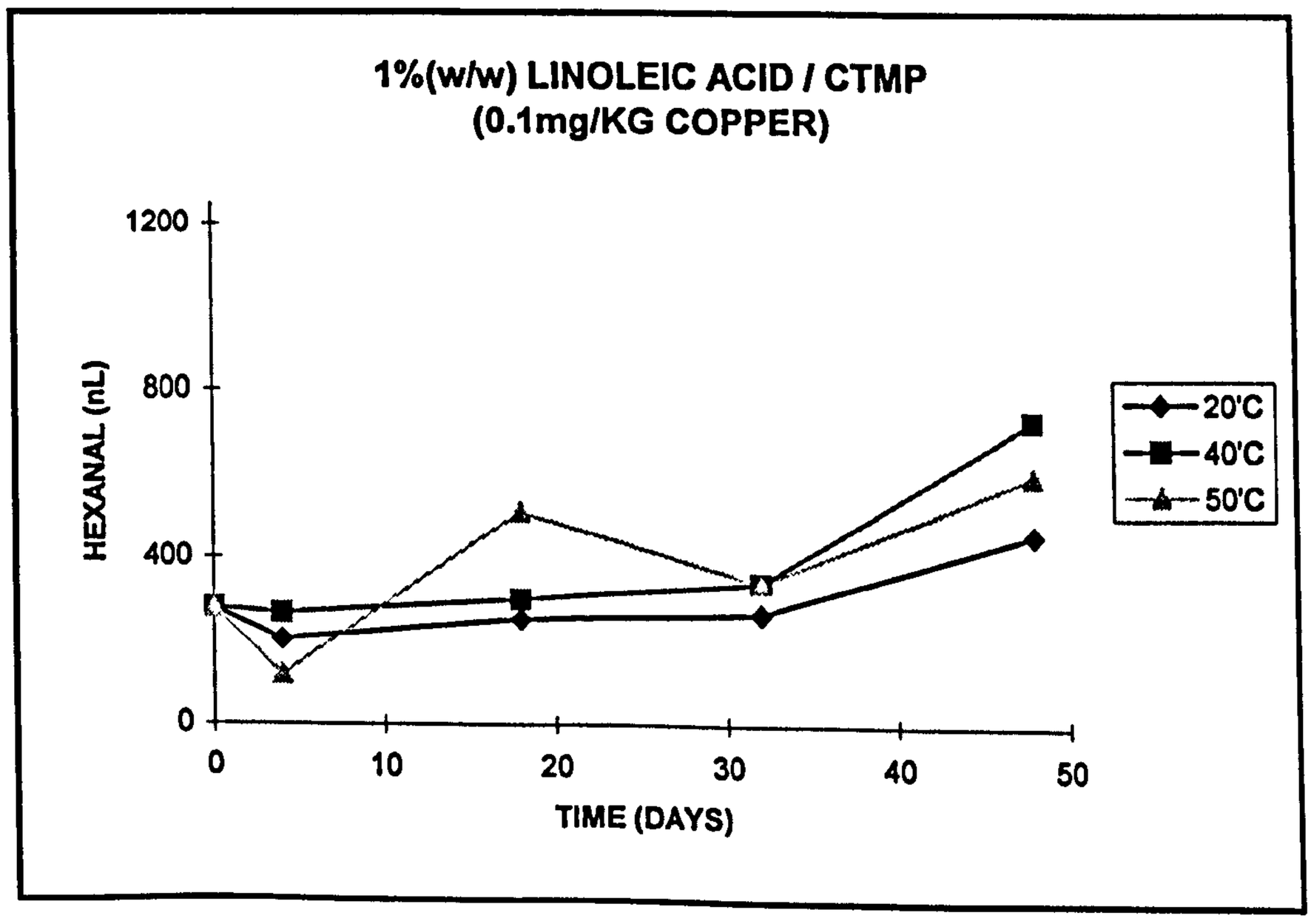


Figure 3.47 Amounts of hexanal recovered (in nL) from samples of linoleic acid/CTMP (+ 0.1mg Copper/kg substrate) stored at 20°C, 40°C and 50°C for up to 48 days (Headspace sampling temperature: 105°C).



This may be due to an increased number of termination reactions following the decomposition of hydroperoxides, or, it may be caused by some other factor. The formation of hexanal from samples, on the addition of 0.05mg/kg of copper ions appears to be, initially, more rapid at higher storage temperatures, than observed for samples where no copper ions had been added (see Figure 3.18). This may reflect the catalytic activity of copper ions even at this low level. Differences may not have been found at longer storage periods due to the subsequent deactivation of the small amounts of catalyst.

At longer storage times, the difference in amounts of hexanal recovered from samples, stored at different temperatures, was greater at the higher copper ion concentration. In addition, the amounts of hexanal recovered from samples stored, for longer periods, at ambient temperature were not in excess of those found in samples stored at higher temperatures, as was the case for linoleic acid/CTMP without copper ion addition. This may indicate that the secondary oxidation products, i.e. hexanal, etc., were being more rapidly decomposed, or oxidised, with the addition of copper ions, particularly at lower temperatures, than if no copper ions were added.

Figure 3.48 shows the amounts of *trans* 2-octenal recovered from samples in which copper ions were added at a concentration of 0.05mg/kg. Figure 3.49 shows the amounts of *trans* 2-octenal recovered from samples in which copper ions were added at a concentration of 0.1mg/kg.

The levels of *trans* 2-octenal recovered from samples of linoleic acid/CTMP, to which copper ions were added, appear to be similar at both copper ion concentrations. In addition, the amounts recovered compare with the levels in samples which had had no copper ions added (see Figure 3.23). One noted exception, however, was the greater amounts of *trans* 2-octenal in the sample stored for 42 days, at an ambient temperature, to which no copper ions were added. This could suggest that the copper ions may be playing a part in the decomposition, or oxidation, of this unsaturated aldehyde.

Figure 3.48 Amounts of *trans* 2-octenal recovered (in nL) from samples of linoleic acid/CTMP (+ 0.05mg Copper/kg substrate) stored at 20°C, 40°C and 50°C for up to 43 days (Headspace sampling temperature: 105°C).

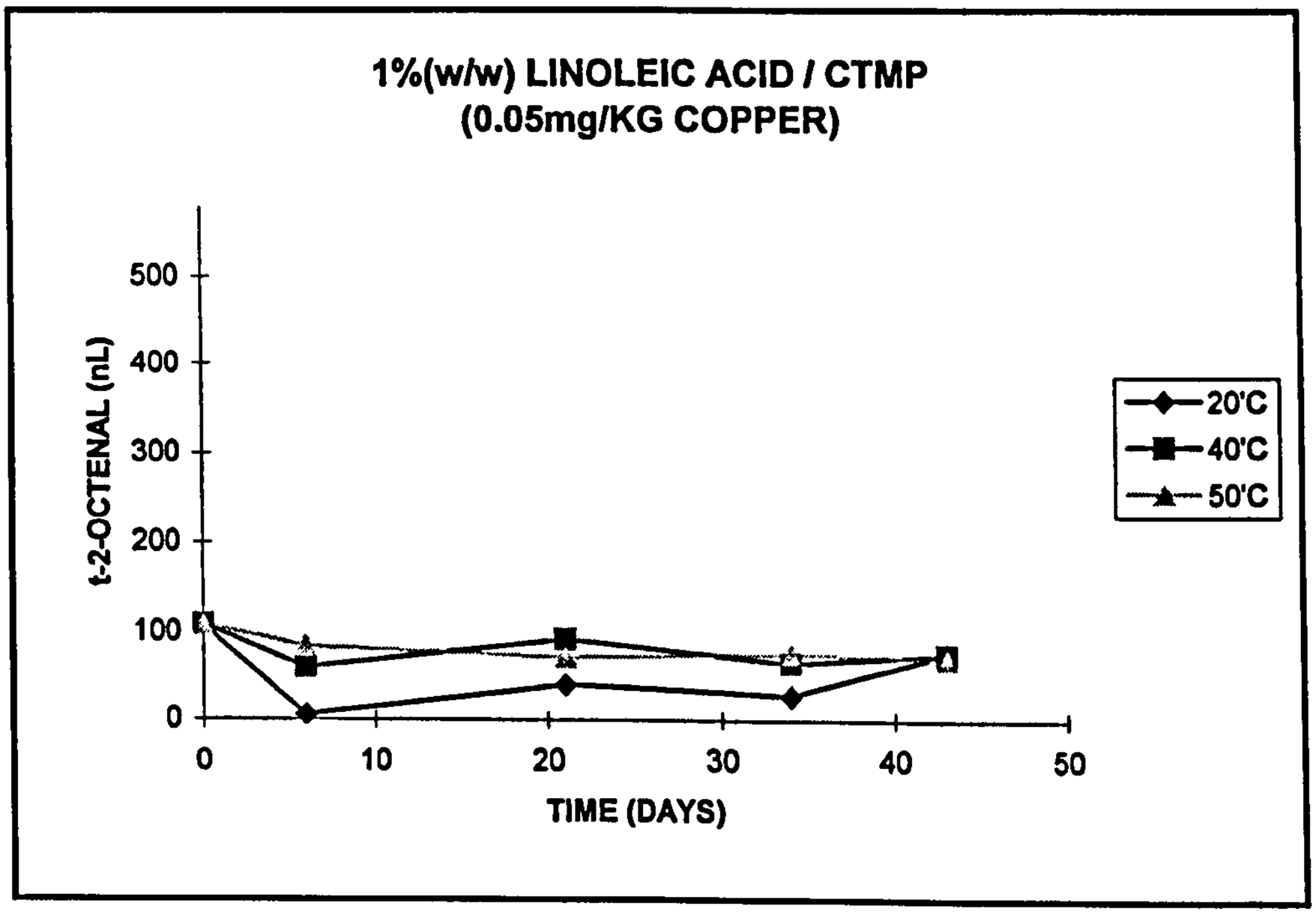


Figure 3.49 Amounts of *trans* 2-octenal recovered (in nL) from samples of linoleic acid/CTMP (+ 0.1mg Copper/kg substrate) stored at 20°C, 40°C and 50°C for up to 48 days (Headspace sampling temperature: 105°C).

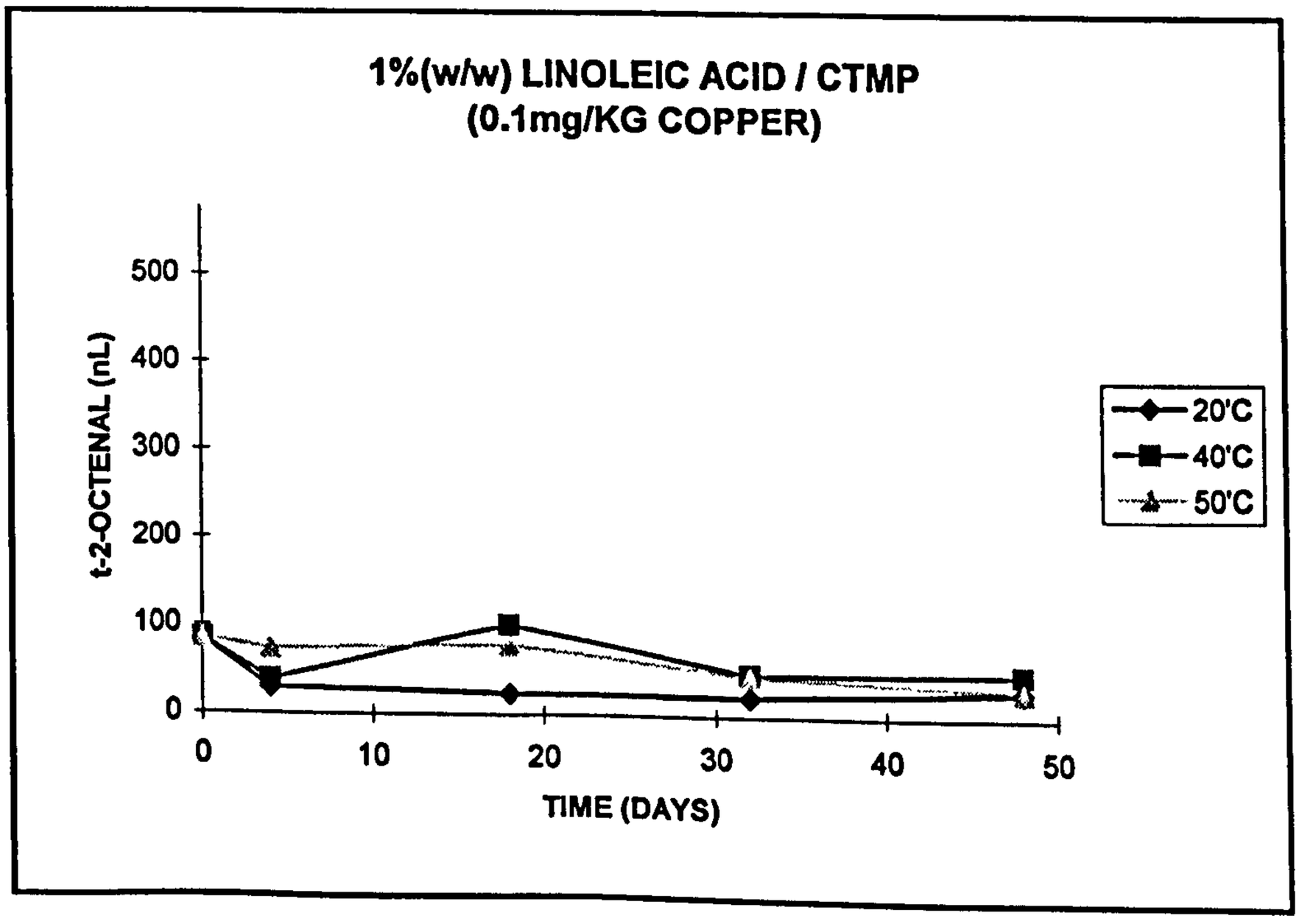
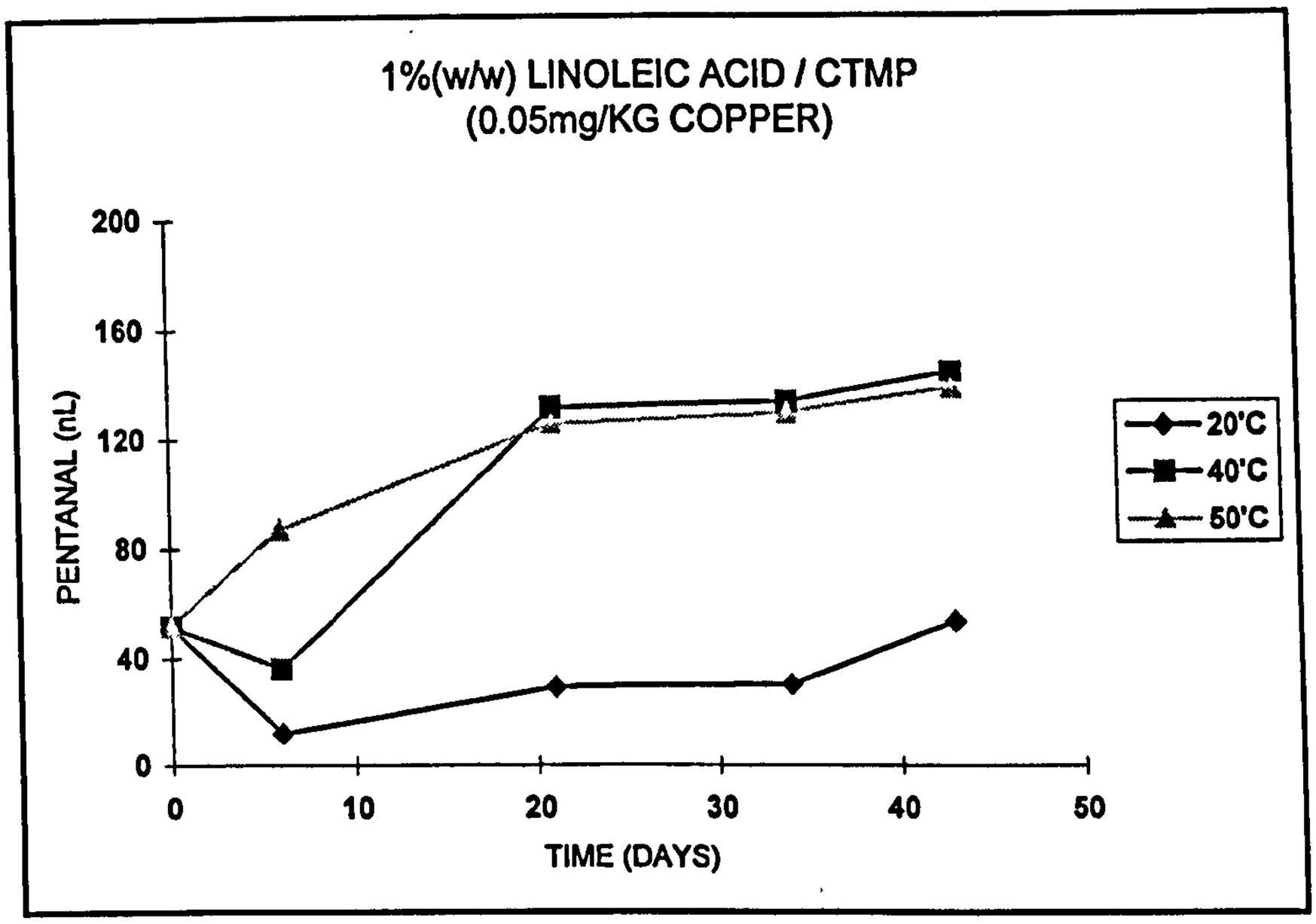


Figure 3.50 shows the amounts of pentanal recovered from samples in which copper ions were added at a concentration of 0.05mg/kg, and Figure 3.51 shows the amounts of pentanal recovered from samples in which copper ions were added at a concentration of 0.1mg/kg.

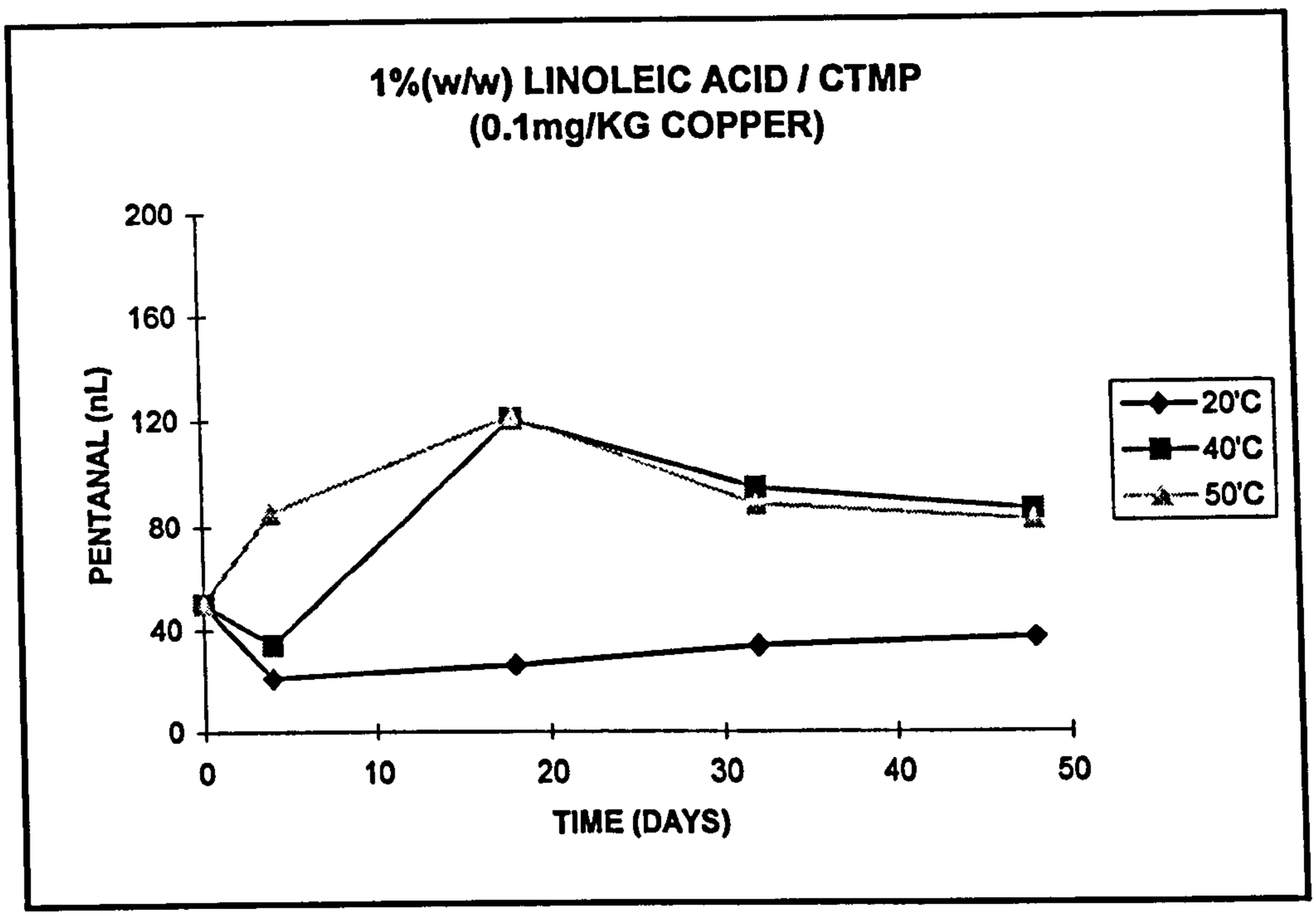
Figure 3.50 Amounts of pentanal recovered (in nL) from samples of linoleic acid/CTMP (+ 0.05mg Copper/kg substrate) stored at 20°C, 40°C and 50°C for up to 43 days (Headspace sampling temperature: 105°C).



The levels of pentanal found at the two copper ion concentrations are not significantly different. However, the amounts found in samples stored at higher temperatures, to which 0.1mg copper ions/kg were added are smaller than those found in samples in which 0.05mg copper ions/kg were added. If one considers the routes to the possible formation of pentanal, in particular the formation via scission route A following the decomposition of methyl linoleate 9-hydroperoxide (see Figure 3.22). In this mechanism, 13-oxo-9,11-tridecadienoate is formed as the principle product of scission route A.

This unsaturated ester is very prone to autoxidation. Therefore, under increased oxidising conditions, the route for the decomposition of methyl linoleate 13-hydroperoxide may be shifted away from scission route A and more towards route B.

Figure 3.51 Amounts of pentanal recovered (in nL) from samples of linoleic acid/CTMP (+ 0.1mg Copper/kg substrate) stored at 20°C, 40°C and 50°C for up to 48 days (Headspace sampling temperature: 105°C).

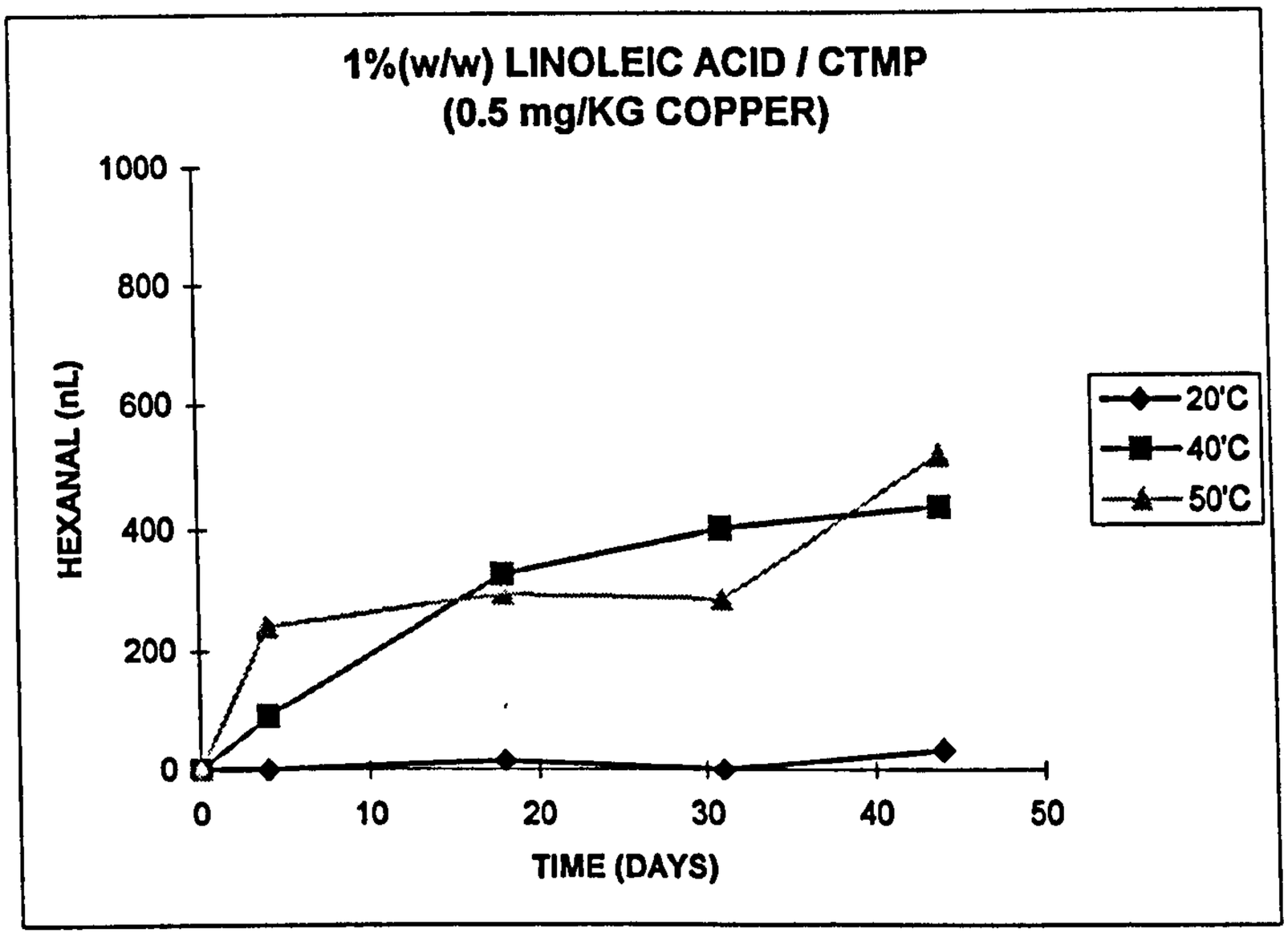


This results in less pentanal being formed, and more hexanal being formed. This theory may be backed up by the fact that smaller amounts of 1-pentanol were found in conjunction with the smaller amounts of pentanal. 1-pentanol, too, is formed by scission route A, following the decomposition of methyl linoleate 9-hydroperoxide.

To determine whether the headspace equilibration period at 105°C was having an effect on the formation of volatile compounds within the, copper ion-enriched, linoleic acid/CTMP system, a test was carried out in which 0.0005 mg of copper ions were added to samples of linoleic acid/CTMP, corresponding to a concentration of 0.5mg copper ions/kg CTMP. These samples were stored for up to 44 days and sampled using an headspace equilibration temperature of 50°C. The amounts of hexanal recovered are shown in Figure 3.52.

The amounts of hexanal recovered are not markedly different to those using the higher equilibration temperature. However, hexanal amounts are significantly less than those for linoleic acid/CTMP samples, to which no copper ions had been added.

Figure 3.52 Amounts of hexanal recovered (in nL) from samples of linoleic acid/CTMP (+ 0.5mg Copper/kg substrate) stored at 20°C, 40°C and 50°C for up to 44 days (Headspace sampling temperature: 50°C).



This suggests that the presence of the copper ions had some effect on oxidation. It is possible that the copper ions may have increased the extent of hydroperoxide decomposition in the system to a level such that termination reactions may have become significant even after short storage periods. These termination reactions result in the combination of radical volatile precursors to produce non-volatile products. Alternatively, the presence of added copper ions may have favoured the formation of higher molecular weight non-volatile species (oligomers) by addition of radical species to an active chain.

During these tests, there may already be copper ions and other transition metal ions present in the system. Any such ions are present in the CTMP as a result of residues from the raw material, i.e. wood, or from pulp processing. In order to determine the background levels of five such transition metals, atomic absorption spectrometric analyses were carried out on samples of CTMP from the same batch as was used throughout the tests. The amounts of each metal ion found, in mg/kg, are shown :

METAL ION	CONCENTRATION (mg/kg of CTMP)			
	1	2	3	AVERAGE
COPPER	66.5	61.5	56.5	61.5
NICKEL	52	55	59.5	55.5
IRON	20	25	25	23.3
MANGANESE	0	0	0	0
COBALT	0	0	0	0

Copper ions, nickel ions, and iron ions were present in the CTMP in significant quantities. One expects rapid catalysed oxidation of linoleic acid at these levels. However, their effect does not seem to be particularly obvious. It is possible that these metals ions may be surrounded by strongly bound ligands, such as chelating anions that effectively deactivate the metal cations as oxidation catalysts. Complexing agents such as EDTA or citric acid may be incorporated in to the CTMP for this very reason. If such agents are present in the CTMP, it is possible that they may, also, partially deactivate any additional metal ions added, such as copper ions. Thus, the effect of copper ions on volatile formation during the tests may have been suppressed. The amounts of hexanal recovered using an equilibration temperature of 50°C indicate that the added copper ions did not increase the amounts of volatile compounds produced. However, the presence of copper ions did have some effect on the oxidation, as indicated by the significant reduction in hexanal recovery at this equilibration temperature, compared to hexanal recovered from samples in to which no copper ions were added.

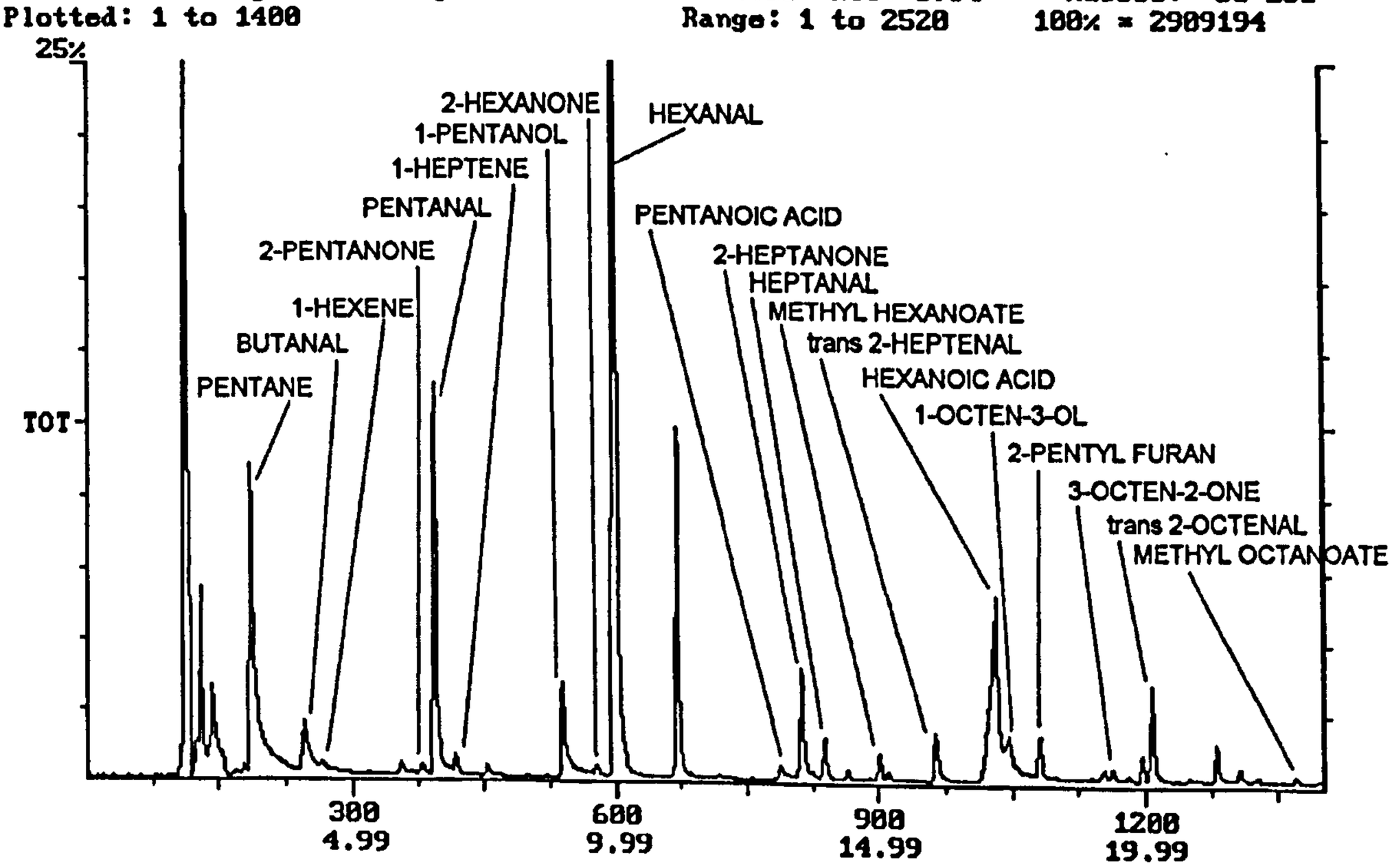
Determination of effect of CTMP on the autoxidation of linoleic acid

A test was carried out on samples of linoleic acid, alone, to determine the nature of the oxidation in the absence of CTMP. 11μL of the acid was placed in each sample vial, corresponding to the amount impregnated into each 1 gram sample of CTMP.

Figure 3.53 shows the chromatogram of the headspace vapours obtained from a sample of linoleic acid stored for 13 days at 40°C. The main observation concerns the presence of pentanoic acid and hexanoic acid. These carboxylic acids had not been detected in any tests, previously.

However, their formation was suspected. Their occurrence may arise as a consequence of the oxidation of aldehydes such as hexanal and 2-octenal. As mentioned before, the lack of their detection may be due to strong interaction between the acid, and the cellulose, and/or lignin, in the CTMP.

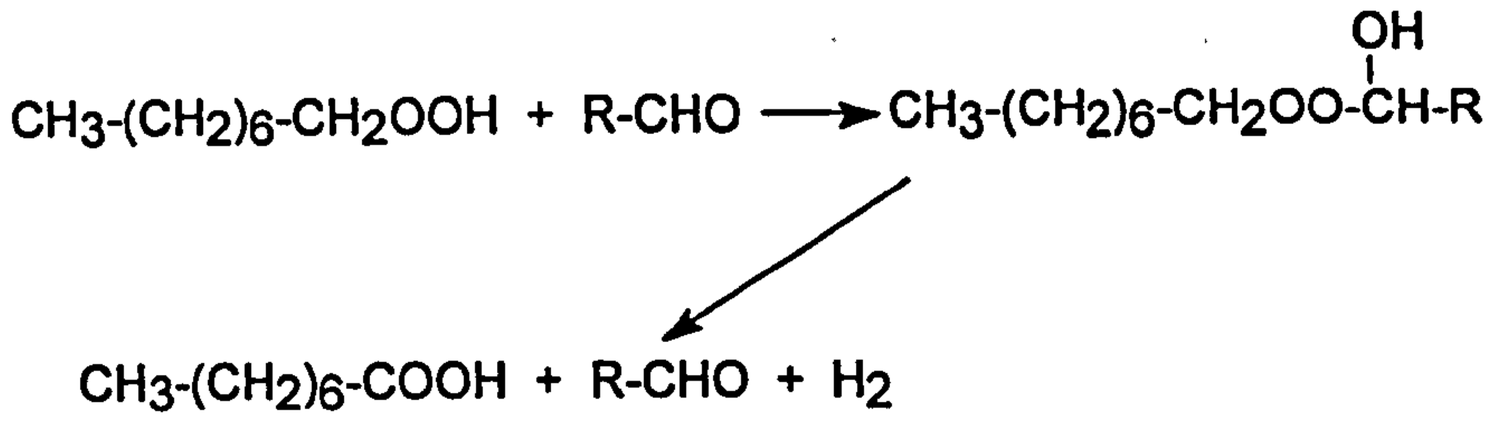
Figure 3.53 Chromatogram acquired from the headspace vapours of a 11μl sample of linoleic acid that had been stored, at 40°C, for 13 days.



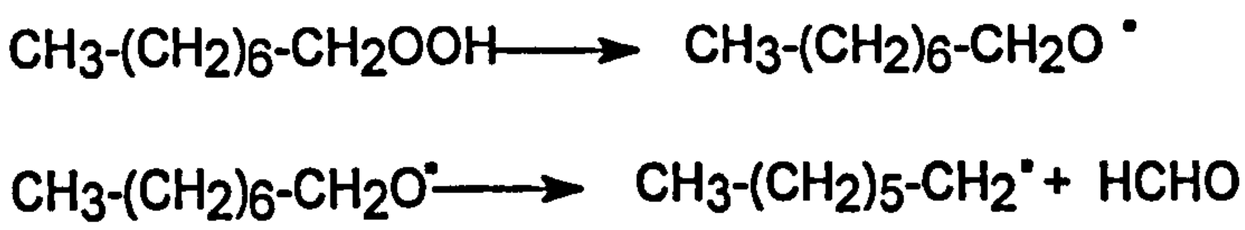
Several reaction pathways have been proposed to explain the formation of short-chain fatty acids. These include the oxidation of the fatty acids to shorter chain lengths, the thermal degradation of esters and the autoxidation of aldehydes and ketones. Fatty acids may be produced by the consecutive shortening of acyl chains that are derived from higher homologue fatty acids.

The primary hydroperoxide formed is converted into a short-chain fatty acid and hydrogen by interaction with an aldehyde, through a peroxy-hydroxy intermediate (Frankel, 1983):

$$CH_3\text{-}(CH_2)_6\text{-}\dot{C}H_2 \xrightarrow{O_2} CH_3\text{-}(CH_2)_6\text{-}CH_2OOH$$



The primary hydroperoxide can, also, lose a hydroxyl radical, to produce formaldehyde and a shorter alkyl radical:



The shorter alkyl radical takes part in the consecutive reactions, which result in the formation of lower fatty acids.

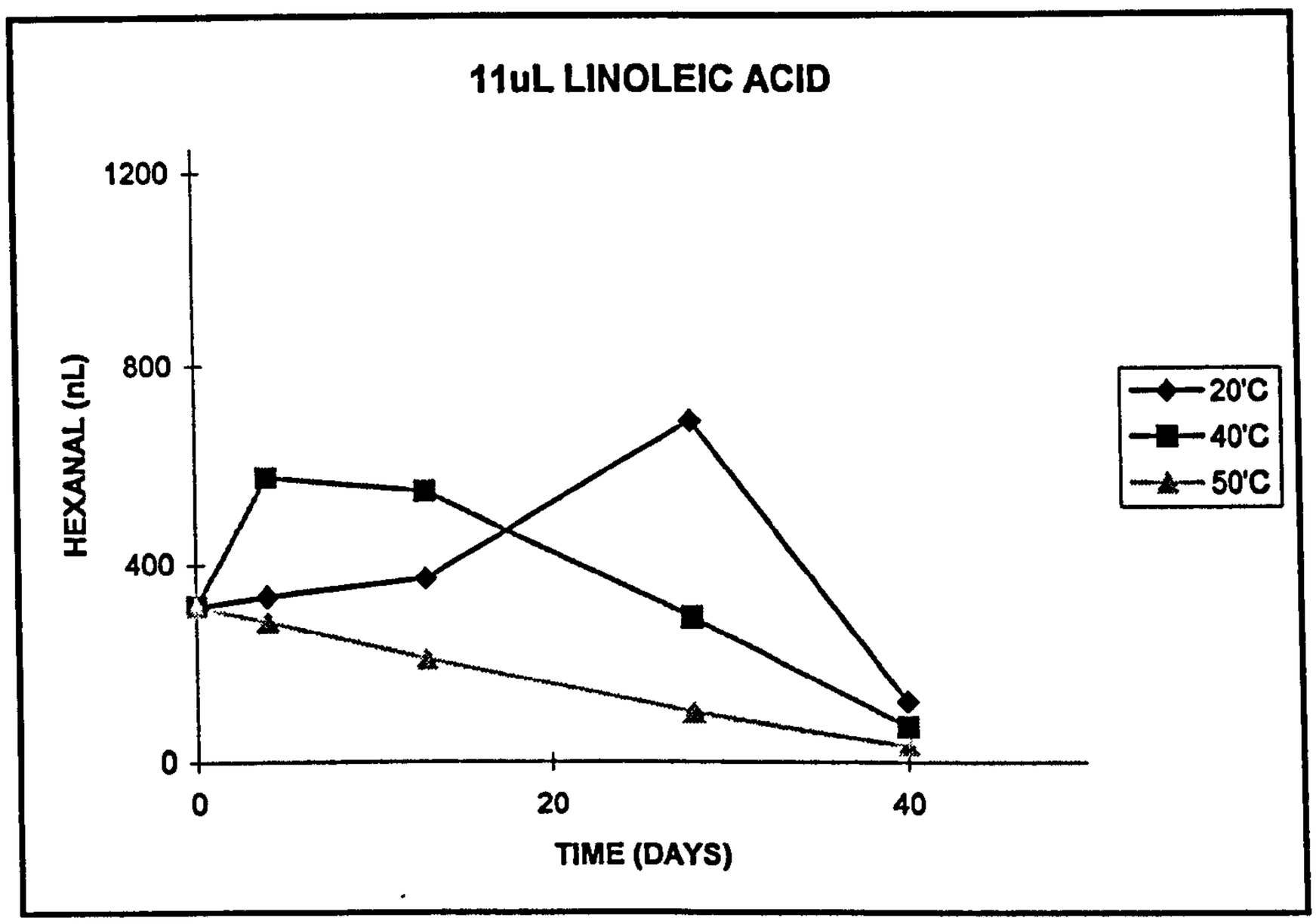
Another reason for the presence of these carboxylic acids may be the lower diffusion limitations, in the linoleic acid medium, for aldehydes needed for the above reaction to take place. In the CTMP medium, diffusion may be limited by the numerous air pockets scattered throughout the matrix.

Figure 3.54 shows the amounts of hexanal recovered in the headspace volatiles from samples of linoleic acid, throughout the test.

The general trend at all three storage temperatures is downwards, as the storage time increases. This tendency is most obvious at 50°C and least obvious at ambient temperature. This pattern may be a result of the vastly reduced surface area between the acid and the oxygen-containing environment, compared to that existing in the CTMP matrix, and the reduced solubility of oxygen as the temperature increases. The surface area available for the adsorption of oxygen into the oxidising medium exists only as the outer film of the linoleic acid droplet, at the bottom of the sample vial. In the CTMP medium, the oxidising medium is impregnated throughout the CTMP which consists of many pores and air pockets, providing a large surface area for oxygen adsorption.

As mentioned earlier, as surface area available for oxygen adsorption increases, the decreased solubility of oxygen at higher temperatures has less effect on reducing the rate of autoxidation.

Figure 3.54 Amounts of hexanal recovered (in nL) from samples of linoleic acid stored at 20°C, 40°C and 50°C for up to 40 days (Headspace sampling temperature: 105°C).



Another explanation of the decline in the amount of hexanal as storage time increases, suggests that, due to the lower diffusion limitations, more radicals may collide with one another, resulting in an increased number of termination reactions.

Figure 3.55 shows the amounts of *trans* 2-octenal recovered from samples of linoleic acid. The levels of *trans* 2-octenal generally show a downward trend with time. However, at a storage temperature of 40°C, an initial peak was observed indicating the rapid build up of 2-octenal, before amounts declined. At 20°C, a gradual build up of 2-octenal was found before amounts began to decline at around four weeks. The build up of *trans* 2-octenal for samples stored at 20°C and 40°C was greater than those amounts for CTMP samples. This may be an indication of the relatively low substrate surface area, leading to a more stable, less oxidising, environment for unsaturated species within the system.

Figure 3.55 Amounts of *trans* 2-octenal recovered (in nL) from samples of linoleic acid stored at 20°C, 40°C and 50°C for up to 40 days (Headspace sampling temperature: 105°C).

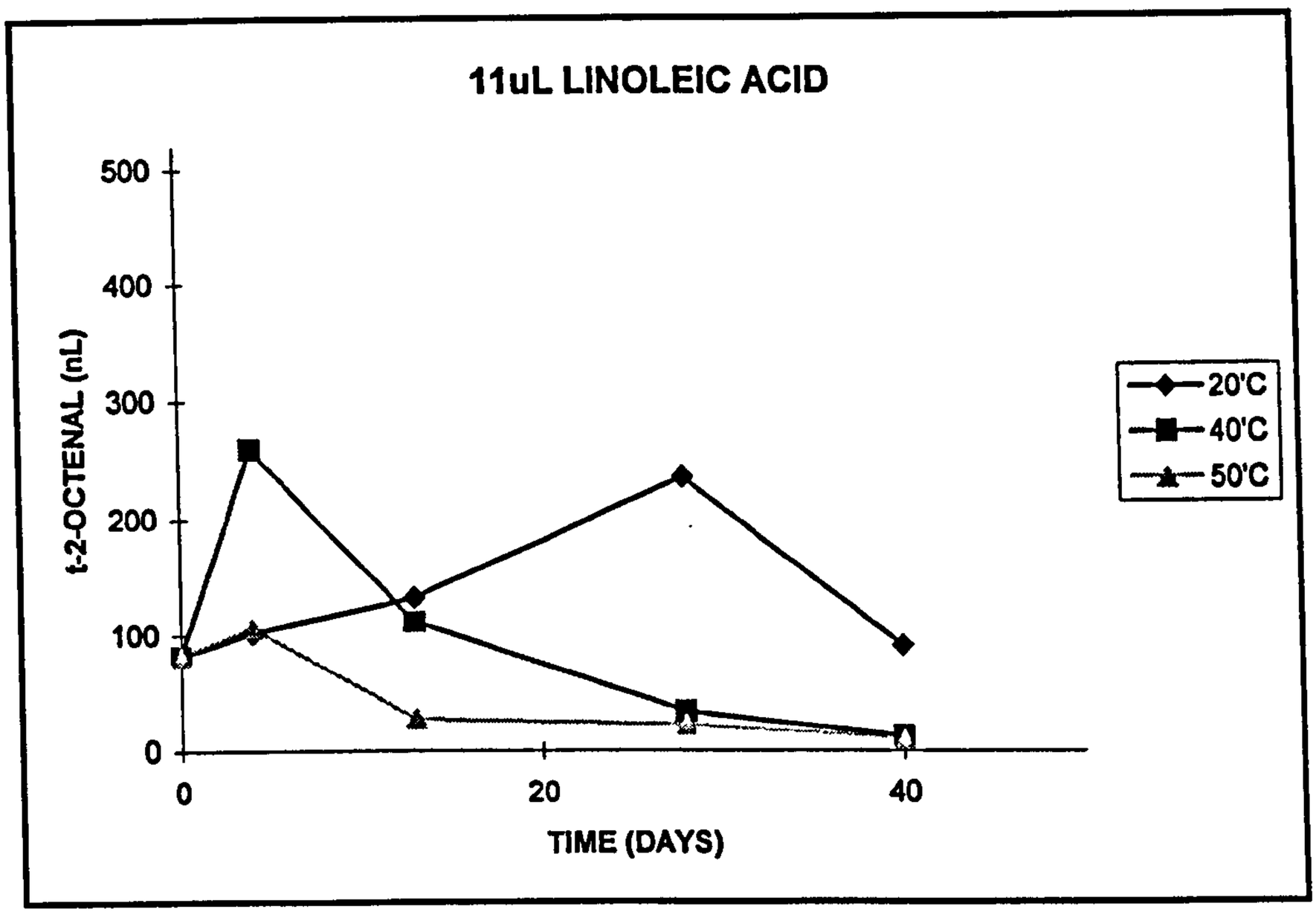
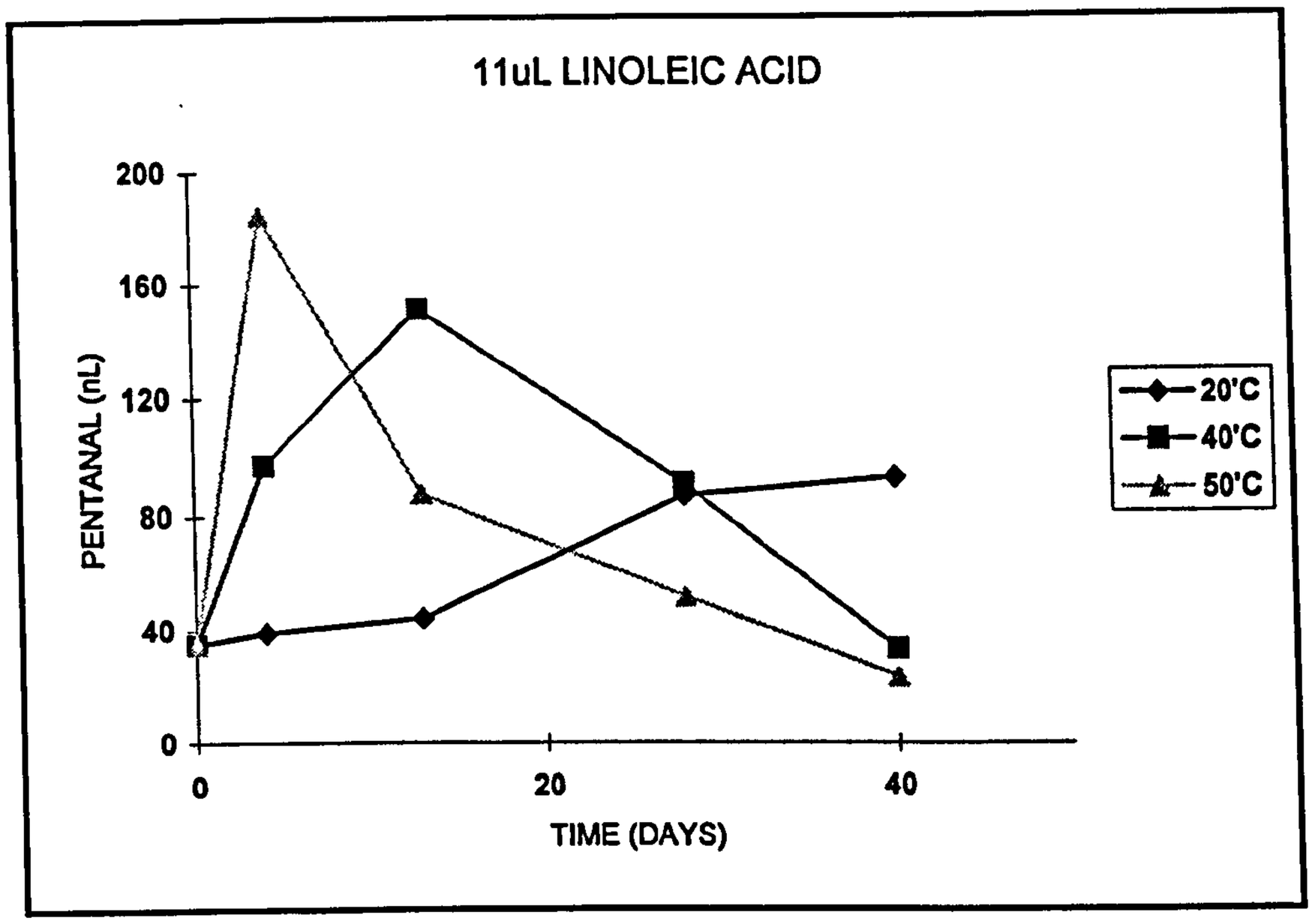


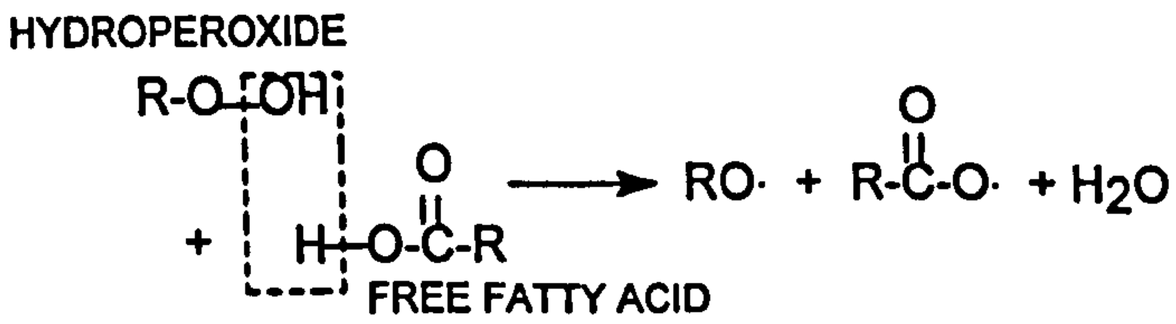
Figure 3.56 shows the amounts of pentanal recovered from samples of linoleic acid. A significant initial build up in pentanal was found for samples stored at 50°C. If pentanal was formed from the decomposition of 2,4-decadienal then the pure linoleic acid system, with its lower surface area for oxygen adsorption, initially favours the formation of 2,4-decadienal from linoleic acid 9-hydroperoxide. Pentanal formation by this route is supported by the fact that pentanal formation does not follow the same trend as seen for hexanal formation, suggesting pentanal formation is not directly linked to the breakdown of linoleic acid 13-hydroperoxide. The amounts of pentanal recovered were relatively large for the shorter storage periods. This may suggest less decomposition of this secondary product, than occurred in the CTMP system. The overall pattern of volatile formation compares favourably with that of samples of linoleic acid/CTMP stored under a reduced oxygen partial pressure. This suggests that, due to the low surface area available for oxygen diffusion, oxygen levels within the oxidising fatty acid system are relatively low.

Figure 3.56 Amounts of pentanal recovered (in nL) from samples of linoleic acid stored at 20°C, 40°C and 50°C for up to 40 days (Headspace sampling temperature: 105°C).



Determination of the effect of time and temperature on the presence of methyl linoleate in CTMP

Free fatty acids (FFAs), such as linoleic acid, oxidise at a slightly higher rate than their corresponding ester moiety. The pro-oxidant effect of FFAs on the autoxidation of many fats has been reported (Kochhar, 1993). A decrease in the induction period for the autoxidation of fats and oils was observed when as little as 0.1% FFA was added. It is thought that this was due to free fatty acids accelerating the decomposition of hydroperoxides. The mechanism is similar to the bimolecular decomposition of hydroperoxides, with the production of water as a by-product:

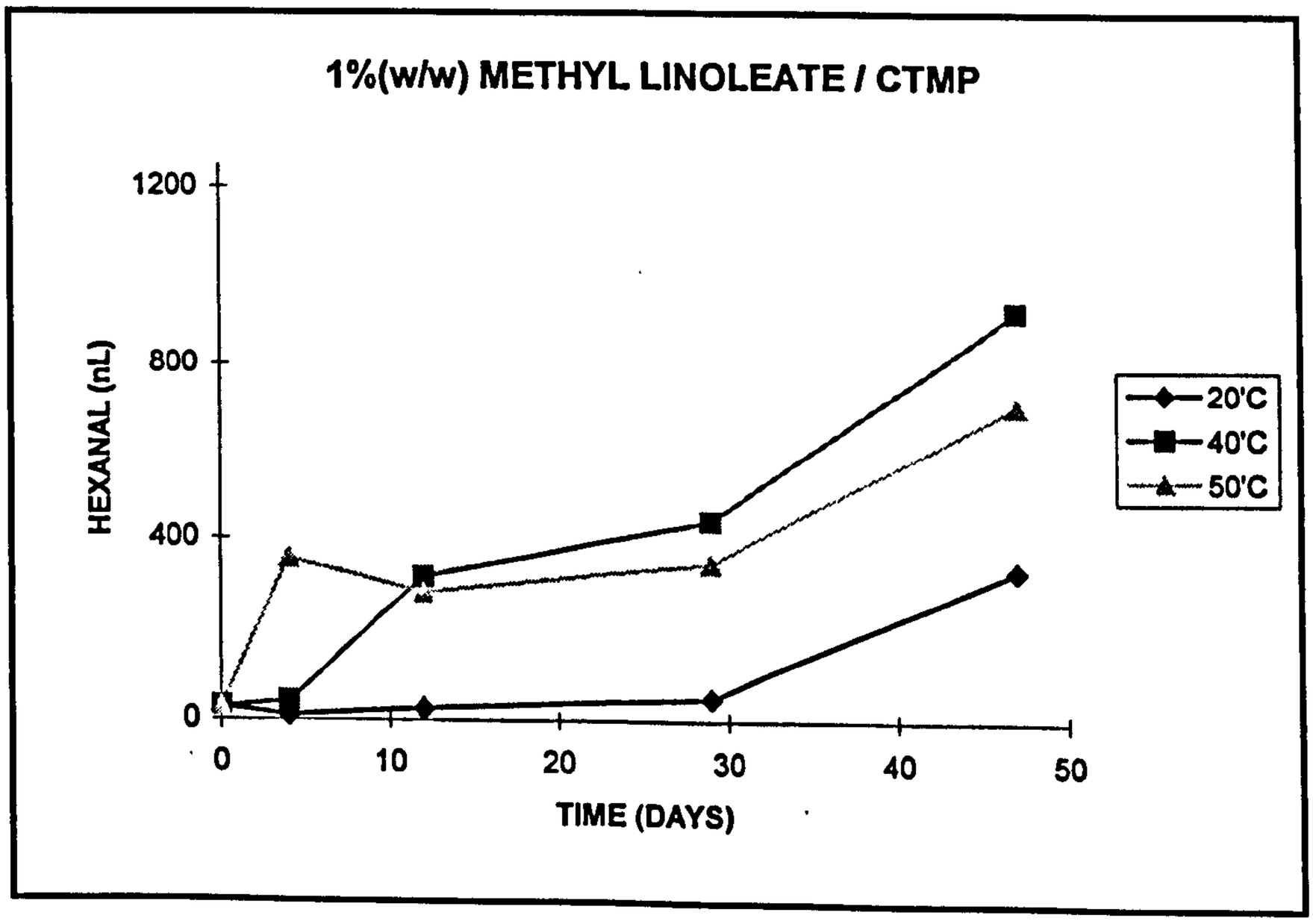


The effectiveness of tocopherol antioxidants is decreased by the addition of FFAs. This maybe due to FFAs accelerating the decomposition of hydroperoxides to the extent that phenolic antioxidants do not function effectively. The presence of relatively large amounts of FFAs in fats and oils can increase the uptake of catalytic trace metals, such as those discussed earlier and, thus, enhance the rate of oxidation in the system further.

To determine the effect that FFAs had previously, a test was carried out in which methyl linoleate, as opposed to linoleic acid, was used.

Chromatograms showed that the compositions of the headspace vapours recovered from samples of methyl linoleate/CTMP were comparable to those for linoleic acid/CTMP. However, the amount of volatiles recovered, in general, tended to be less than those for linoleic acid/CTMP. Figure 3.57 shows the amounts of hexanal recovered from samples of methyl linoleate/CTMP.

Figure 3.57 Amounts of hexanal recovered (in nL) from samples of methyl linoleate/CTMP stored at 20°C, 40°C and 50°C for up to 47 days (Headspace sampling temperature: 105°C).



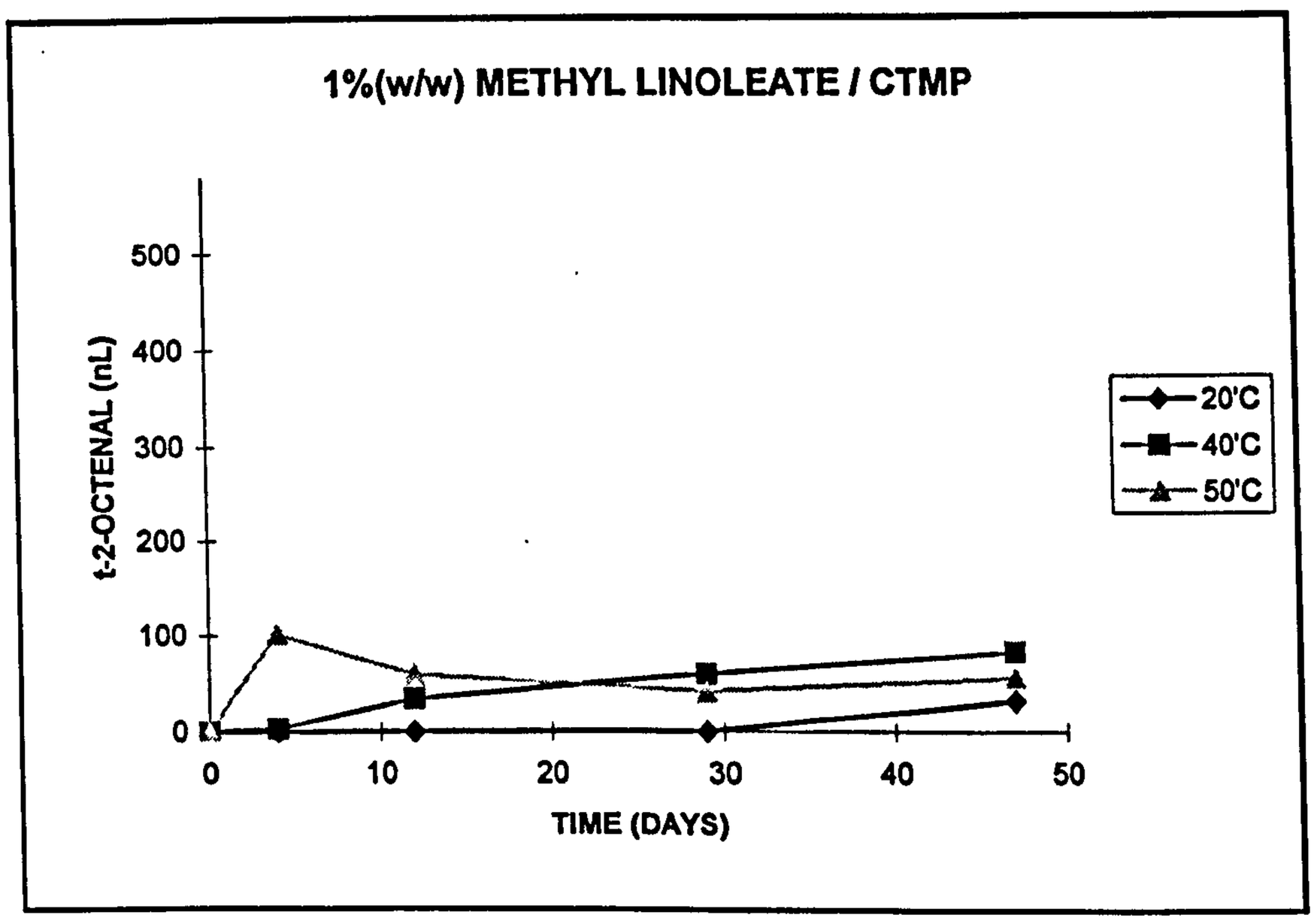
At the higher temperatures, hexanal formation is greater than for the linoleic acid/CTMP system. This formation does not decline at longer storage periods. This may be a result of a slower rate of hydroperoxide decomposition in the

system leading to a lower number of free radicals and termination reactions, resulting in the lower decomposition extent of the secondary oxidation products.

Samples stored at ambient temperature contained smaller amounts of hexanal than the linoleic acid/CTMP. This may reflect the slower rate of peroxide decomposition in the ester system, than occurred in the free acid.

Figure 3.58 shows the amounts of *trans* 2-octenal recovered from samples of methyl linoleate/CTMP.

Figure 3.58 Amounts of *trans* 2-octenal recovered (in nL) from samples of methyl linoleate/CTMP stored at 20°C, 40°C and 50°C for up to 47 days (Headspace sampling temperature: 105°C).

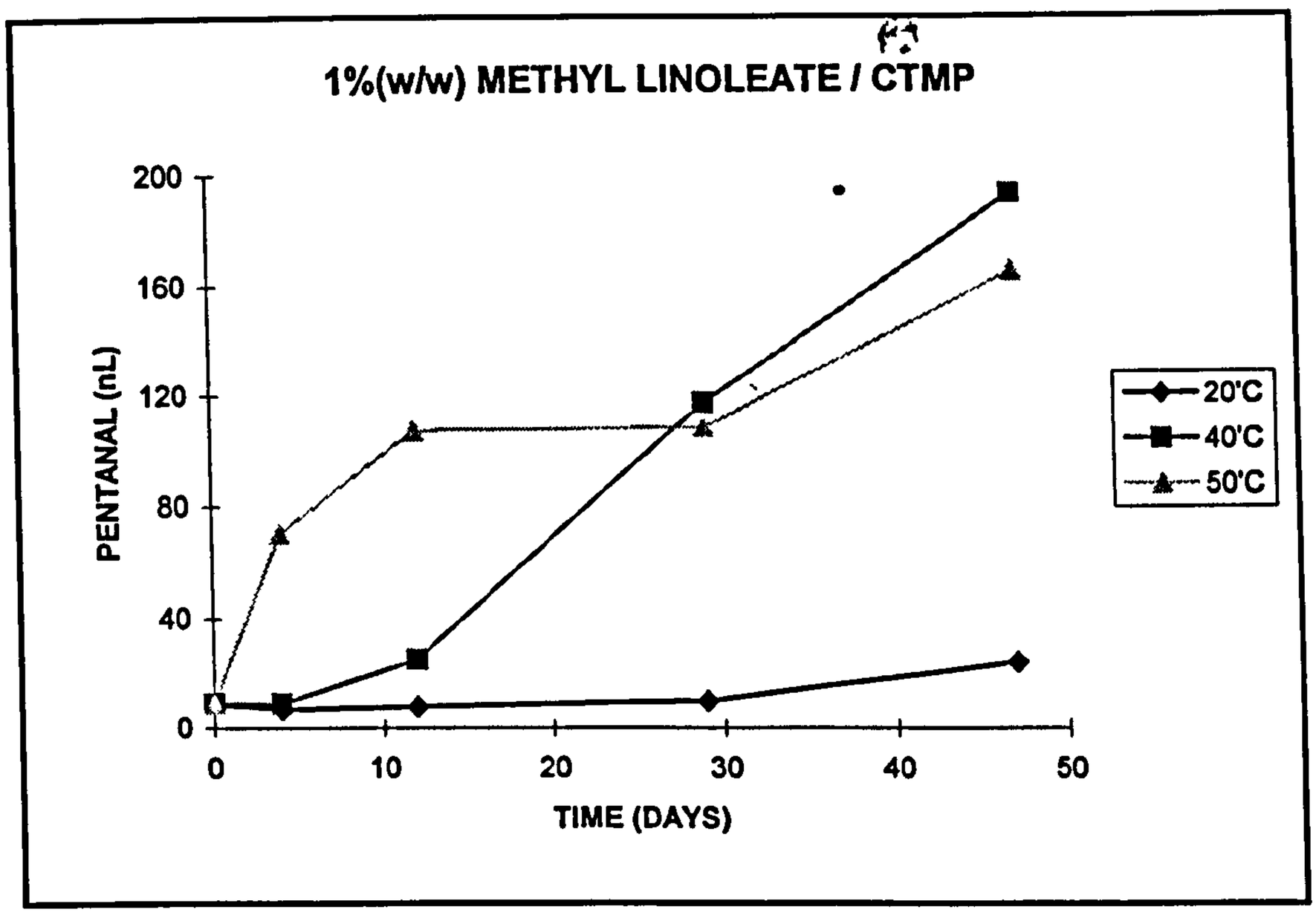


The levels of *trans* 2-octenal are not significantly different to those for linoleic acid/CTMP. This could suggest that 2,4-decadienal is formed in similar amounts in the ester form and the free acid form. Therefore, the nature of the acid/ester group may play no obvious part in the isomerisation of monohydroperoxides formed from methyl linoleate. One minor difference between Figure 3.58 and that obtained for linoleic acid/CTMP (Figure 3.23) is the greater amounts of *trans* 2-octenal for the linoleic acid/CTMP sample stored for 42 days at 20°C. This, again, may indicate the lower extent of

hydroperoxide decomposition at ambient temperature, for the ester compared to the free acid.

Figure 3.59 shows the amounts of pentanal recovered from samples of methyl linoleate/CTMP.

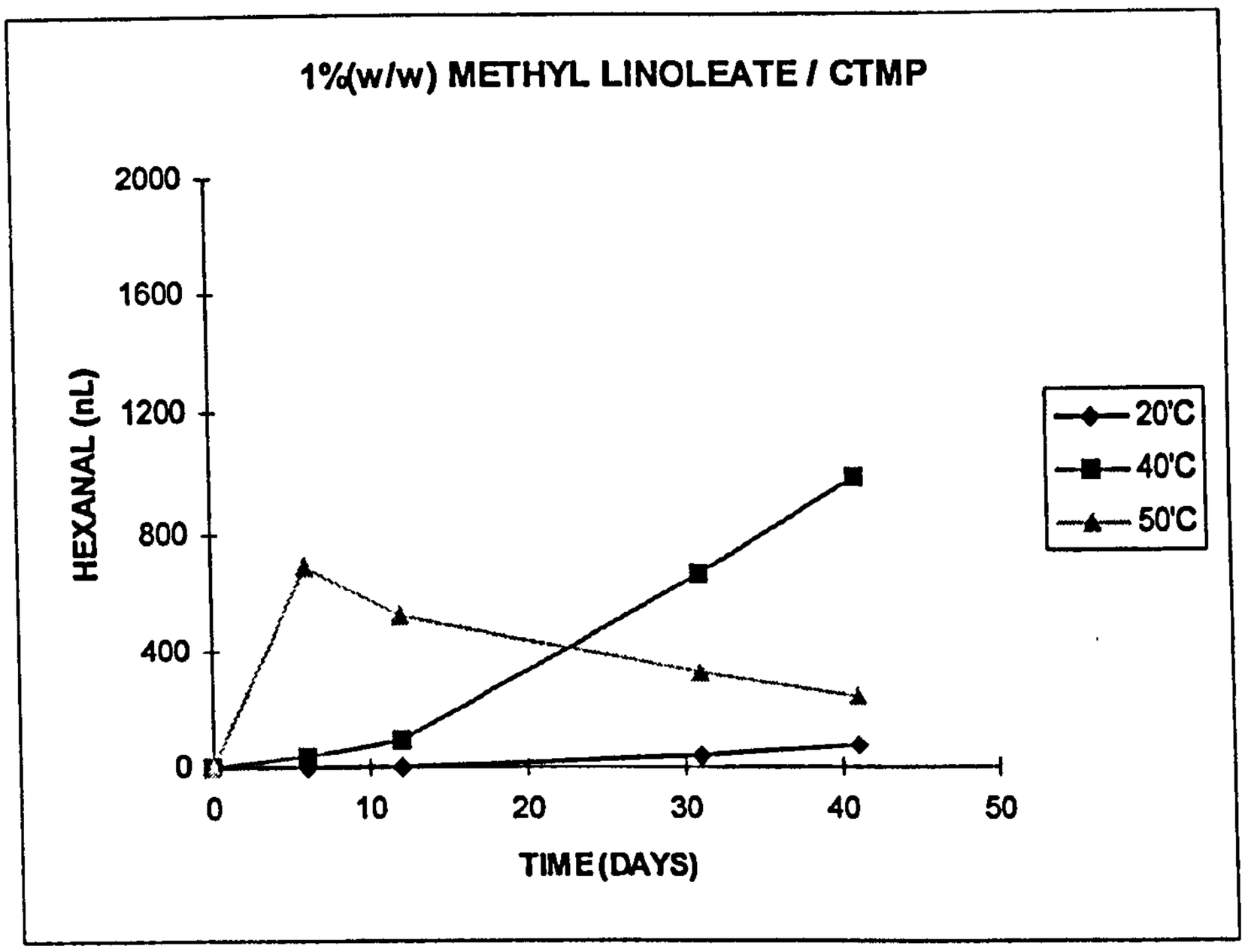
Figure 3.59 Amounts of pentanal recovered (in nL) from samples of methyl linoleate/CTMP stored at 20°C, 40°C and 50°C for up to 47 days (Headspace sampling temperature: 105°C).



The levels of pentanal recovered at higher temperatures are much greater than those for linoleic acid/CTMP. This could suggest that the number of termination reactions in this system is smaller than those found in the free acid system. Pentanal can form from methyl linoleate 13-hydroperoxide via scission route A, (see Figure 3.19), in which the subsequent pentyl radicals react with oxygen to form a hydroperoxide that decomposes to form pentanal. The pentyl radical must survive long enough to react with an oxygen molecule. Thus, a lower density of radicals could exist in the system, reducing the frequency of termination reactions.

The test with methyl linoleate was repeated using a headspace sampling temperature of 50°C. Figure 3.60 shows the amounts of hexanal recovered during the test.

Figure 3.60 Amounts of hexanal recovered (in nL) from samples of methyl linoleate/CTMP stored at 20°C, 40°C and 50°C for up to 47 days (Headspace sampling temperature: 50°C).



The amounts of hexanal recovered from samples stored at 40°C were not significantly different to those from samples acquired using an equilibration temperature of 105°C. The amounts of hexanal recovered from samples stored at this temperature continue to increase throughout the duration of the test, suggesting that termination reactions are not so significant within the ester system, using an equilibration temperature of 50°C. At a storage temperature of 50°C, these termination reactions appear to be more significant and coupled to a slower rate of hydroperoxide decomposition, using an equilibration temperature of 50°C, result in a decline in hexanal recoveries after one week of storage. Hexanal amounts in samples stored at 20°C were significantly less than those for the free acid equilibrated at 50°C. This indicates that decomposition of hydroperoxides occurs at a lower level in the ester system, resulting in much slower build up of volatiles.

At ambient temperatures, the rate of hydroperoxide formation and decomposition were found to be very similar for the both the free acid and methyl ester, as indicated by the amount of hexanal recovered.

Determination of the significance of the formation of *trans,trans* 2,4-decadienal as a precursor in the formation of volatile oxidation products from linoleic acid/ alkyl linoleate in CTMP

One of the major products of the decomposition of linoleic acid 9-hydroperoxide is *trans*, *trans* 2,4-decadienal. Although this compound was not detected in any previous tests, largely due to its rapid autoxidation in the presence of oxygen, especially at the elevated temperatures used during the headspace equilibration period, *trans* 2-octenal has been an assumed indicator of the involvement of this compound.

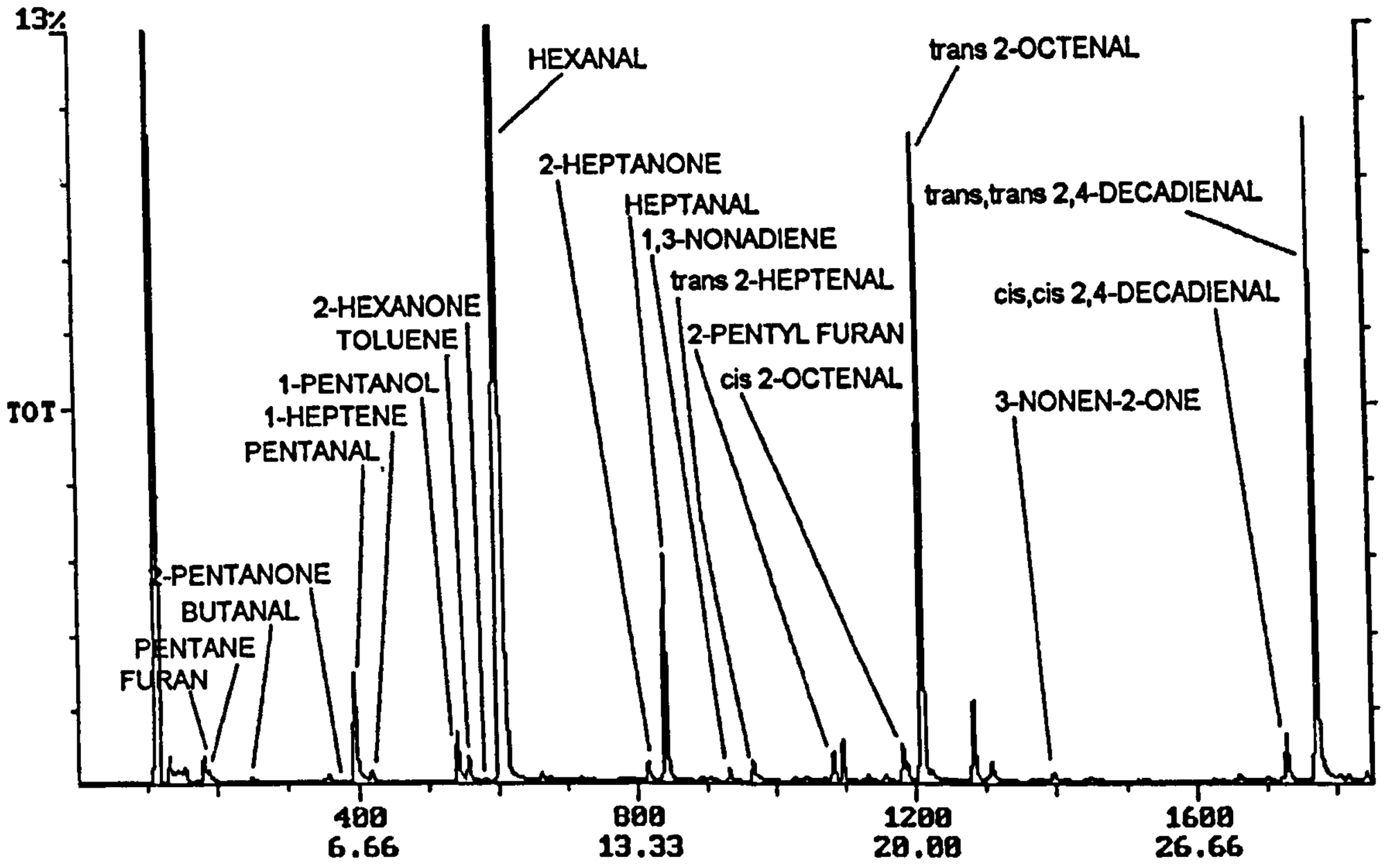
To determine whether this assumption was valid and, in addition, to determine the nature of other volatile breakdown products of the unsaturated aldehyde, a test was carried out in which *trans*, *trans* 2,4-decadienal was impregnated into samples of CTMP at a level of 1% (w/w). These samples were stored in a similar way to those conditioned with fatty acids.

The chromatogram in Figure 3.61 shows the headspace volatiles recovered from a sample of 2,4-decadienal/CTMP that had undergone thermal degradation during the headspace equilibration period. The chromatogram is similar to those for linoleic acid and its ester derivative, suggesting that 2,4-decadienal is responsible for a large proportion of the volatiles found in samples of linoleic acid. The two dominant volatiles found are hexanal and *trans* 2-octenal. Other volatiles included furan, pentanal, 1-pentanol, toluene, 2-heptanone, heptanal, *cis* 2-octenal, and *cis* and *trans* isomers of 2,4-decadienal.

Figure 3.62 shows the chromatogram for headspace volatiles recovered from a sample of *trans*, *trans* 2,4-decadienal stored for 47 days at 20°C. The amount of *trans* 2-octenal recovered is significantly less and the amount of hexanal significantly greater than those amounts for the sample that had undergone thermal degradation, suggesting that *trans* 2-octenal readily breaks down to form hexanal. Only a trace of *trans*, *trans* 2,4-decadienal was recovered. This illustrates the instability of this compound. The amount of heptanal recovered was markedly less than that obtained from the unstored sample. Small amounts of 6-undecanone and pentyl hexanoate were indicated by the chromatogram. These two compounds have been found as oxidation

products of some linoleic acid samples. Their presence suggests the involvement of 2,4-decadienal in these systems.

Figure 3.61 Chromatogram acquired from the headspace vapours of a one gram sample of CTMP/1%(w/w) *trans, trans* 2,4-decadienal that had undergone thermal degradation, at 105°C, for 60 minutes.



Interestingly, significant amounts of 1-pentanol were found, in addition to smaller amounts of pentane. Up to now it has been assumed that these two volatiles were principally formed from the decomposition of linoleic acid 13-hydroperoxide. It was also suggested that pentanal may be formed by addition of oxygen to the pentyl radical, produced from the decomposition of linoleic acid 13-hydroperoxide. These three compounds may still form via a pentyl radical, though the route to the formation of this radical is unclear.

The amounts of *trans* 2-heptenal in samples of 2,4-decadienal/CTMP were less than those found in fatty acid samples, suggesting that this volatile is formed principally by another mechanism that does not involve 2,4-decadienal.

Figure 3.62 Chromatogram acquired from the headspace vapours of a one gram sample of CTMP/1%(w/w) *trans, trans* 2,4-decadienal that had been stored, at 20°C, for 47 days.

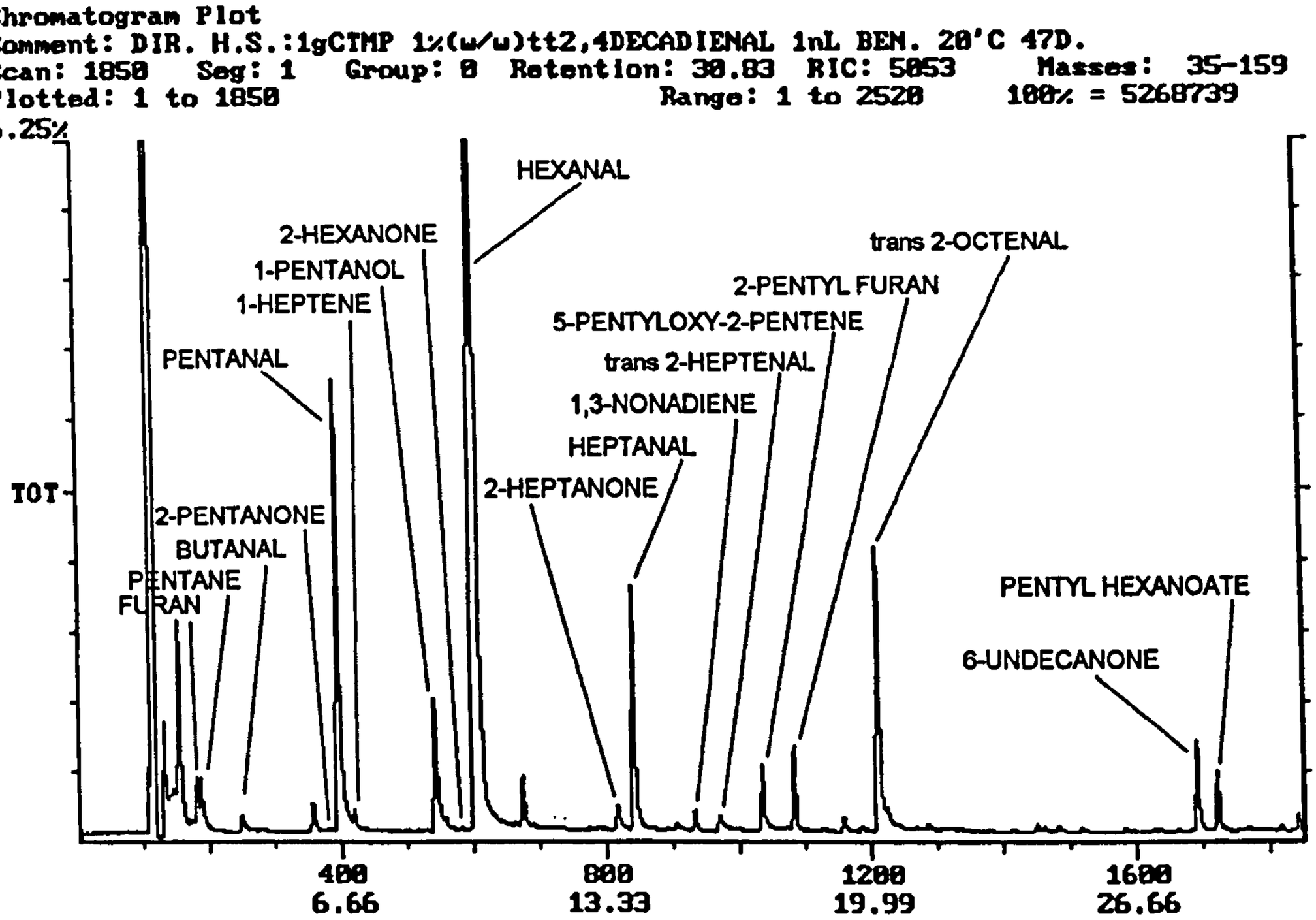


Figure 3.63 shows the amounts of hexanal recovered from samples of *trans, trans* 2,4-decadienal/CTMP.

The amounts of hexanal are smaller than those for linoleic acid. In addition, the storage temperature has less effect on the amount of hexanal recovered.

Figure 3.64 shows the amounts of *trans* 2-octenal recovered from samples of *trans, trans* 2,4-decadienal/CTMP.

The amounts of *trans* 2-octenal recovered initially were very large, indicating that this compound is a major breakdown product of 2,4-decadienal, as previously assumed. The levels recovered decline sharply during the first four days of storage, mainly due to oxidation and the decomposition of this volatile into other products. However, the levels rose again as the storage time increased. This may be due to decreased oxygen levels, following extensive uptake in the system, thus retarding the oxidation of 2-octenal into the corresponding fatty acid. Throughout the test, levels of *trans* 2-octenal were greater than those for linoleic acid samples.

Figure 3.63 Amounts of hexanal recovered (in nL) from samples of *trans*, *trans* 2,4-decadienal/CTMP stored at 20°C, 40°C and 50°C for up to 47 days (Headspace sampling temperature: 105°C).

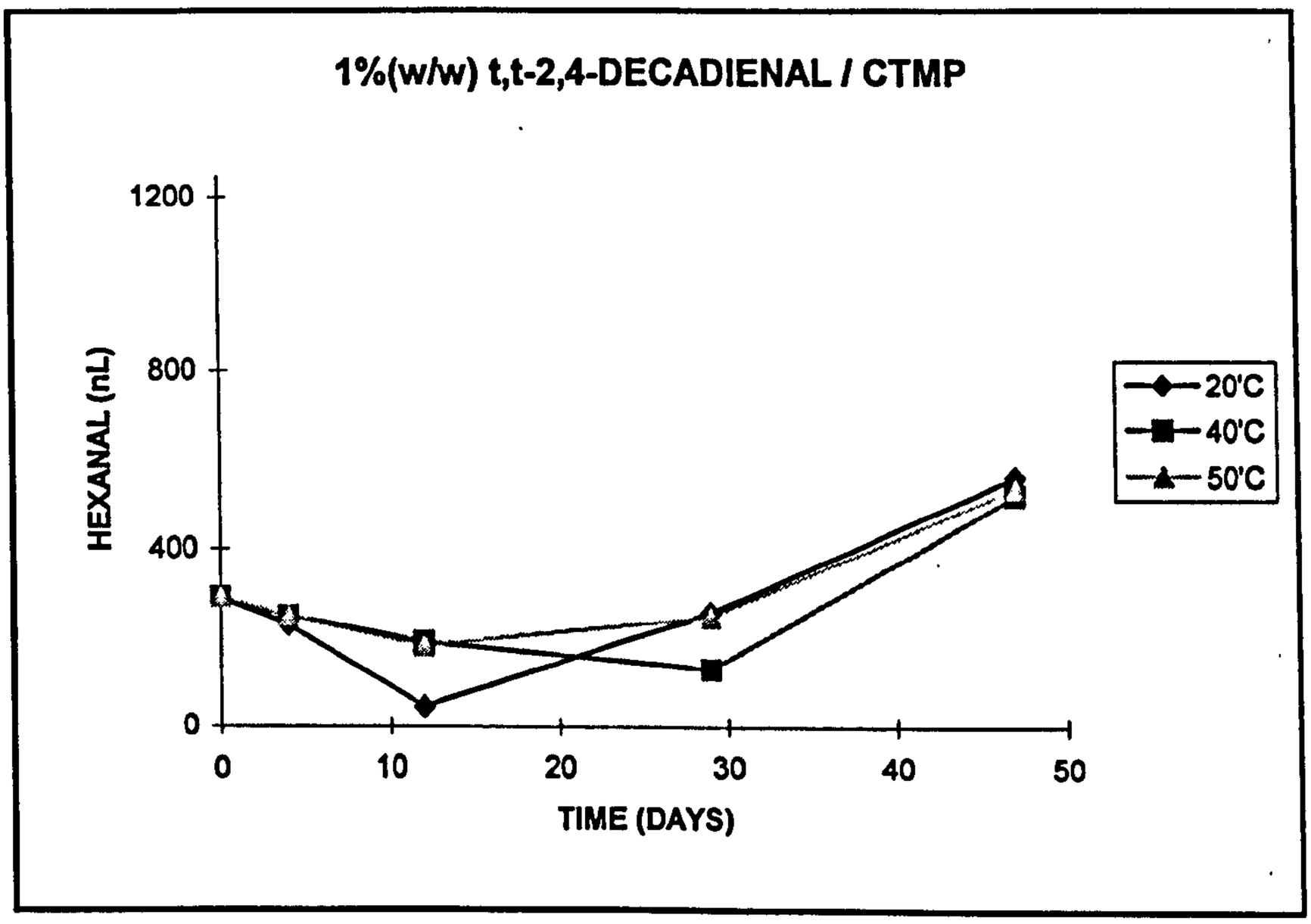


Figure 3.64 Amounts of *trans* 2-octenal recovered (in nL) from samples of *trans*, *trans* 2,4-decadienal/CTMP stored at 20°C, 40°C and 50°C for up to 47 days (Headspace sampling temperature: 105°C).

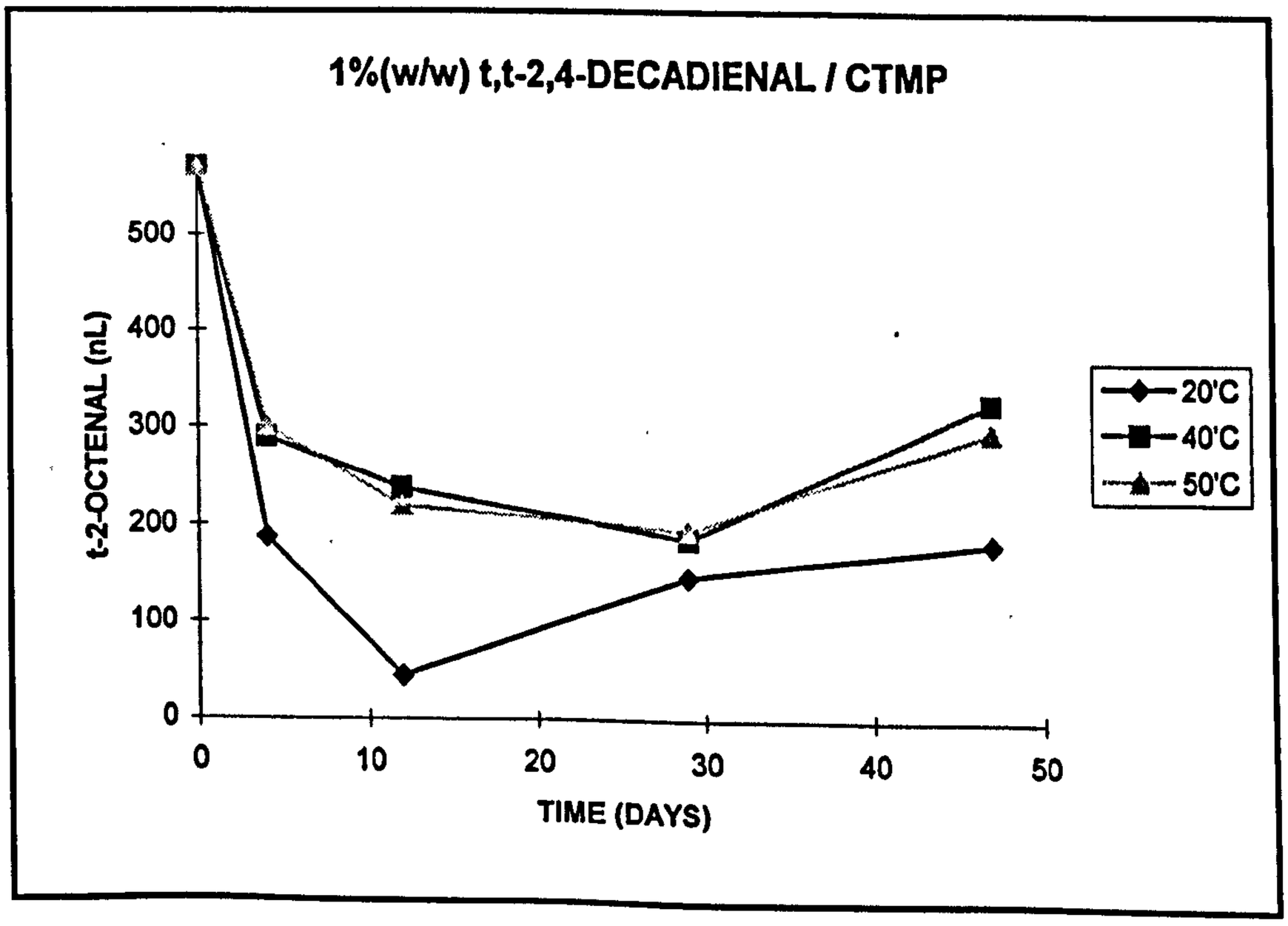
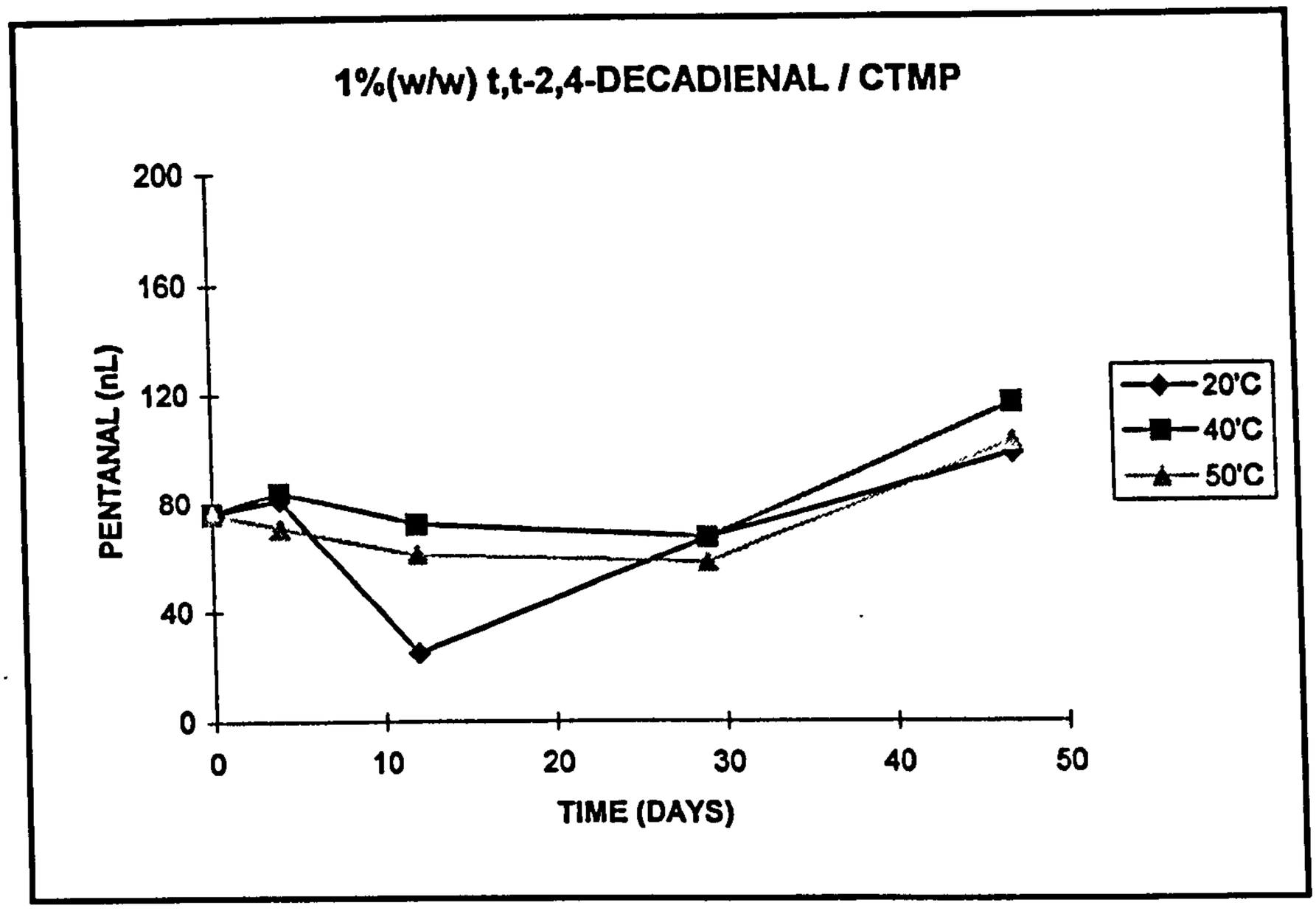


Figure 3.65 shows the amounts of pentanal recovered from samples of *trans*, *trans* 2,4-decadienal/CTMP.

Figure 3.65 Amounts of pentanal recovered (in nL) from samples of *trans*, *trans* 2,4-decadienal/CTMP stored at 20°C, 40°C and 50°C for up to 47 days (Headspace sampling temperature: 105°C).

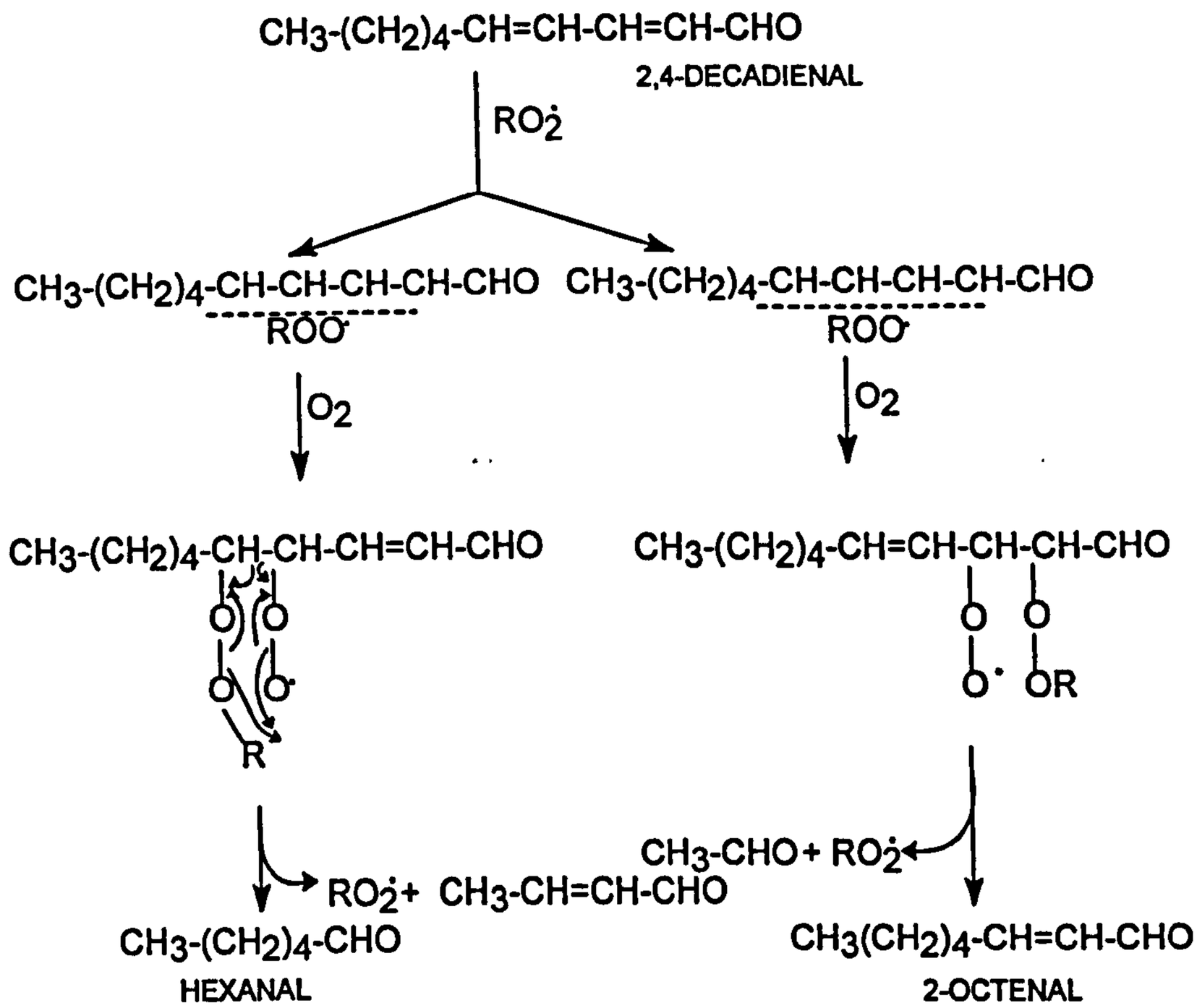


The amounts of pentanal recovered are comparable to those in the tests involving linoleic acid, reinforcing the argument that 2,4-decadienal is a major precursor of pentanal. Similar amounts of pentanal were found at the three storage temperatures, suggesting that if pentanal is formed predominantly from the breakdown of 2,4-decadienal, then variation in the amounts of pentanal found from linoleic acid samples, at different temperatures, is due to variation in formation of 2,4-decadienal itself. In other words, the formation of linoleic acid 9-hydroperoxide and its subsequent decomposition, has a degree of temperature dependence.

Figure 3.66 shows the mechanism involved in the formation of hexanal and 2-octenal from 2,4-decadienal.

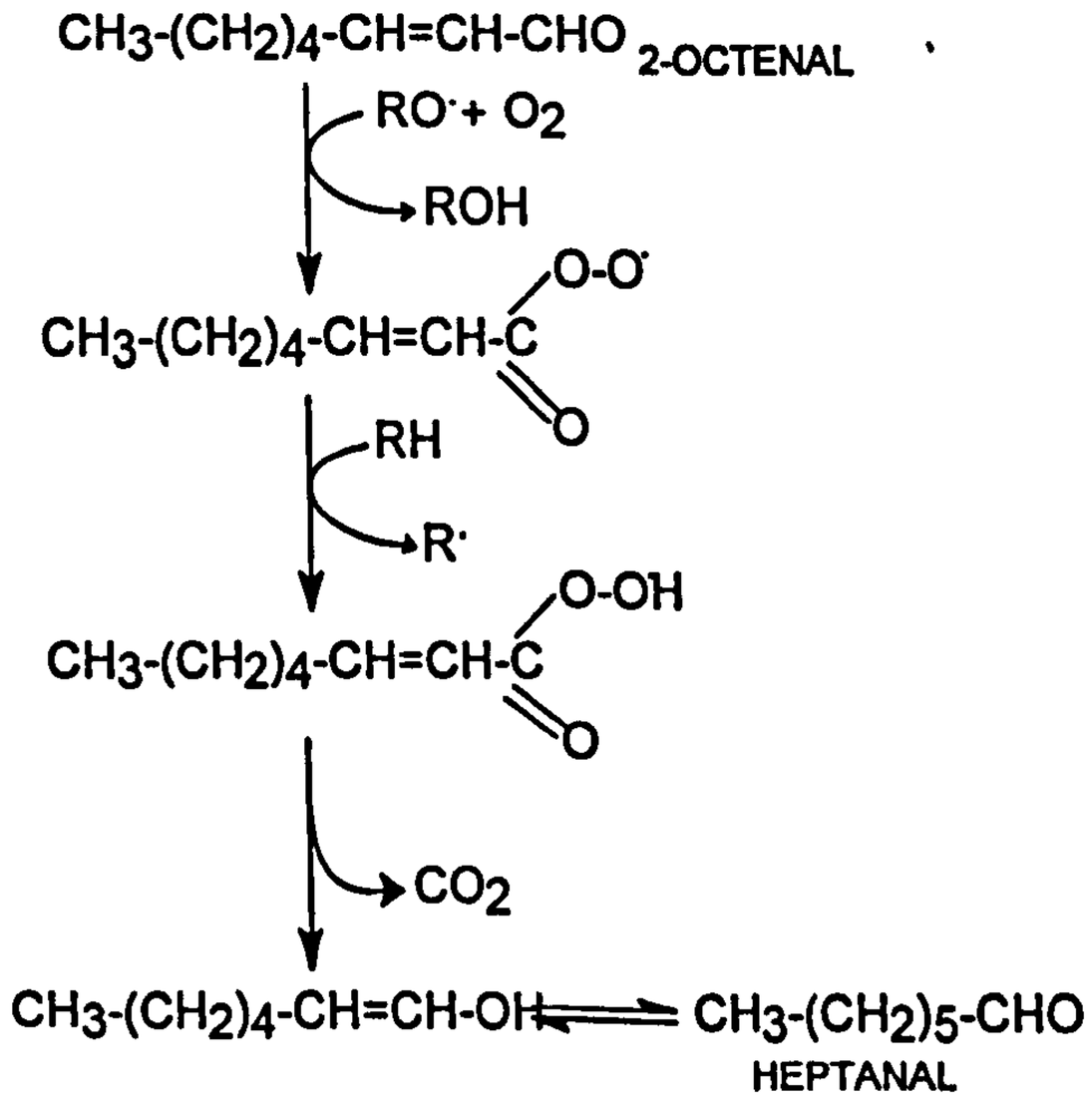
Relatively large amounts of heptanal were found in samples of 2,4-decadienal/CTMP that had undergone thermal degradation.

Figure 3.66 The mechanism for the formation of hexanal and 2-octenal from 2,4-decadienal.



Schieberle and Grosch (1981) have proposed that this formation is brought about by the autoxidation of 2-octenal, via a per-acid intermediate (Figure 3.67).

Figure 3.67 The mechanism for the formation of heptanal from 2-octenal, via a per-acid intermediate.



It can, therefore, be expected that, as the amount of 2-octenal in the system decreases, the amount of heptanal formed will also decrease. This point is demonstrated by the initial sharp drop in heptanal accompanying the drop in 2-octenal recovered from samples, between the 0 and 4 days storage times.

Determination of the effect of singlet oxygen on the oxidation of linoleic acid in CTMP

Experiments involving the irradiation of samples of linoleic acid/CTMP with UV light, generally, yielded slightly smaller amounts of volatile compounds to those of non-irradiated samples. This suggests that the system had no photosensitisers that, if present, on irradiation, play a part in photoxidation on the unsaturated fatty acid to yield volatile compounds.

The photoxidation process involves interaction between a double bond and an excited singlet oxygen unit, produced from ordinary triplet oxygen ($^3O_2$) by electromagnetic radiation of suitable energy (e.g. visible, ultraviolet or X-ray) in the presence of a sensitiser. The photo-sensitised oxidation process is illustrated below:

$$\text{Sens} \xrightarrow[h\nu]{\text{light}} {}^1\text{Sens}^* \longrightarrow {}^3\text{Sens}^*$$

$$^3\text{Sens}^* + {}^3O_2 \longrightarrow {}^1O_2^* + {}^1\text{Sens}$$

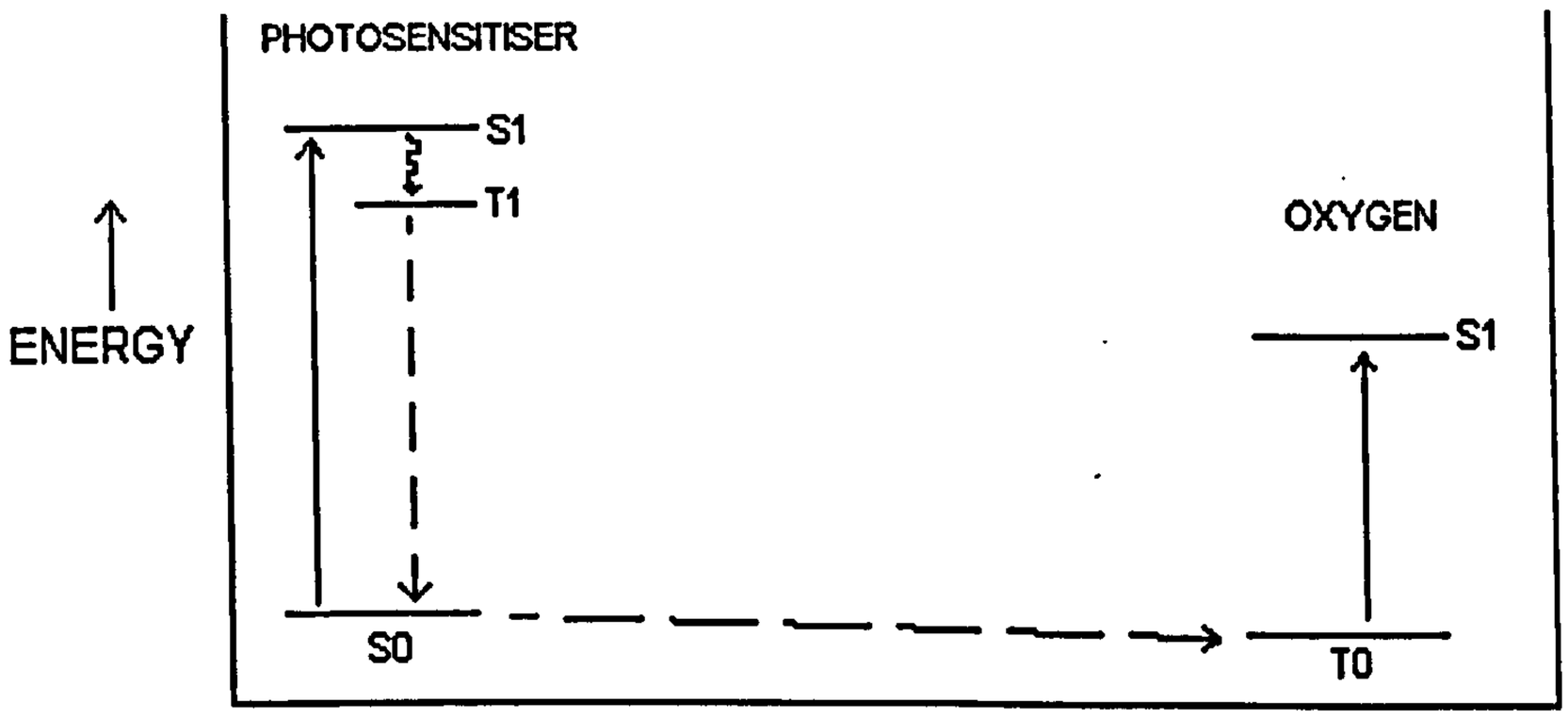
$$^1O_2^* + \text{RH} \longrightarrow \text{ROOH}$$

where Sens, $^1\text{Sens}^*$ and $^3\text{Sens}^*$ represent sensitiser, excited singlet state of the sensitiser and excited triplet state of the sensitiser, respectively. The oxygen molecule becomes activated to the singlet state by the transfer of energy from the excited triplet state photosensitiser. This process can be illustrated in the state diagram representing the energy levels of the photosensitiser molecule and the oxygen molecule (Figure 3.68).

On excitation with UV light, the photosensitiser molecule undergoes a (n,π^*) transition from the singlet ground state (S_0) to the first singlet excited state (S_1).

The energy gap, ΔE_{ST}, between the first excited singlet state (S_1) and first excited triplet state (T_1) of the photosensitiser molecule is small.

Figure 3.68 State diagram representing the energy transfer from the photosensitiser molecule to the oxygen molecule.



Therefore, intersystem crossing between the excited singlet state (S_1) and the excited triplet state (T_1) is favourable. Energy transfer then occurs rapidly between the excited triplet state (T_1) of the photosensitiser molecule and the oxygen molecule in the triplet ground state (T_0). This results in the excitation of the oxygen molecule to the singlet excited state (S_1). The process is fast because there is no overall change in electron spin between the excited singlet state (S_1) of the photosensitiser and the excited singlet state of the oxygen molecule. Reversal of electron spin is a forbidden process and processes involving electron spin reversal tend to be very slow. For the process to take place it is essential that the energy of first singlet excited state of oxygen is lower than that of the first excited triplet state of the photosensitiser. A good photosensitiser should have a first excited triplet state of at least 92 KJ mol^{-1} above the ground state.

The excited singlet oxygen ($^1O_2^*$), thus produced, reacts like a highly reactive alkene and not like ground state triplet oxygen, which behaves like a diradical. Singlet oxygen reacts with methyl linoleate 10^3 to 10^4 times faster than does normal oxygen (Kochhar, 1993). Decomposition of hydroperoxides formed in

this manner, possibly being catalysed by heavy metals, may initiate free radical autoxidation.

Foote (1976) has postulated another mechanism, whereby the triplet sensitiser forms a Sens-oxygen complex. This reacts with the substrate to produce a hydroperoxide and regenerates the sensitiser as:

$$^3Sens + {}^3O_2 \longrightarrow {}^1[Sens\text{-}O_2]$$

$$^1[Sens\text{-}O_2] + RH \longrightarrow ROOH + {}^1Sens$$

As mentioned earlier, singlet oxygen reacts with double bonds by concerted addition and, thus, gets attached at either carbon of a double bond, to produce an allylic hydroperoxide in a *trans* configuration.

Linoleic acid produces a mixture of 9-, 10-, 12-, and 13-hydroperoxides. The proportions of hydroperoxide isomers formed by autoxidation and photo-oxygenation of linoleic acid are given below (Figure 3.69).

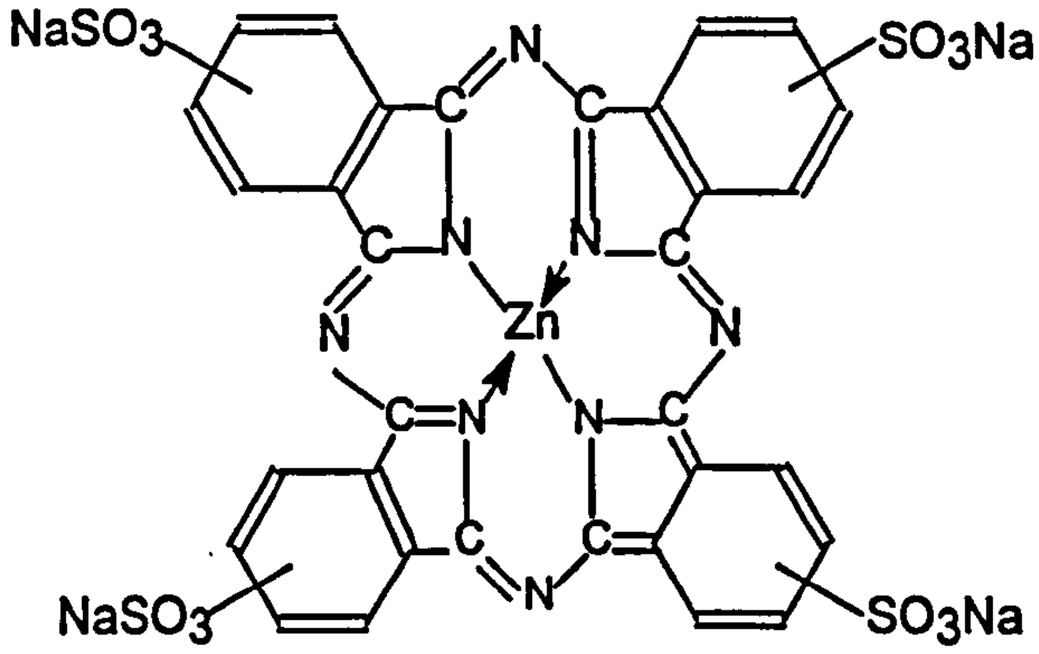
Figure 3.69 Proportions of monohydroperoxides formed by autoxidation and photoxidation of linoleic acid (Belitz and Grosch, 1987; Gunstone, 1984; Kochhar, 1993).

Position of		Proportion (%)	
-OOH group	Double bond	Autoxidation	Photoxidation
8	9,12	1.5	
9	10,12	46.5	31
10	8,12	0.5	18
12	9,13	0.5	18
13	9,11	49.5	33
14	9,12	1.5	

The proportions of internal isomers of the photoxidised linoleic acids (10- and 12-OOH) are produced in significantly lower concentrations than are the external isomers, due to 1,3-cyclisation of the internal isomers to produce

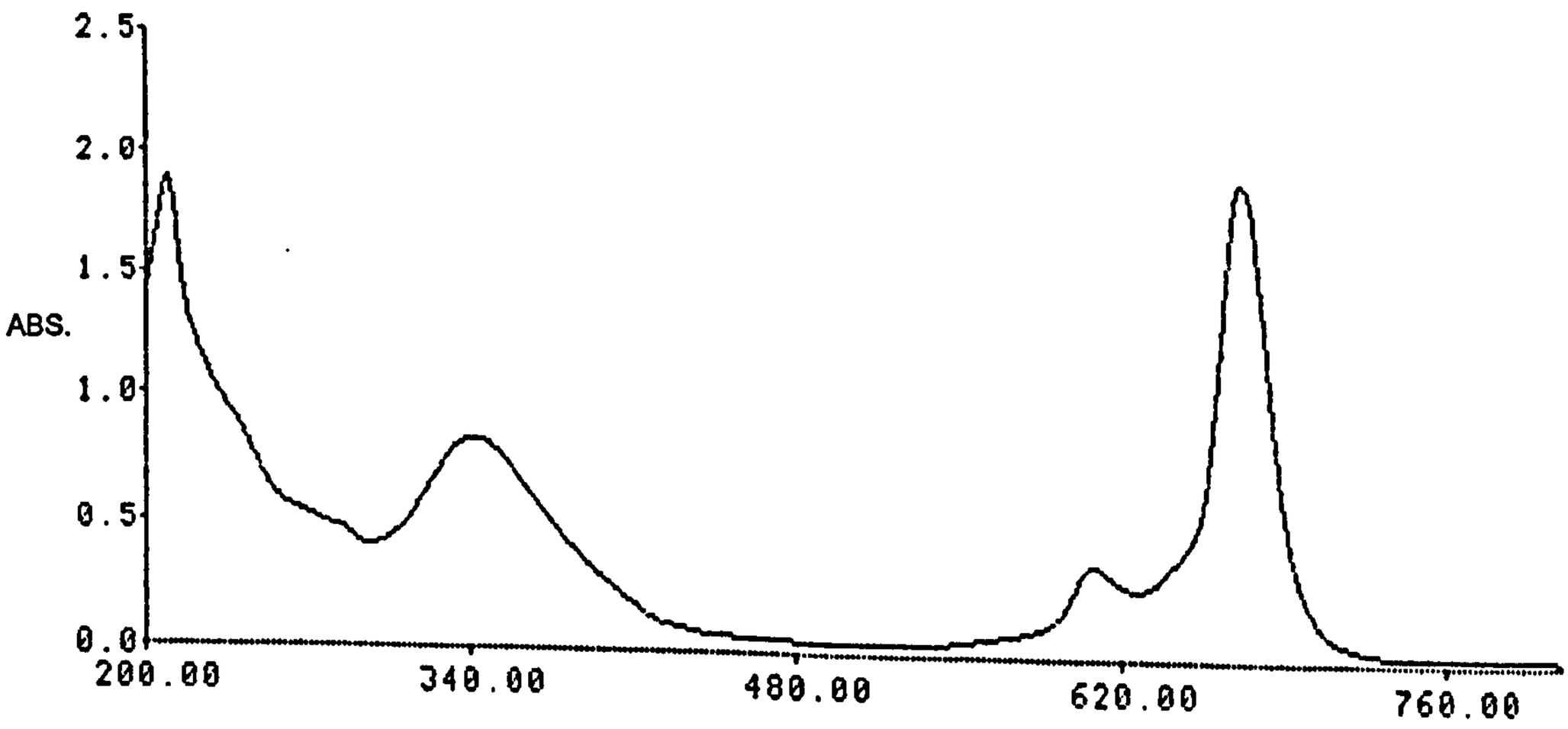
hydroperoxy cyclic peroxides. However, these internal isomers are produced in significantly greater amounts than during autoxidation.

The photosensitiser used during this test was tetrasulphozincphthalocyanine (TSZnPh.), as a 0.2 grams solution in 100 cm^3 of water. The formula of this compound is illustrated below. The UV/visible absorbance spectrum is shown in Figure 3.70.



Tetrasulphozincphthalocyanine

Figure 3.70 UV/Visible Absorbance Spectrum of Tetrasulphozincphthalocyanine in Water



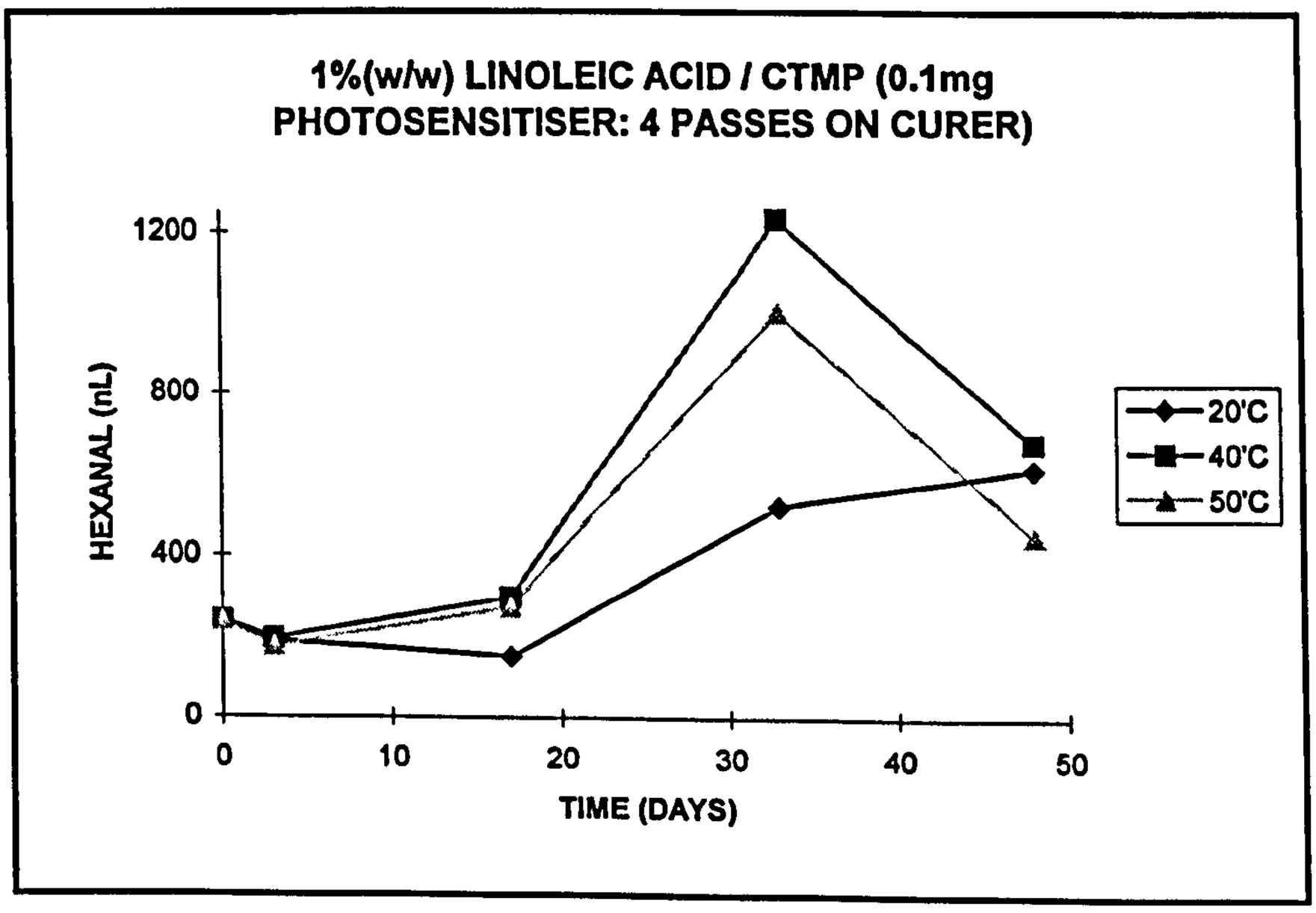
The absorbance spectrum of the tetrasulphozincphthalocyanine shows two major absorbance bands, one at 210nm and the other at 673nm. Thus, this

photosensitiser absorbs in the UV region (<400nm) and should adopt an excited state on irradiation with UV light.

The headspace vapour chromatograms obtained from samples of linoleic acid/CTMP + TSZnPh, show that the volatile product compositions were not too different to those where no photosensitiser was present. Thus, it is concluded on this evidence that no significant differences in reaction pathway take place in the presence of singlet oxygen.

Figure 3.71 shows the amounts of hexanal recovered from samples of linoleic acid/CTMP in the presence of TSZnPh.

Figure 3.71 Amounts of hexanal recovered (in nL) from samples of linoleic acid/CTMP + 0.1mg TSZnPh photosensitiser stored at 20°C, 40°C and 50°C for up to 48 days (Headspace sampling temperature: 105°C).

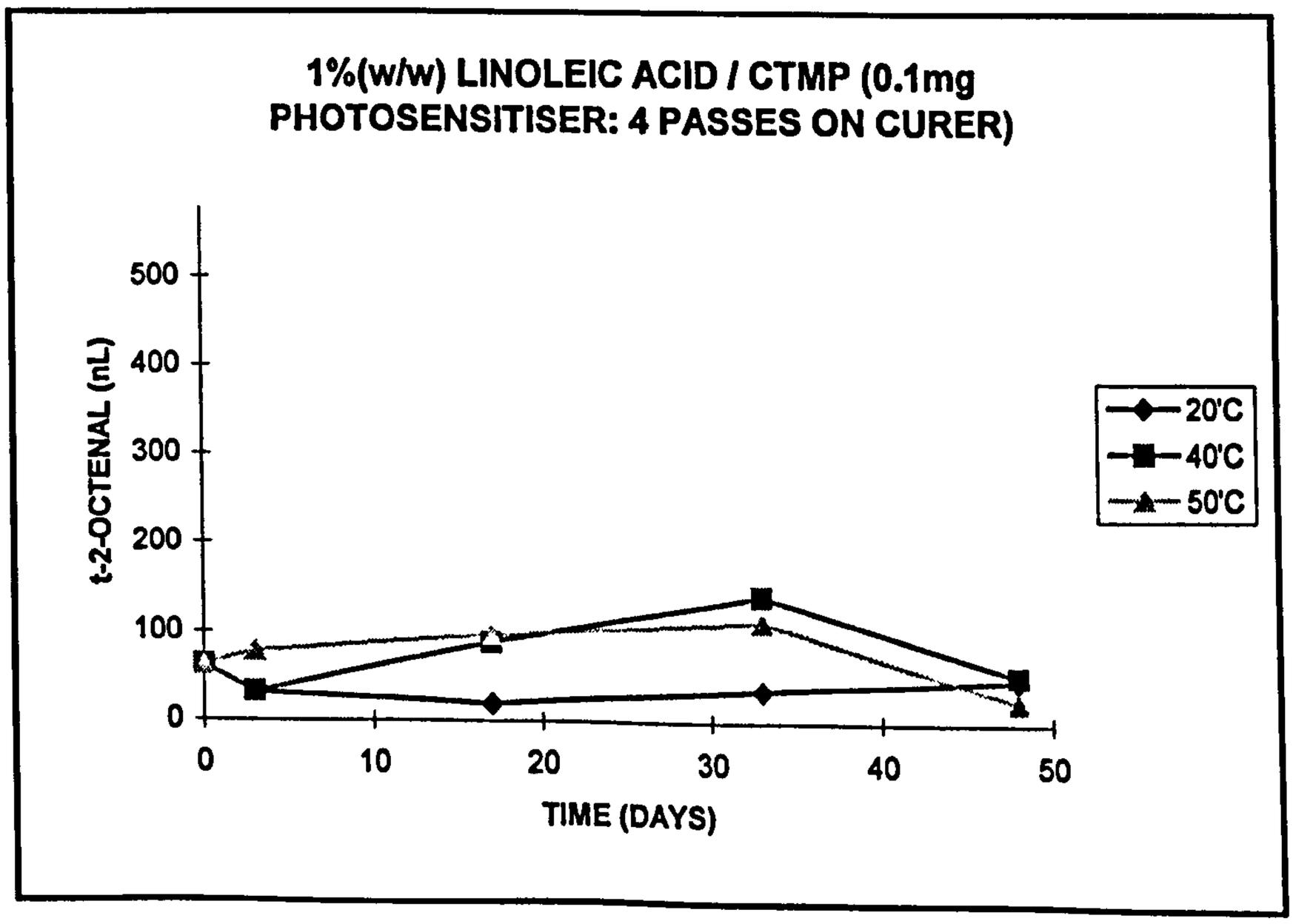


After approximately three weeks storage, the amounts of hexanal recovered increases sharply. The amount of hexanal reaches a maximum after 33 days storage, at 40°C and 50°C. After this high level, the amounts of hexanal declined. The amounts of hexanal recovered from samples stored at ambient temperature were comparable with those of samples stored at ambient temperatures, in the absence of TSZnPh.

The large amounts of hexanal are due to the increased extent of hydroperoxide formation, resulting from photosensitised oxidation. As previously discussed, photoxidation is not subject to an induction period and singlet oxygen may add to the fatty acid substrate much more rapidly than during autoxidation. The hydroperoxides formed via photoxidation decompose as a function of time and temperature. The amounts of hexanal monitored after approximately five weeks, suggests that termination reactions, in particular $ROO^{\bullet} + ROO^{\bullet}$, have not suppressed volatile compound formation. The reason for this may lie in the rapid formation of hydroperoxides from peroxy radicals, via hydrogen abstraction, thus, reducing the number of peroxy radicals available for combination. In autoxidation, the formation of hydroperoxides from peroxy radicals is the slowest step and is the rate determining step in the oxidation sequence, therefore, no appreciable volatile compound formation can occur until there are sufficient hydroperoxides in the system that decompose.

Figure 3.72 shows the amounts of *trans*-2-octenal recovered from samples of linoleic acid/CTMP in the presence of TSZnPh.

Figure 3.72 Amounts of *trans* 2-octenal recovered (in nL) from samples of linoleic acid/CTMP + 0.1mg TSZnPh photosensitiser stored at 20°C, 40°C and 50°C for up to 48 days (Headspace sampling temperature: 105°C).



Levels of *trans* 2-octenal recovered were comparable to those for linoleic acid/CTMP samples in the absence of photosensitiser. If 2,4-decadienal is considered to be the main precursor of this compound, it appears that photoxidation has little effect on the formation of the linoleic acid 9-hydroperoxide isomer.

Tressl et al. (1981) found that the amounts of 2,4-decadienal formed from photoxidation of methyl linoleate were much reduced relative to those found from autoxidation. However, the data presented above suggest that the formation of 2,4-decadienal from linoleic acid in CTMP is not significantly affected by photoxidation. The same author reported a significant increase in the amount of *trans* 2-heptenal that was recovered from photoxidised methyl linoleate.

trans 2-Heptenal is formed principally via a hydroperoxy cyclic peroxide intermediate. Frankel et al. (1982) suggested that photoxidation of methyl linoleate yields 13-hydroperoxy-10,12-epidioxy-*trans*-8-octadecenoate and 9-hydroperoxy-10,12-epidioxy-*trans*-13-octadecenoate. The major products, from the pyrolysis at 210°C, of the latter intermediate were found to be 2-heptenal and methyl 9-oxononanoate (Figure 3.73).

Figure 3.73 Reaction scheme proposed for the thermal decomposition of methyl 9-hydroperoxy-10,12-epidioxy-*trans*-13-octadecenoate.

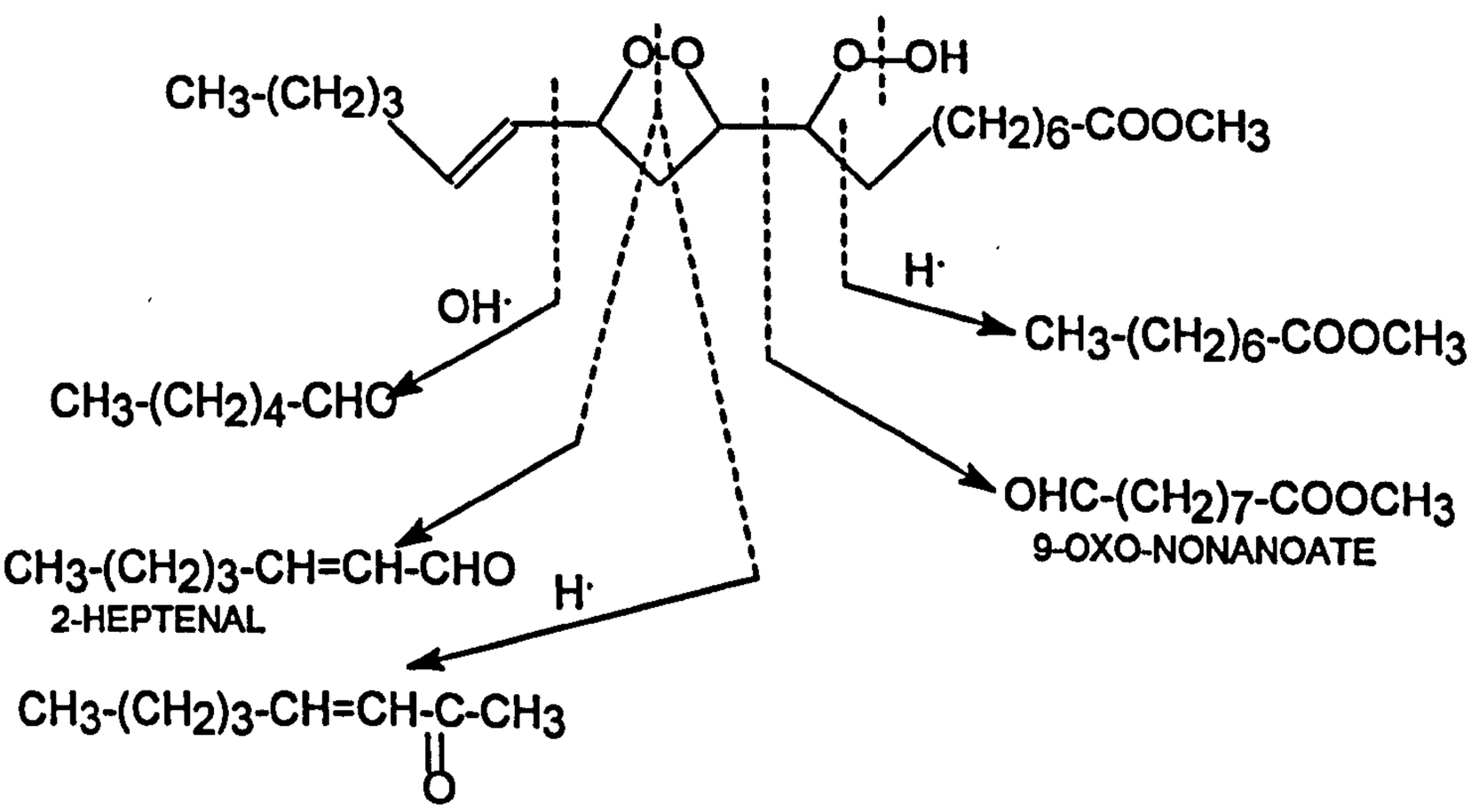
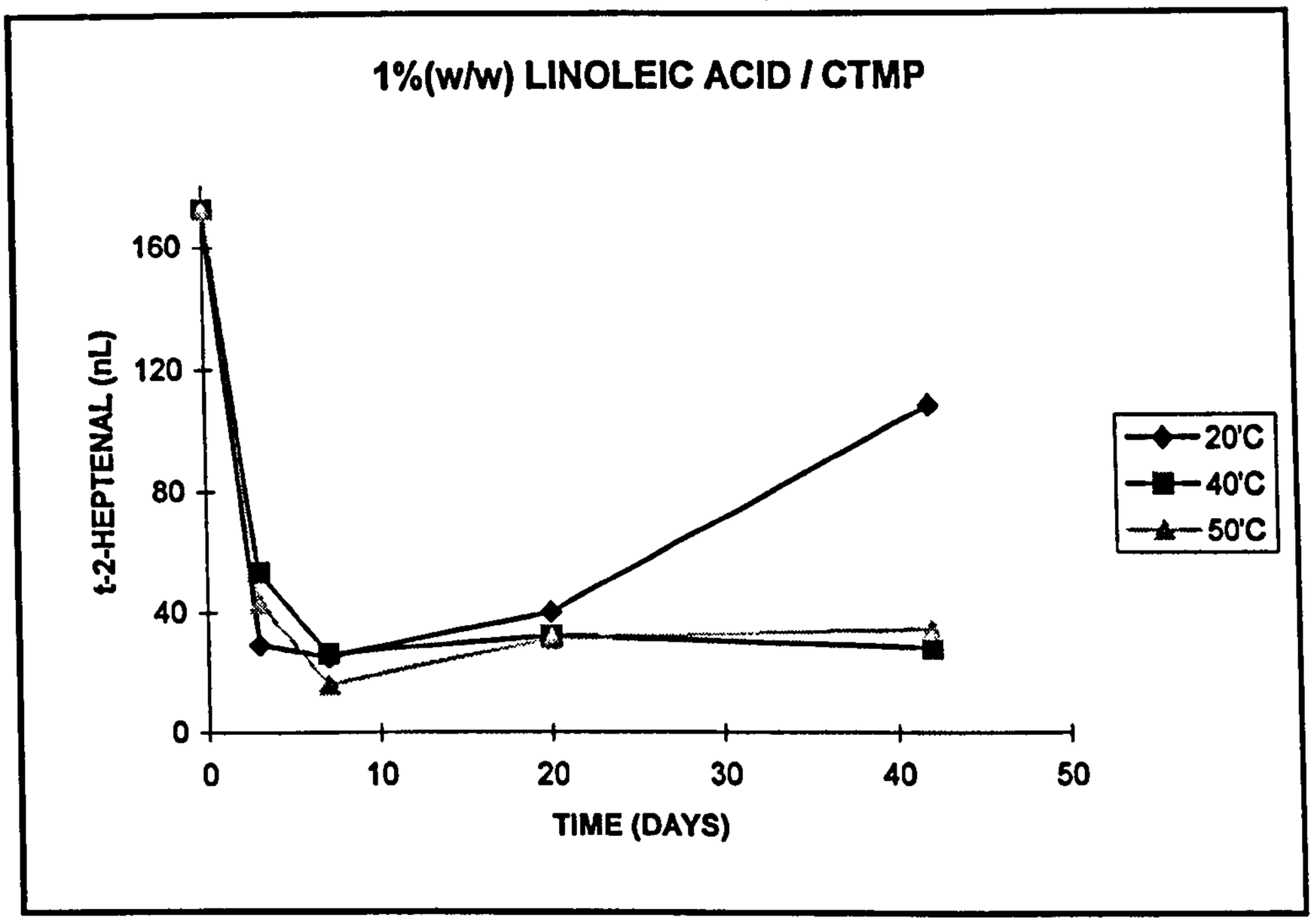


Figure 3.74 shows the amount *trans* 2-heptenal recovered from samples of linoleic acid/CTMP in the absence of photosensitiser and Figure 3.75 shows the

amounts of *trans*-2-heptenal recovered from samples of linoleic acid/CTMP in the presence of TSZnPh photosensitiser.

Figure 3.74 Amounts of *trans* 2-heptenal recovered (in nL) from samples of linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 42 days (Headspace sampling temperature: 105°C).



The amounts of 2-heptenal recovered from samples of linoleic acid/CTMP in the presence of photosensitiser are generally no greater than those recovered from samples in its absence. This suggests that photosensitisation does not bring about a significant increase in the formation of hydroperoxy epidioxides.

From tests carried out, there is evidence that hydroperoxy epidioxides form in linoleic acid/CTMP systems during autoxidation. This is indicated by the presence of 3-octen-2-one recovered, in the headspace volatiles of samples, in the presence and in the absence of photosensitiser. 3-octen-2-one is reported to be unique to the decomposition of cyclic peroxides (Frankel et al., 1982).

Figure 3.76 shows the amounts of pentanal recovered from samples of linoleic acid/CTMP in the presence of photosensitiser.

Figure 3.75 Amounts of *trans* 2-heptenal recovered (in nL) from samples of linoleic acid/CTMP + 0.1mg TSZnPh. photosensitiser stored at 20°, 40°C and 50°C for up to 48 days (Headspace sampling temperature: 105°C).

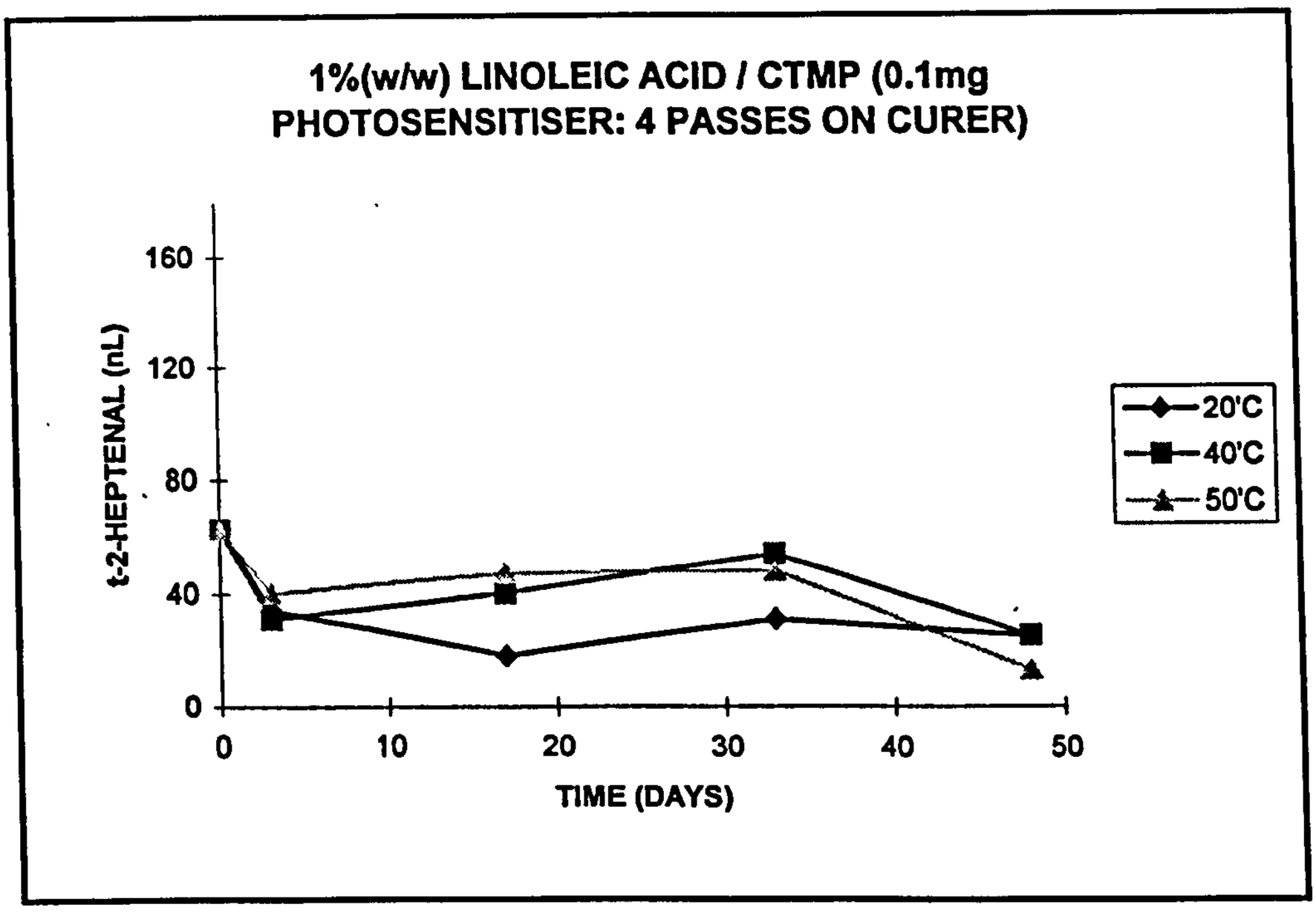
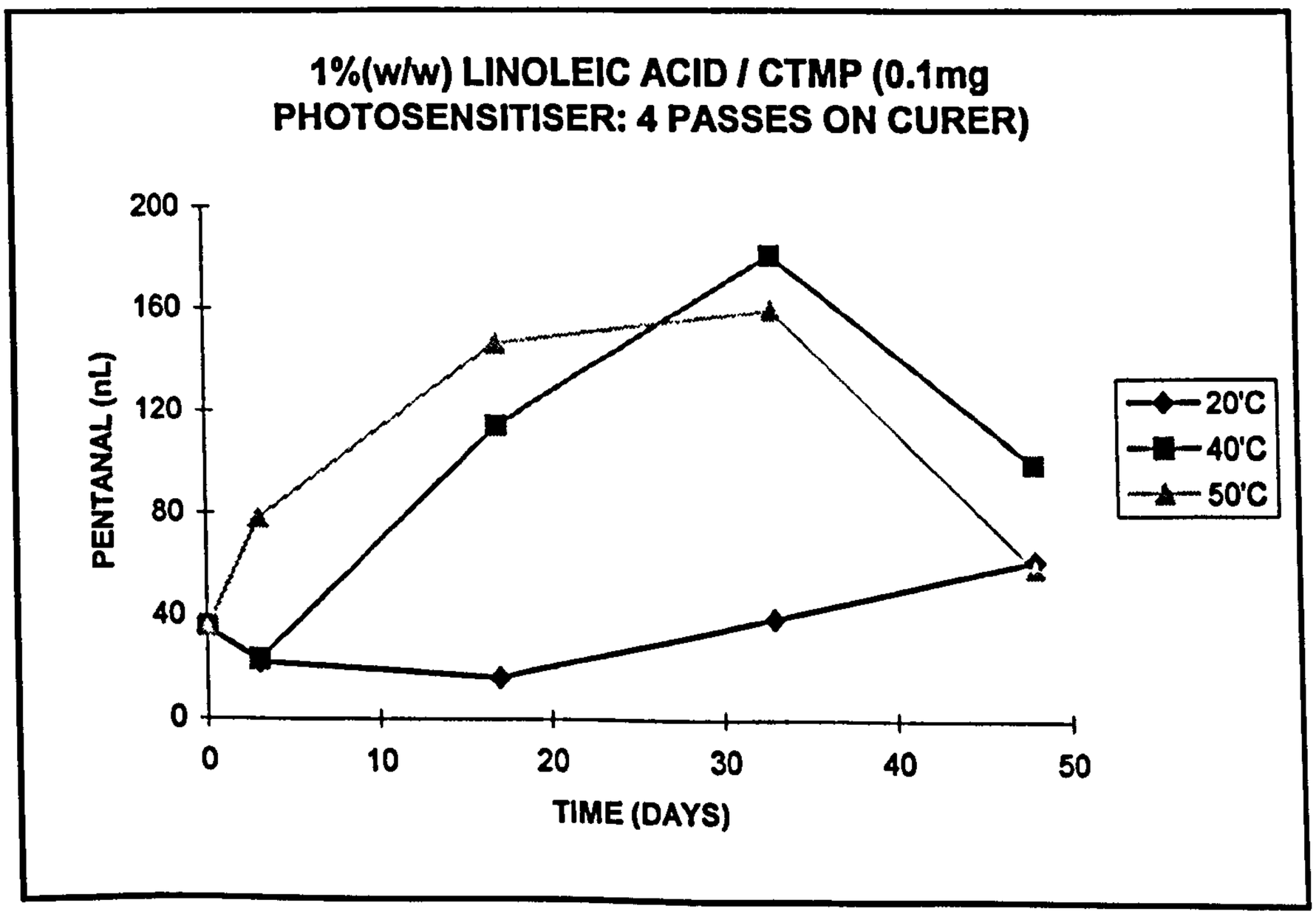


Figure 3.76 Amounts of pentanal recovered (in nL) from samples of linoleic acid/CTMP + 0.1mg TSZnPh. photosensitiser stored at 20°C, 40°C and 50°C for up to 48 days (Headspace sampling temperature: 105°C).

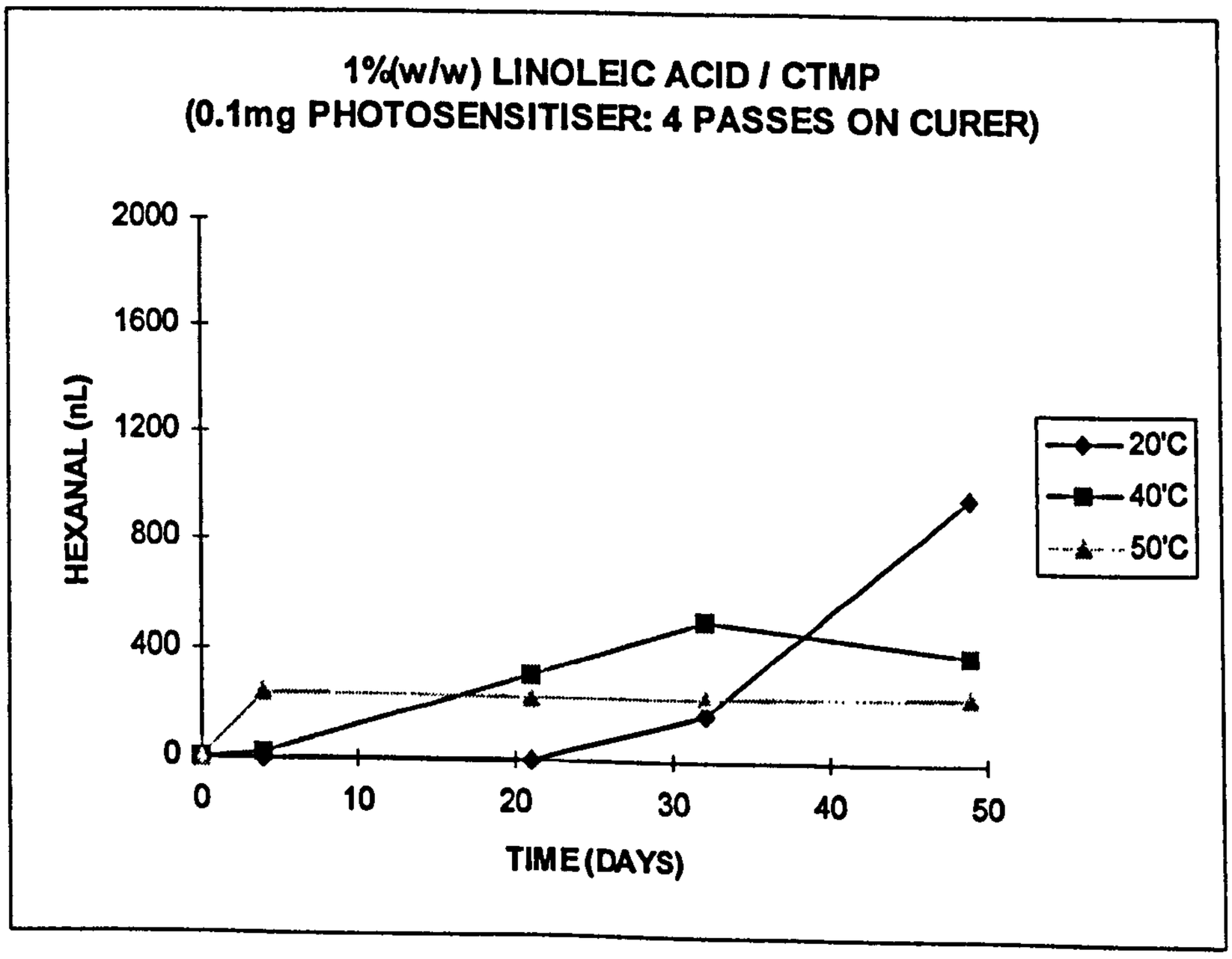


The amounts of pentanal recovered after five weeks of storage at 40°C and 50°C are large, corresponding to the large amounts of hexanal recovered. This suggests that pentanal is linked in some way to the formation of hexanal. The most likely route to its formation is via the pentyl radical, produced from the decomposition of linoleic acid 13-hydroperoxide.

The previous test involving the autoxidation of 2,4-decadienal in CTMP suggested that pentanal was produced in significant amounts from the decomposition of this unsaturated aldehyde. However, during this test 2,4-decadienal was not formed in significant amounts, based on the amounts of *trans* 2-octenal recovered.

Samples of linoleic acid/CTMP impregnated with a 0.2 grams solution of TSZnPh in 100 cm^3 of water were irradiated and stored, as before, and sampled using a headspace equilibration temperature of 50°C. Figure 3.77 shows the amounts of hexanal recovered during the test.

Figure 3.77 Amounts of hexanal recovered (in nL) from samples of linoleic acid/ CTMP + 0.1mg TSZnPh. photosensitiser stored at 20°C, 40°C and 50°C for up to 49 days (Headspace sampling temperature: 50°C).



The hexanal levels are generally less than those found for the corresponding samples using an equilibration temperature of 105°C. They are also less than

the amounts of hexanal found in linoleic acid/CTMP samples in the absence of photosensitiser, equilibrated at 50°C.

The presence of photosensitiser, increased the formation of the volatile component, resulting from oxidation, quite significantly at an equilibration temperature of 105°C. Therefore, the presence of the photosensitiser results in a higher degree of hydroperoxide formation. The greater number of hydroperoxides should be present in the samples equilibrated at the lower headspace temperature. The smaller amounts of hexanal from samples equilibrated at 50°C may be due to the formation of non-volatile products being favoured. This was thought to be the case in the linoleic acid/CTMP system in the absence of photosensitiser. Alternatively, the higher concentration of hydroperoxides within the system may result in a higher concentration of radicals on decomposition, causing the number of termination reactions to become significant.

Another possibility lies in the nature of the hydroperoxides formed. Photoxidation results in the formation of greater amounts of the 10- and 12-isomers of linoleic acid hydroperoxide, which will be less prone to isomerisation at the lower equilibration temperature. These isomers are susceptible to 1,3-cyclisation to produce hydroperoxy cyclic peroxides. These cyclic peroxides are quite stable at 50°C, resulting in lower decomposition.

The levels of hexanal, recovered for samples stored at ambient temperature, continued to build up throughout the test duration. After six weeks, the hexanal levels were significantly greater than the levels for the corresponding system, in the absence of photosensitiser. This confirms the that the presence of the photosensitiser resulted in the formation of a larger number of hydroperoxides within the system. At ambient temperature, these hydroperoxides slowly decompose to yield volatile compounds that are at a low risk from thermal degradation at ambient temperature. Slow decomposition of hydroperoxides prevents a large concentration of radicals forming and, thus, termination is not as significant as at higher temperatures.

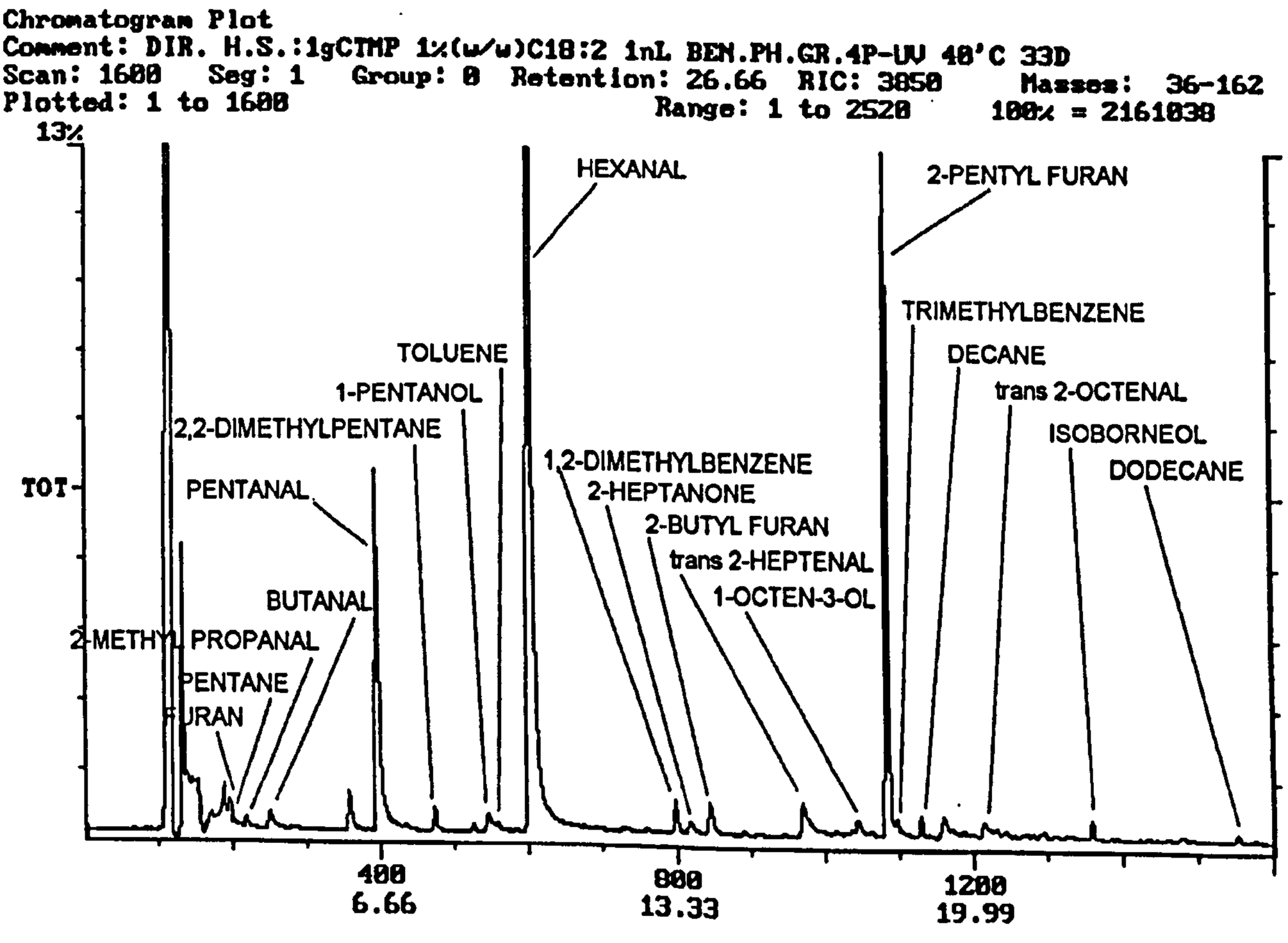
Determination of the effect of UV-cured ink on the presence of linoleic acid in CTMP

Samples of CTMP, impregnated with linoleic acid, were print proofed with a thin film of a Unicure 'Phthalo Green' UV-curable ink, irradiated, sealed and then stored as in previous tests.

The test was meant to simulate the printing of board material on an actual printing line. However, the CTMP substrate surface was far different from that of finished carton-board. The CTMP had no surface size, fillers, pigments or binders, unlike the carton-board. Therefore, ink absorption and penetration into the substrate were much greater. This could be seen as a benefit, since any effect imparted by the irradiated ink on the linoleic acid/CTMP system could be attenuated and a clearer picture gained.

Figure 3.78 shows the chromatogram obtained from a sample of linoleic acid/ CTMP print proofed with a thin film of Phthalo Green ink, irradiated, and stored for 33 days at 40°C.

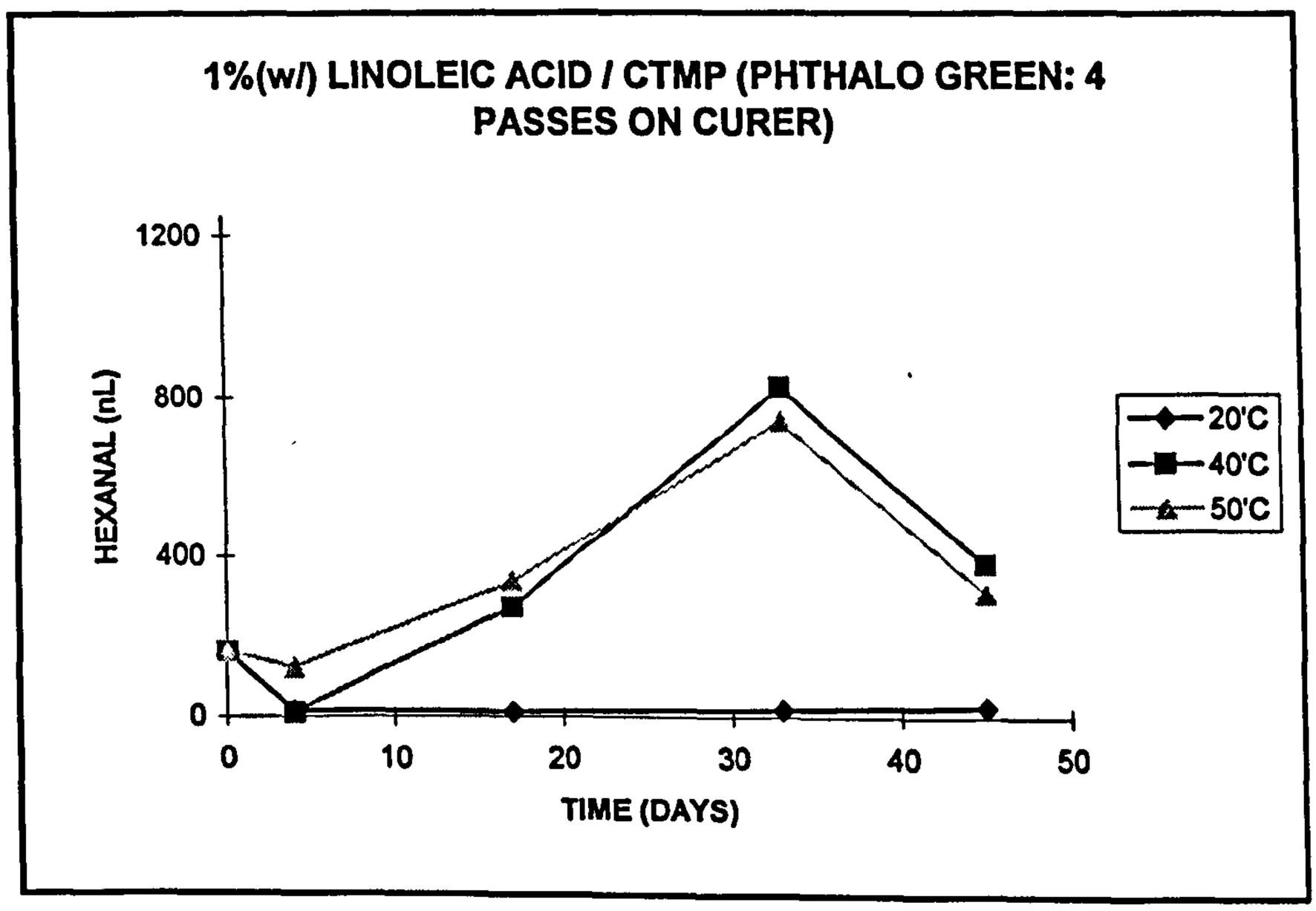
Figure 3.78 Chromatogram acquired from the headspace vapours of an irradiated one gram sample of CTMP/1%(w/w) linoleic acid, print proofed with a film of Phthalo Green UV ink, that had been stored, at 40°C, for 33 days.



The dominant volatiles are hexanal and 2-pentyl furan. Other compounds recovered in the headspace volatiles can be associated to the ink. These include: 2,2-dimethylpentane, toluene, 1,2-dimethylbenzene, trimethylbenzene, decane, isoborneol, dodecane and small amounts of additional hydrocarbons and alkylated benzenes.

Figure 3.79 shows the amounts of hexanal recovered from samples of linoleic acid/CTMP print proofed with the Phthalo Green ink and irradiated.

Figure 3.79 Amounts of hexanal recovered (in nL) from samples of linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 40 days (Headspace sampling temperature: 105°C).

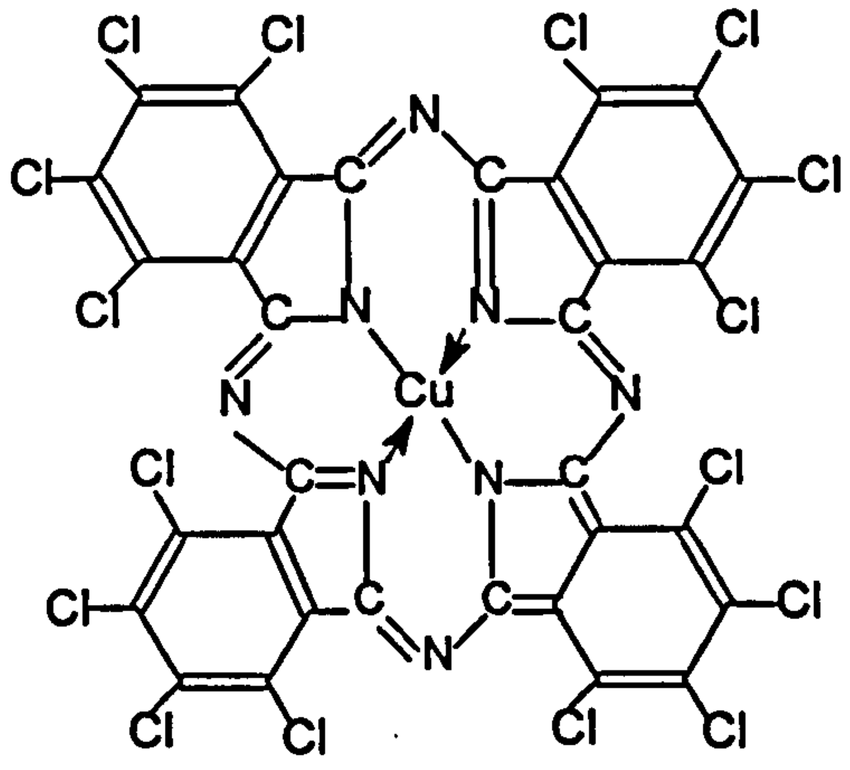


At 40°C and 50°C the amount of hexanal recovered follows the pattern seen for samples of linoleic acid/CTMP, in the presence of photosensitiser, though amounts recovered from the inked samples were smaller. At 20°C, the amount of hexanal recovered was very small. The reason for this is unclear. The ink film may have operated as a barrier, preventing the diffusion of volatiles through the CTMP substrate. This affects the inked side of the substrate, but not the un-inked side. Thus, diffusion of volatiles through the un-inked areas should have occurred. This did not happen. At higher temperatures, the

diffusion of volatiles through the inked substrate is impeded to a lesser extent. Hence, a higher recovery of volatiles was achieved.

A possible explanation for the small amounts of hexanal recovered at ambient temperature may lie in the interaction of volatiles, or volatile precursors, formed from the decomposition of linoleic acid with components of the ink. Radical precursors may readily react with the diluent and prepolymer components of the ink and be bound into the ink matrix. At higher temperatures, the lifetimes of radical species involved in the formation of volatiles are much shorter and may not be sufficient for migration and combination with radicals within the ink film.

The hexanal levels recovered from samples at 40°C and 50°C suggest that the ink film contains a photosensitiser. This photosensitiser could be the pigment used in ink formulation. The pigment used in the Phthalo Green ink is C.I. Pigment Green 7, which is a fully chlorinated copper phthalocyanine. The structure for this pigment molecule is illustrated below:



The possibility of this pigment molecule acting as a photosensitiser is very remote, since copper phthalocyanines have characteristically very short lifetime excited triplet states. This is due to the unpaired electrons of the paramagnetic copper within the phthalocyanine molecule being available to couple with the promoted electrons of the excited singlet and triplet states, leading to their rapid deactivation. The triplet state lifetime is so short that insufficient time is available for interaction with normal triplet state oxygen. Thus, no significant amounts of singlet oxygen are produced.

The photoinitiator(s) is (are) the obvious choice for singlet oxygen generation. Benzophenone is present in the Phthalo Green ink and its function is to generate radicals for initiation during the curing process.

Benzophenone, and its derivatives, act as photoinitiators by a hydrogen abstraction mechanism, known as a Norrish II mechanism.

Singlet oxygen is not directly involved in the initiation process, though, it may form by the interaction of benzophenone, in the excited triplet state, with normal triplet oxygen:

$$PI \longrightarrow \underset{\text{triplet}}{(PI)} \longrightarrow \underset{\text{singlet}}{(PI^*)}$$

$$\underset{\text{triplet}}{(PI^*)} + \underset{\text{triplet}}{(O_2)} \longrightarrow PI + \underset{\text{singlet}}{(O_2)}$$

Where PI is the photoinitiator, benzophenone in this case.

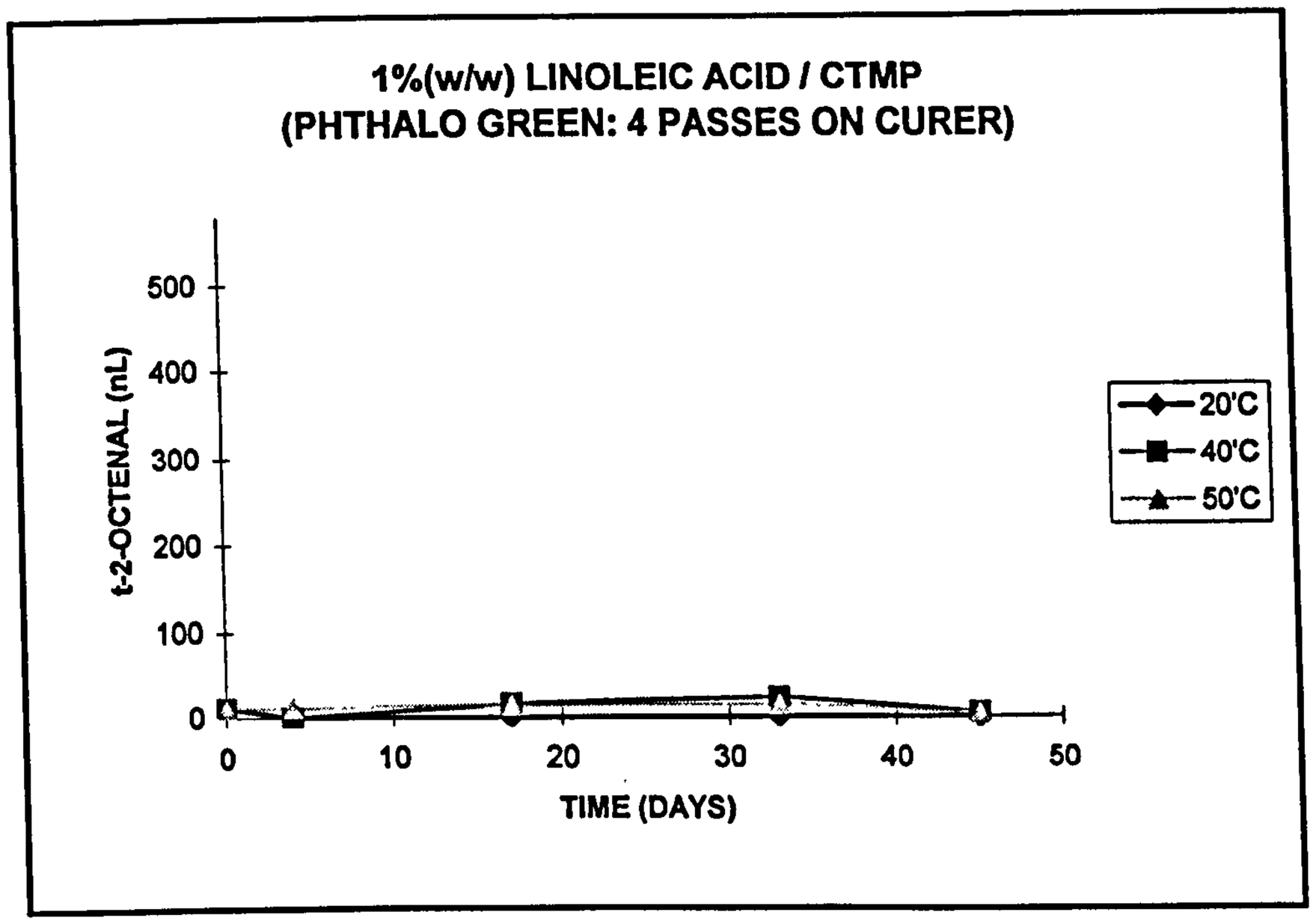
If formed, singlet oxygen is available for photoxidation in the fatty acid system. The magnitude of this effect depends upon the singlet oxygen lifetime and on its migration through the CTMP substrate. The singlet oxygen lifetime is affected by factors such as the humidity. The higher the humidity, the shorter its lifetime.

Figure 3.80 shows the amounts of *trans* 2-octenal recovered from samples of linoleic acid/CTMP print proofed with Phthalo Green ink and irradiated.

The amounts of *trans*-2-octenal recovered are very small. This may be a result of low extent of formation of 2,4-decadienal and/or the binding of this unsaturated aldehyde or other precursors, if formed, into the ink resin matrix, during the curing process.

If the small amounts of *trans* 2-octenal were due to the low formation of 2,4-decadienal, then these findings are in agreement with those of Tressl et al. (1981) who found that smaller amounts of 2,4-decadienal were obtained from photoxidised methyl linoleate compared to autoxidised methyl linoleate. As mentioned earlier, the amounts of *trans* 2-octenal found in photoxidised linoleic acid in the presence of TSZnPh were no less than the amounts recovered from samples of autoxidised linoleic acid in CTMP.

Figure 3.80 Amounts of *trans* 2-octenal recovered (in nL) from samples of linoleic acid/CTMP proofed with Phthalo Green ink, irradiated, and stored at 20°C, 40°C and 50°C for up to 45 days (Headspace sampling temperature: 105°C).

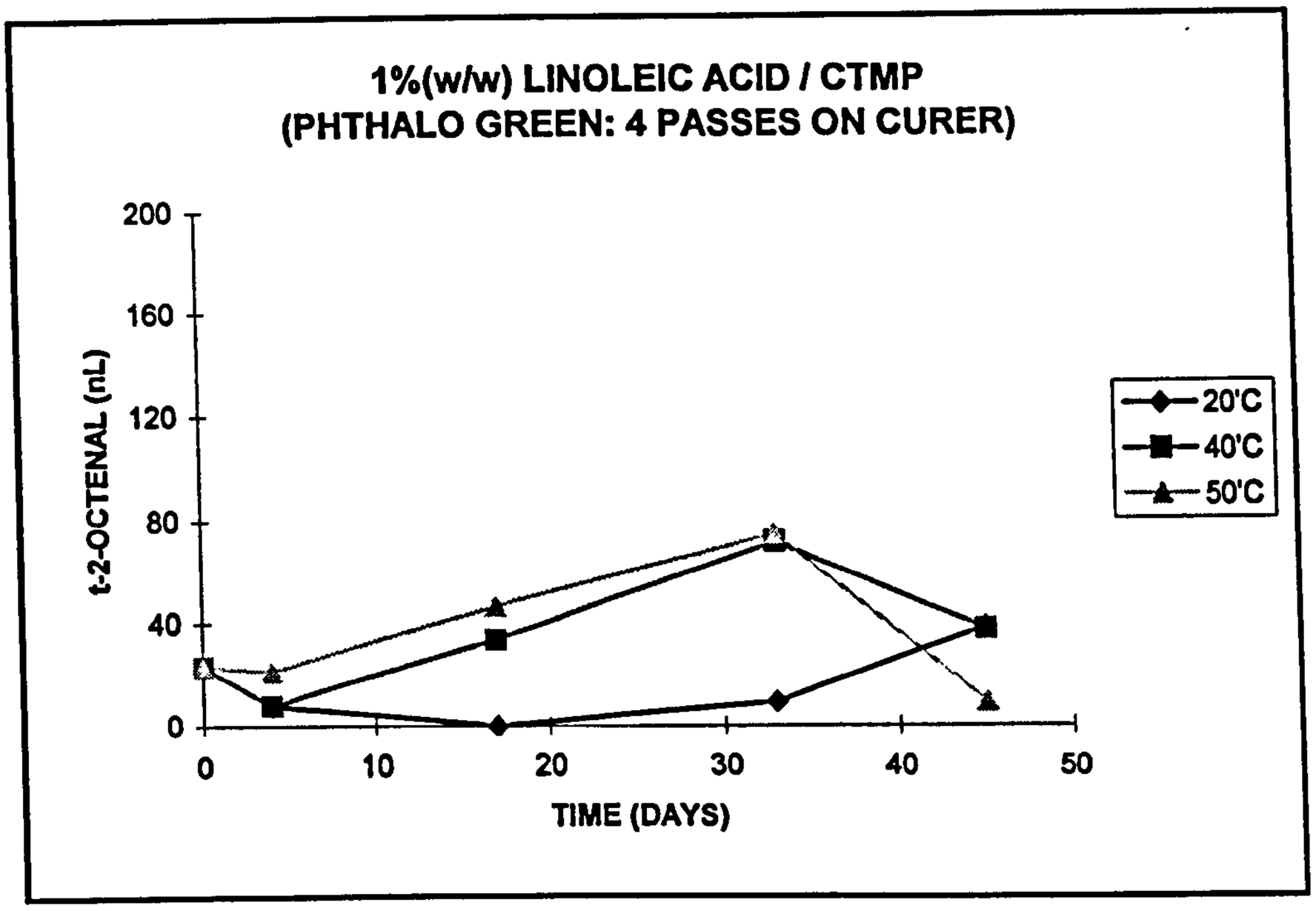


The amounts of pentanal recovered from samples of linoleic acid/CTMP, print proofed with Phthalo Green ink and irradiated, are shown in Figure 3.81.

Amounts of pentanal are much smaller than those recovered from samples of linoleic acid/CTMP that had undergone autoxidation. This may be due, again, to the binding of radical precursors to the ink resin matrix.

As far as the binding of particular species to the ink resin matrix is concerned, the extent of ultraviolet exposure to the ink system is significant. A limited exposure results in an under-curing of the ink. This leads to the existence of a larger number of unreacted sites in the ink matrix, capable of reacting with radicals produced from fatty acid oxidation. An extensive ultraviolet exposure results in a relatively small number of unreacted sites remaining in the ink, capable of reacting with oxidation associated radicals.

Figure 3.81 Amounts of pentanal recovered (in nL) from samples of linoleic acid/CTMP print proofed with Phthalo Green ink, irradiated, and stored at 20°C, 40°C and 50°C for up to 45 days (Headspace sampling temperature: 105°C).



Amounts of 2-heptenal found in samples of linoleic acid/CTMP proof printed with Phthalo Green ink, and irradiated, were slightly less than the corresponding amounts found in un-inked samples. This supports the findings of the test that showed that hydroperoxy epidioxide formation does not increase during photoxidation of linoleic acid/CTMP samples, beyond the formation that occurs during autoxidation.

As mentioned earlier, 2-pentyl furan was present in greater amounts in proofed samples than in unproofed samples. Figure 3.82 shows the amounts of 2-pentyl furan recovered from samples of linoleic acid/CTMP that had not been proofed, or irradiated, and Figure 3.83 shows the amounts of 2-pentyl furan recovered from samples of linoleic acid/CTMP proofed with Phthalo Green ink and irradiated.

At 40°C and 50°C the amounts of 2-pentyl furan recovered from inked samples, after approximately five weeks storage, are significantly greater than those for the non-inked samples. These amounts, however, deteriorate beyond this storage time.

Figure 3.82 Amounts of 2-pentyl furan recovered (in nL) from samples of linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 42 days (Headspace sampling temperature: 105°C).

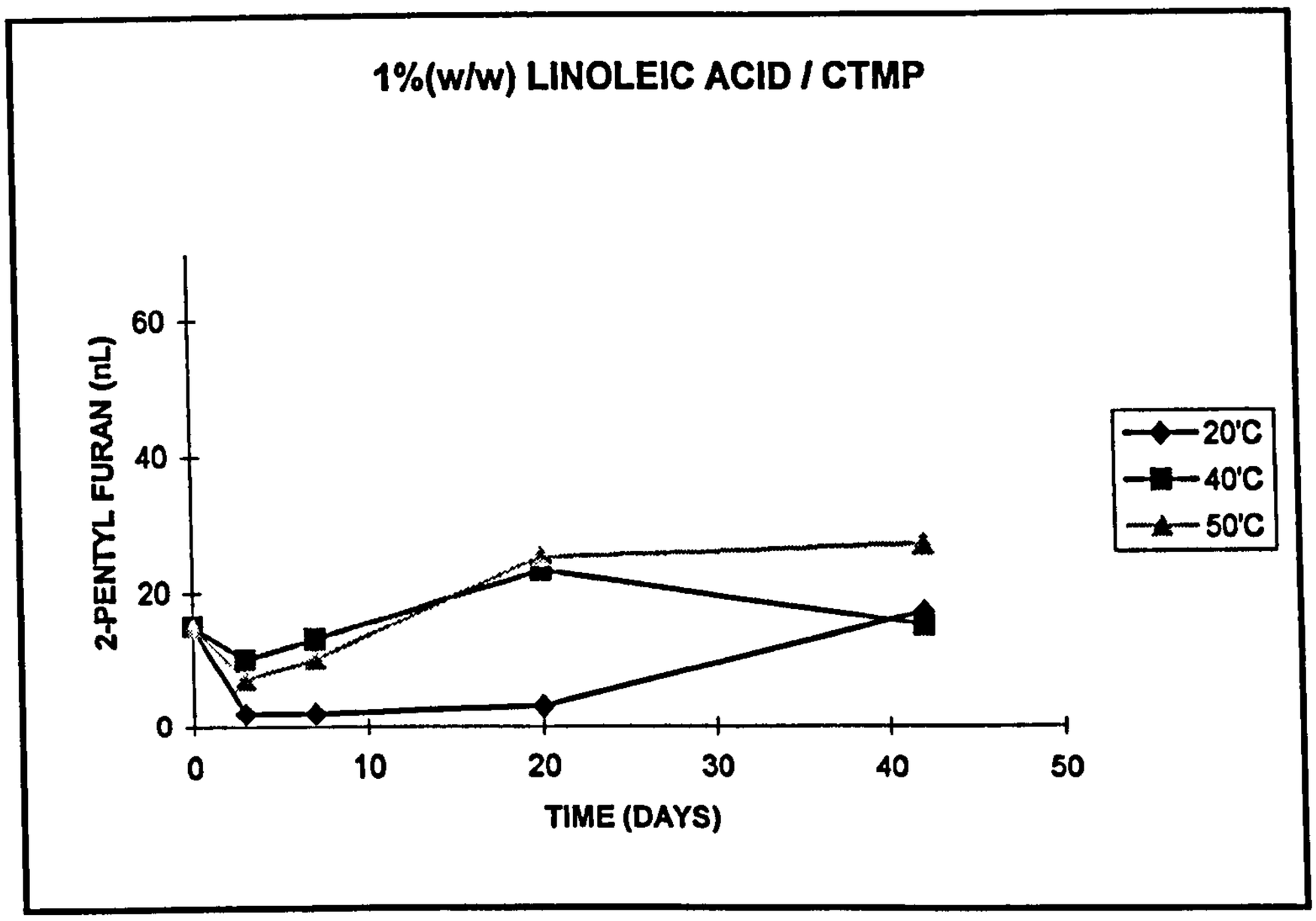
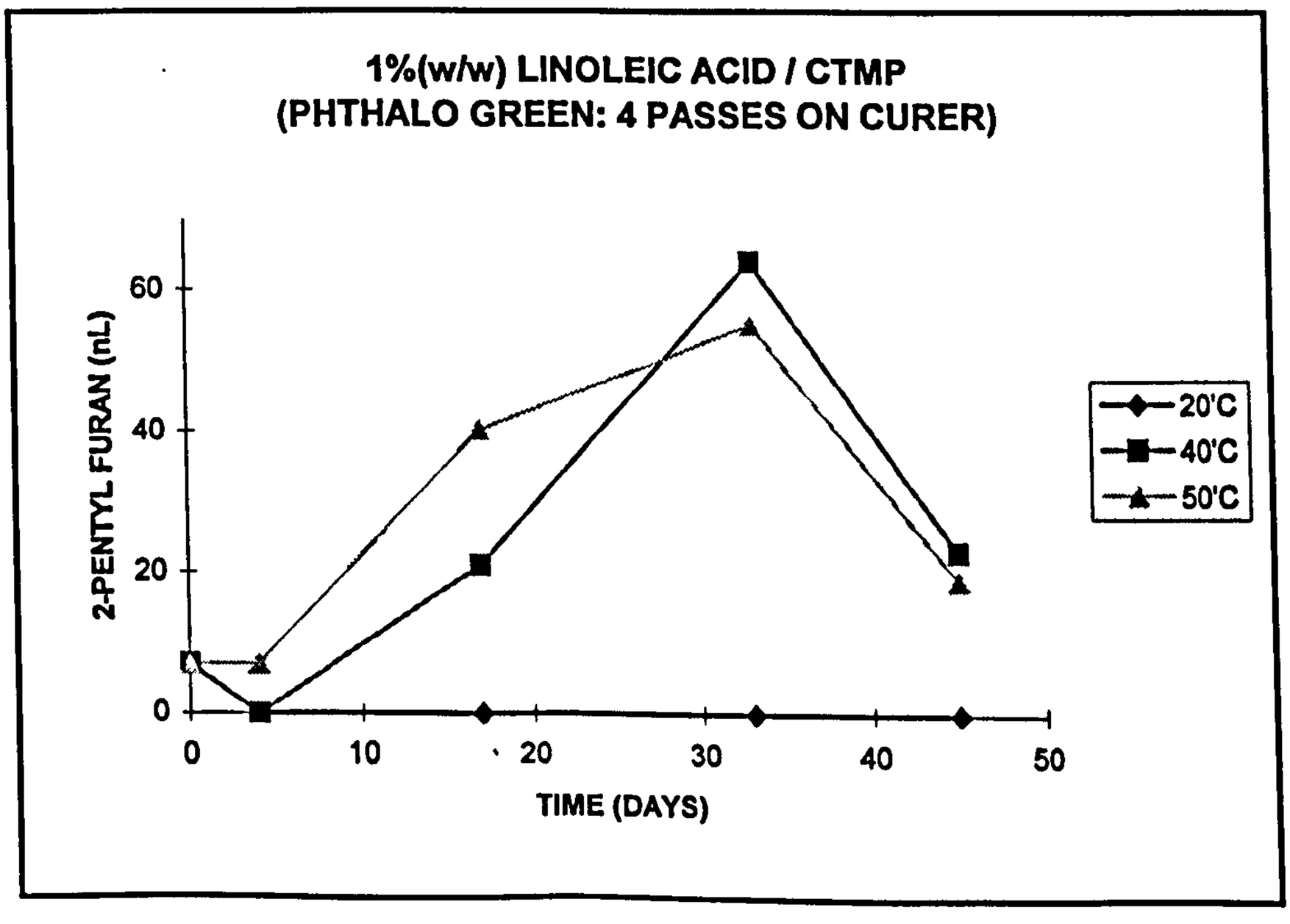


Figure 3.83 Amounts of 2-pentyl furan recovered (in nL) from samples of linoleic acid/CTMP proofed with Phthalo Green ink, irradiated, and stored at 20°C, 40°C and 50°C for up to 45 days (Headspace sampling temperature: 105°C).



The reason for these larger amounts of 2-pentyl furan may lie in the formation route of this compound. As mentioned earlier, this compound may be formed via a cyclic peroxide intermediate of linoleic 9-hydroperoxide and/or via linoleic 10-hydroperoxide. The small amounts of 2-heptenal recovered from inked samples suggested that cyclic peroxide formation was not enhanced during photoxidation of linoleic acid/CTMP. Therefore, the introduction of ink in to the system and its subsequent irradiation may have brought about an increase in the formation of linoleic acid 10-hydroperoxide.

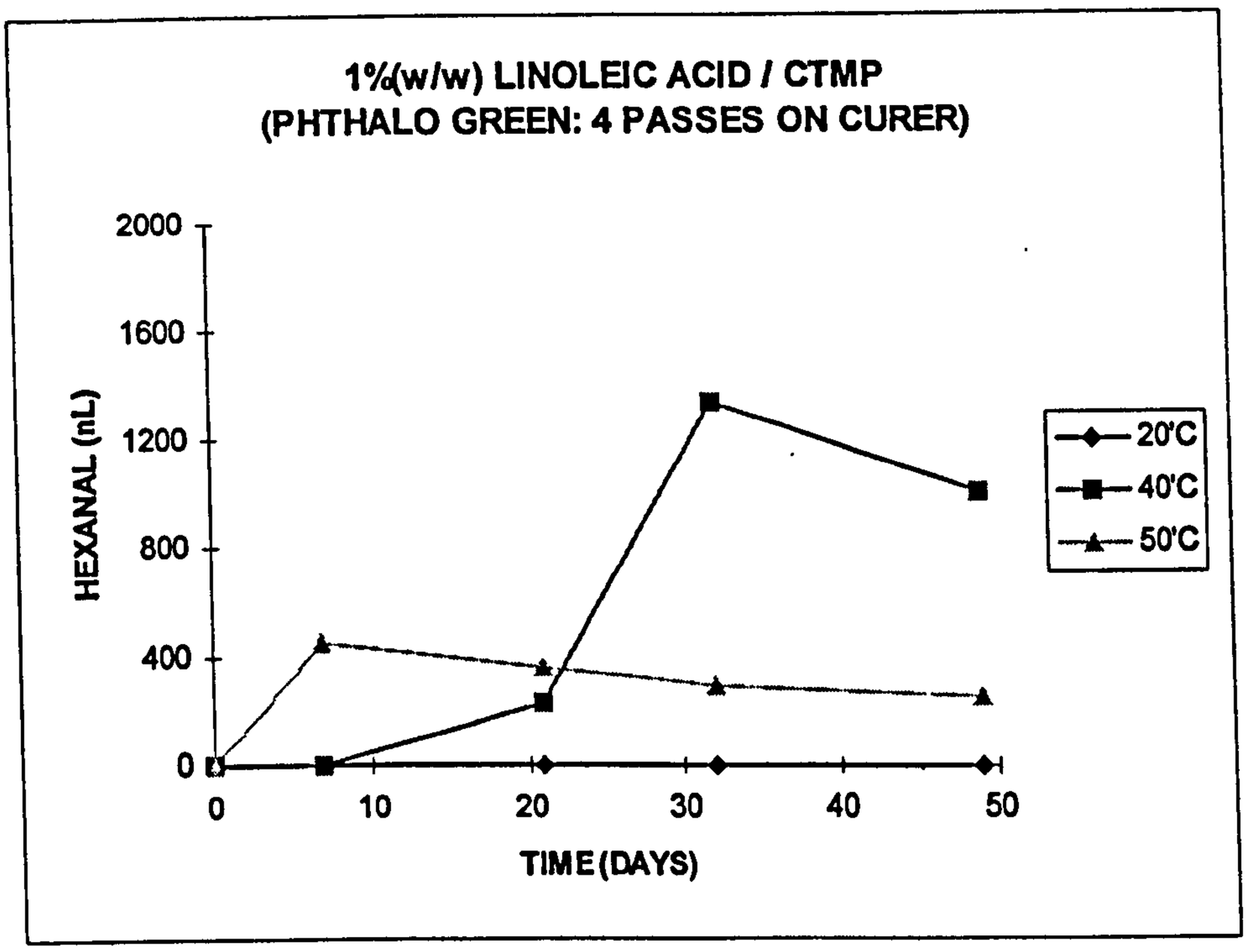
At ambient storage temperatures, the amounts of 2-pentyl furan recovered were negligible for the inked samples. For the non-inked samples, the amount increased with storage time. These smaller amounts recovered from inked samples may be due, firstly from combination of intermediates with the ink components, or secondly, to the lesser formation of linoleic acid 10-hydroperoxide at ambient temperature.

To determine the effect of a lower headspace equilibration temperature on the amount of volatiles recovered from samples of linoleic acid/CTMP print proofed with the Phthalo Green ink and irradiated, the test was repeated using a headspace equilibration temperature of 50°C. Figure 3.84 shows the amounts of hexanal recovered during this test.

The amounts of hexanal recovered from samples stored at ambient temperature are very small and are comparable to those for the corresponding samples, headspaced at 105°C. If a high concentration of hydroperoxides was formed as a result of photoxidation during irradiation, the low concentration of radicals resulting from their decomposition at ambient temperature may have easily been quenched by interaction with the active sites within the ink matrix.

At 40°C, the amounts of hexanal produced are significantly greater than those for the corresponding samples equilibrated at 105°C. This could suggest that hexanal is broken down at the higher equilibration temperature or undergoes a higher degree of reaction into the ink matrix. The levels of hexanal are still somewhat less than those for samples of linoleic acid/CTMP, equilibrated at 50°C, in the absence of the ink. This suggests that hydroperoxide decomposition occurred at a lower level in the inked system and that termination reactions were more significant.

Figure 3.84 Amounts of hexanal recovered (in nL) from samples of linoleic acid/ CTMP proofed with Phthalo Green ink, irradiated, and stored at 20°C, 40°C and 50°C for up to 49 days (Headspace sampling temperature: 50°C).



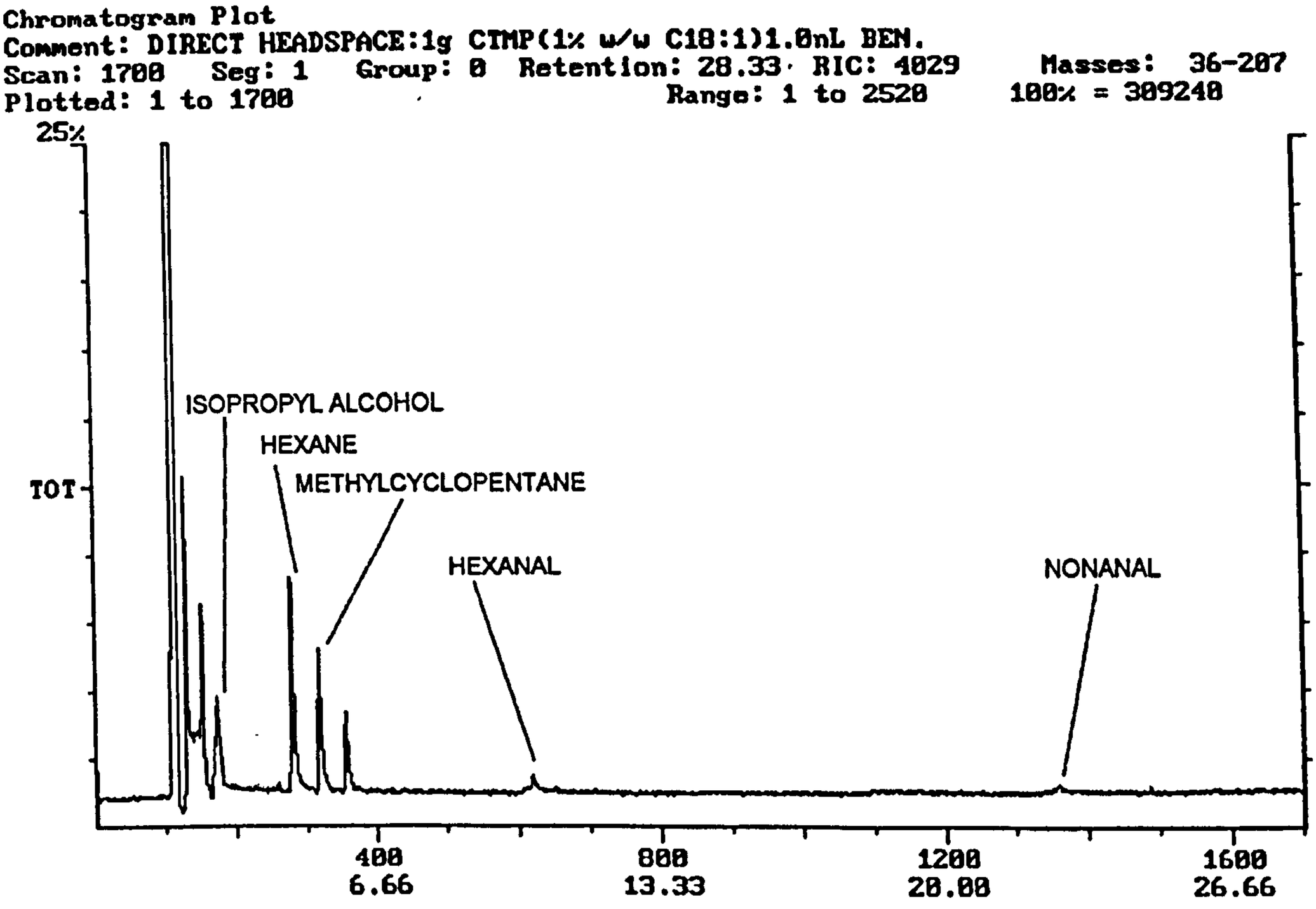
At 50°C, the amounts of hexanal reach a maximum amount then slowly decline. These amounts are fairly small, indicating that termination reactions and hexanal break down are likely to be of importance.

Determination of the effect of time and temperature on the presence of oleic acid in CTMP

To this stage of the investigation the experiments have involved the oxidation of linoleic acid, or its methyl ester derivative, principally in CTMP. Another unsaturated fatty acid that is present in significant amounts in wood-pulp of softwoods is oleic acid, or *cis* 9-octadecenoic acid. This free fatty acid is derived from the triglyceride, triolein. According to work reported by Gunstone (1984), oleic acid autoxidises at a rate that is 27 times slower than for linoleic acid. Thus, the presence of oleic acid in a carton-packaging system and its subsequent oxidation, has implications after longer periods of time than apply in the case of linoleic acid.

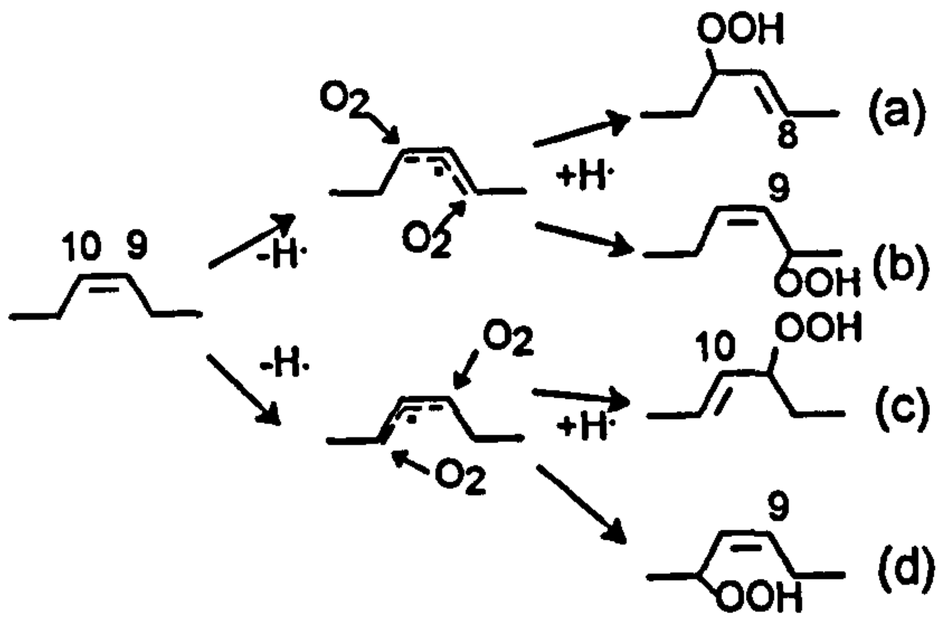
Figure 3.85 shows the chromatogram of the headspace volatiles collected from a sample of oleic acid/CTMP that had undergone thermal degradation at 105°C for 60 minutes. In contrast to linoleic acid/CTMP samples, hexane, methylcyclopentane and very small amounts of hexanal and nonanal were recovered. This implies that the autoxidation process was still in the induction stage and hydroperoxide concentrations were still low.

Figure 3.85 Chromatogram acquired from the headspace vapours of a one gram sample of CTMP/1%(w/w) oleic acid that had undergone thermal degradation, at 105°C, for 60 minutes.



Autoxidation of oleic acid proceeds via hydroperoxide intermediates as with linoleic acid. However, the proportions of the hydroperoxide isomers formed are different for oleic acid. In this case, hydrogen abstraction occurs on C-8 and C-11 producing two allylic radicals. These intermediates react with oxygen at the end carbons to produce a mixture of 8-,9-,10- and 11-allylic hydroperoxides. Figure 3.86 shows the reaction scheme for the formation of these four isomeric hydroperoxides.

Figure 3.86 Reaction scheme for the formation of four isomeric hydroperoxides from oleic acid autoxidation: (a) 10-OOH; (b) 8-OOH; (c) 9-OOH; (d) 11-OOH.



Studies by Frankel et al. (1977) and by Chan and Levett (1977) have shown that the mechanism of methyl oleate autoxidation is more complicated than that presented above. The amounts of 8- and 11-hydroperoxides are slightly greater than those of 9- and 10-isomers. The proportions of monohydroperoxides formed by autoxidation of oleic acid are shown below.

Position of		Proportion (%)
-OOH group	Double bond	Autoxidation
8	9	27
9	10	23
10	8	23
11	9	27

Source: Belitz and Grosch (1987) and Gunstone (1984).

The above data suggest that there is a somewhat greater reactivity of C-8 and C-11 with $^3O_2$. In reality, free-radical autoxidation of oleic acid produces a mixture of all eight isomers of 8-, 9-, 10- and 11-hydroperoxides. At 25°C, the amounts of *cis* and *trans* 8- and 11-isomers are similar, but the 9- and 10-isomers are mainly *trans* in configuration.

Hydroperoxide decomposition occurs by the same mechanism as with linoleic acid, involving the homolytic cleavage of the -OOH group, giving rise to an

alkoxy radical and a hydroxyl radical. This alkoxy radical then undergoes ß-scission of the C-C bond, with the formation of an aldehyde group and an alkyl (or vinyl) radical.

Figure 3.87 shows the mechanism and products resulting from the decomposition of methyl oleate 8-hydroperoxide.

Figure 3.87 The mechanism for the decomposition of methyl oleate 8-hydroperoxide.

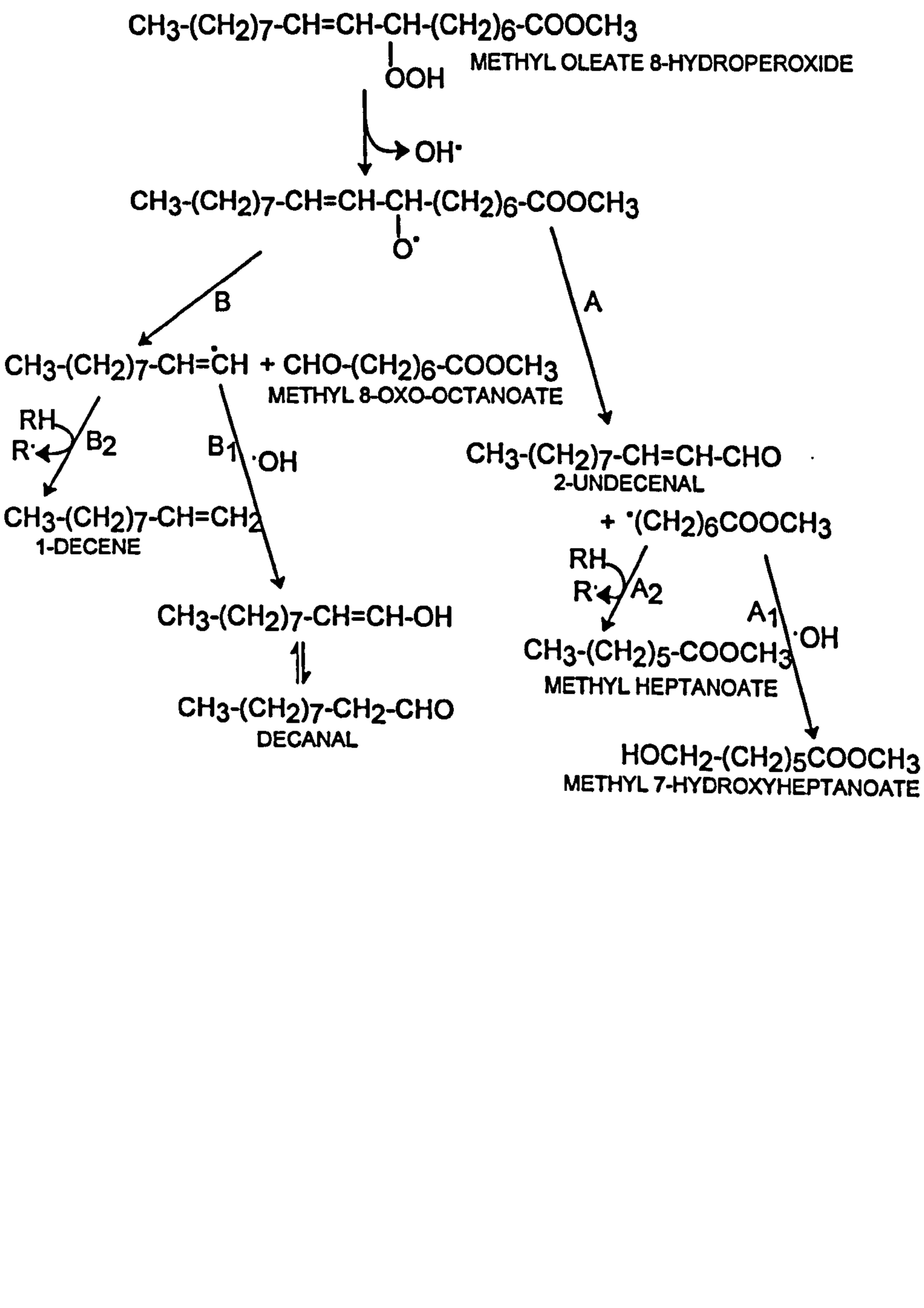


Figure 3.88 shows the mechanism and products resulting from the decomposition of methyl oleate 9-hydroperoxide.

Figure 3.88 The mechanism for the decomposition of methyl oleate 9-hydroperoxide.

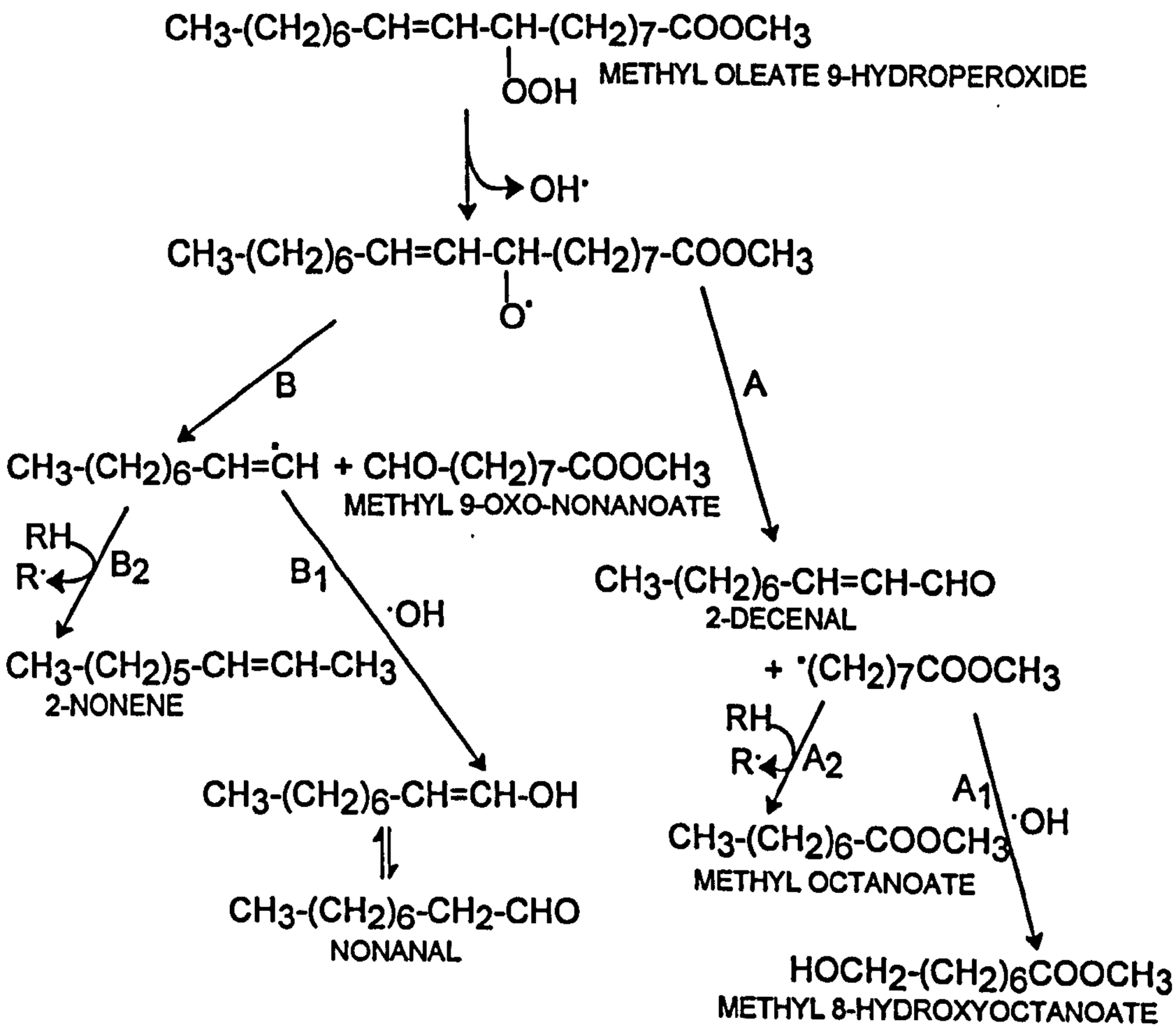


Figure 3.89 shows the mechanism and products resulting from the decomposition of methyl oleate 10-hydroperoxide.

Figure 3.89 The mechanism for the decomposition of methyl oleate 10-hydroperoxide.

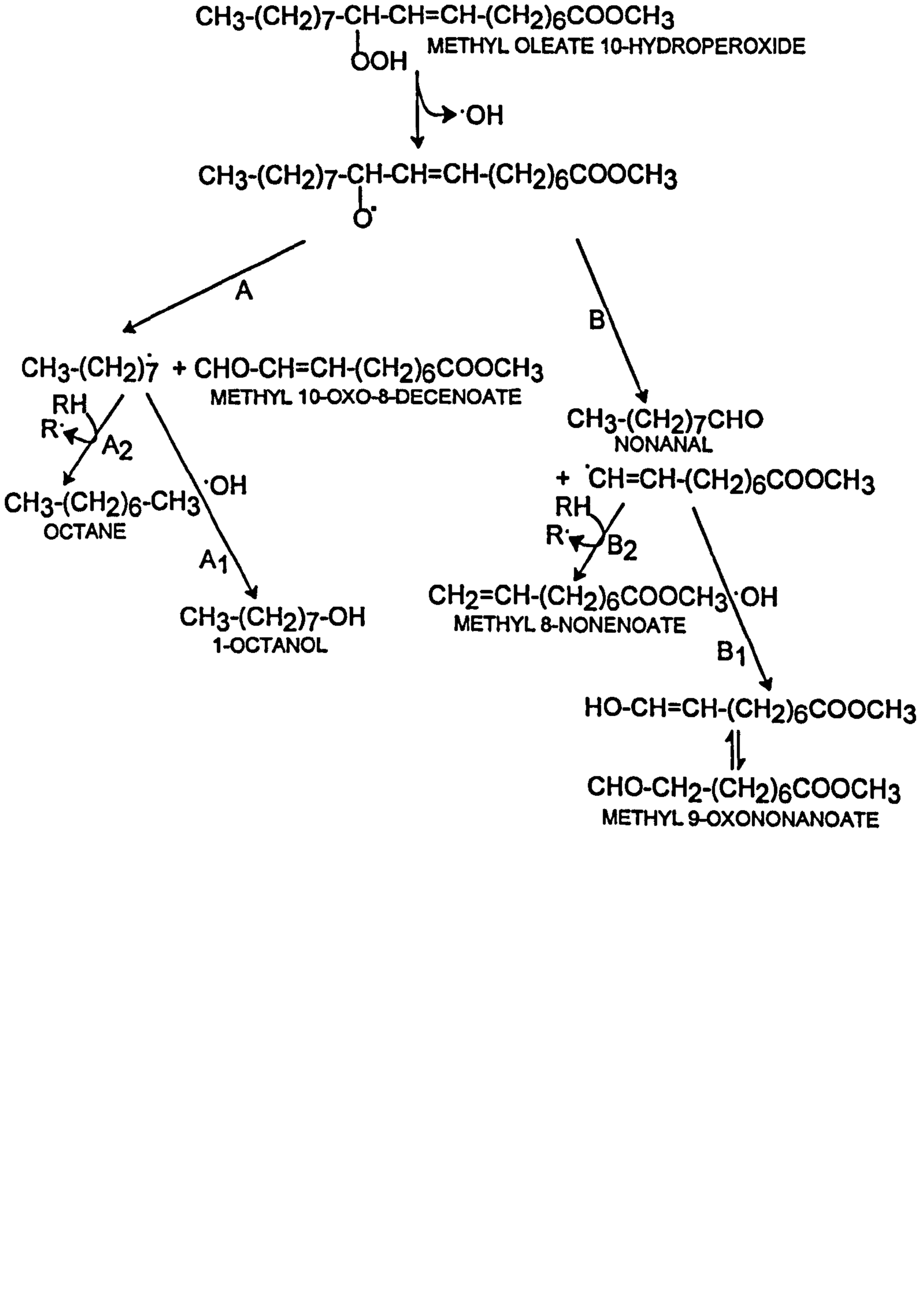
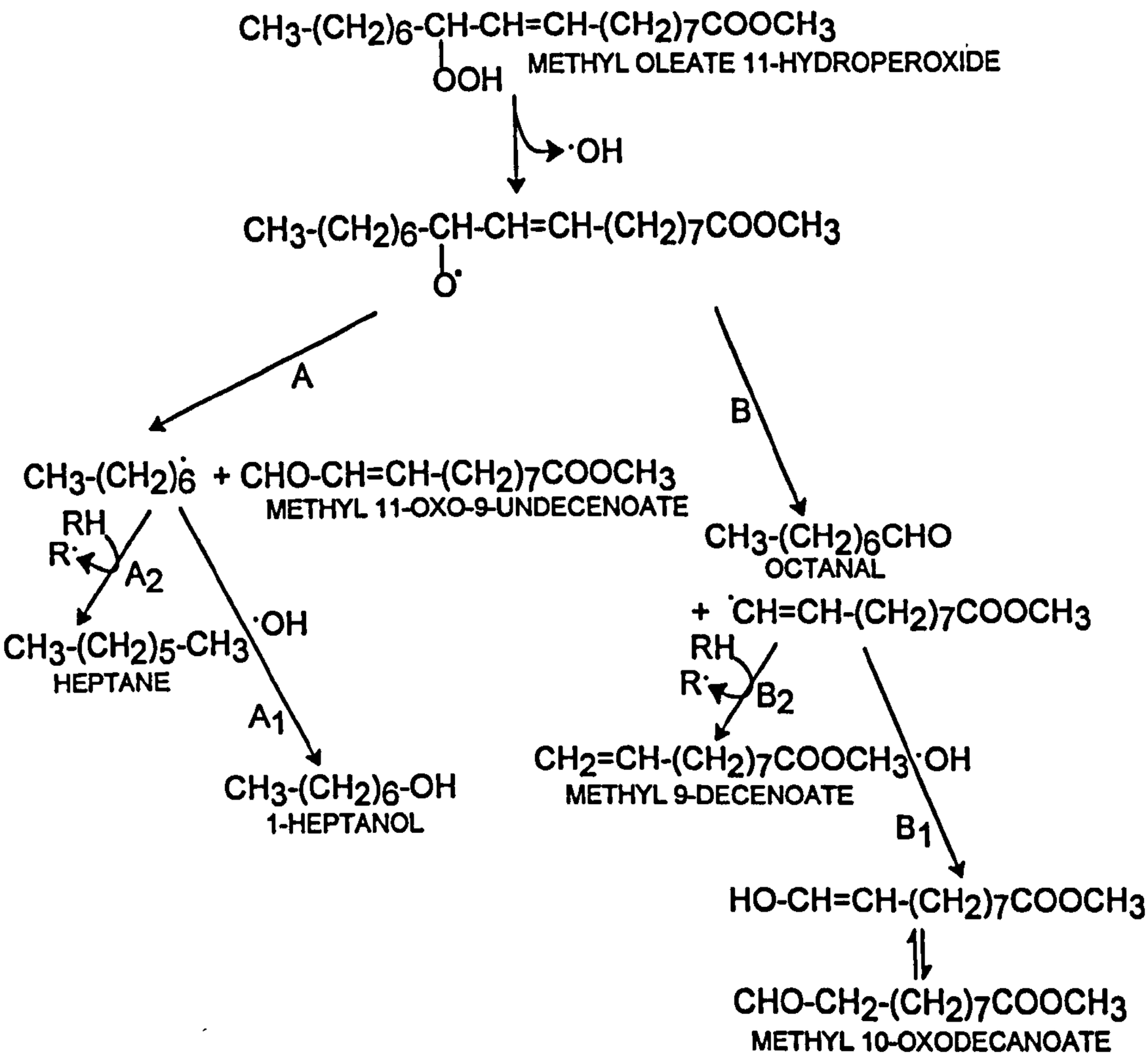


Figure 3.90 shows the mechanism and products resulting from the decomposition of methyl oleate 11-hydroperoxide.

Figure 3.90 The mechanism for the decomposition of methyl oleate 11-hydroperoxide.



It is accepted that nonanal is the major aldehyde produced from the autoxidation of oleic acid at ambient temperatures (Ellis et al., 1968). Badings (1970) found that octanal was produced in greater amounts with more extensive degrees of oxidation. It has reported that methyl oleate autoxidation is accompanied by the formation of water and carbon dioxide (Grosch, 1987).

Heptanal, decanal, undecanal, *trans*-2-decenal, and *trans* 2-undecenal have also been found as major compounds resulting from the autoxidation of methyl oleate at ambient temperature (Ellis et al., 1968; Grosch, 1987).

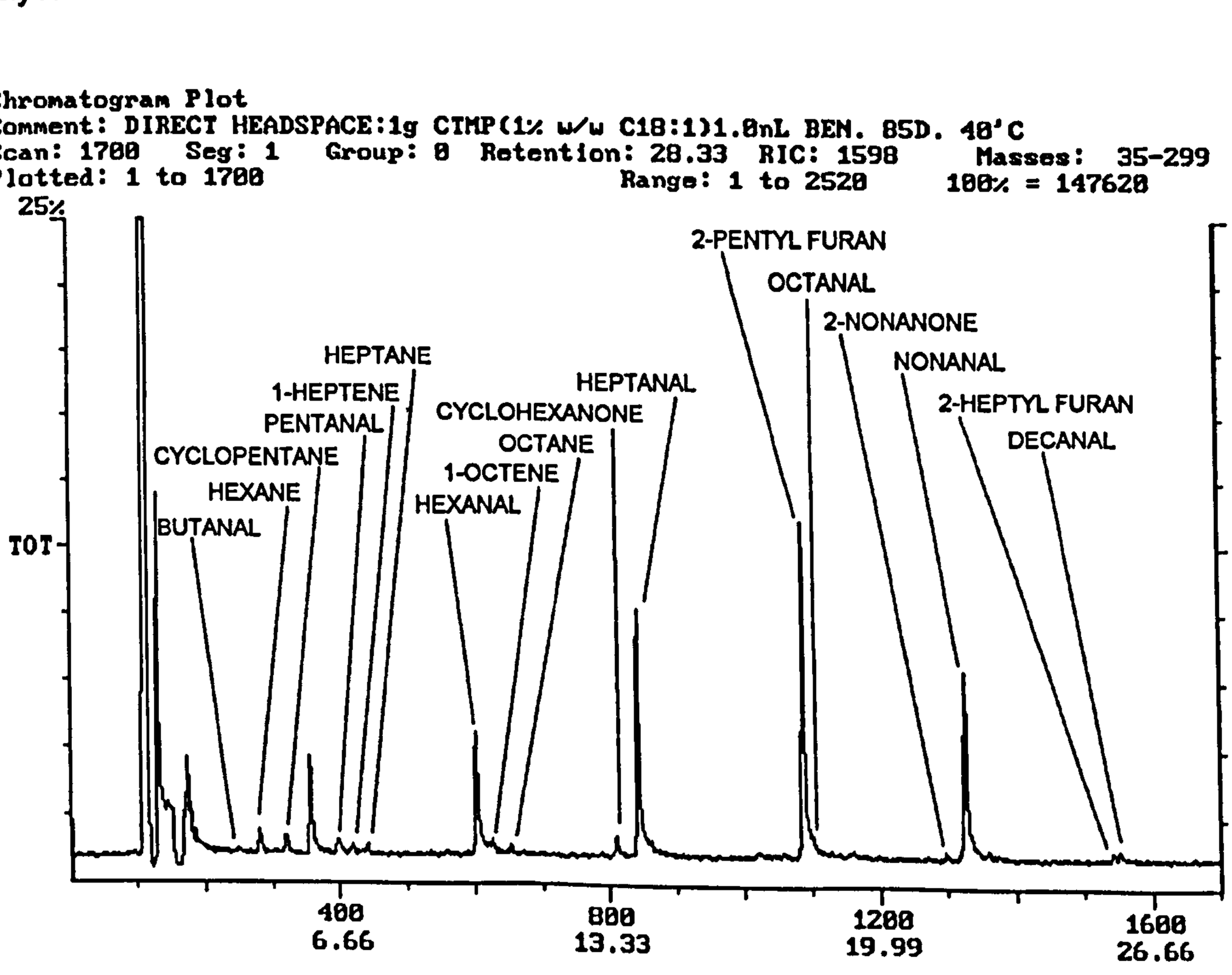
At elevated temperatures, 82% of the total volatile compounds resulting from the autoxidation of methyl oleate, at 192°C for 10 minutes, were heptane,

octane, heptanal, octanal, nonanal, 2-decenal, and 2-undecenal (Grosch, 1987). Minor products resulting from the autoxidation of methyl oleate, at elevated temperatures, include alkanes (C3-6), alkenes (C9, 10, 17), alkanals (C2-6, 10), 2-alkenals (3, 7-11), methyl ketones (C6-8), 1-alkanols (C5-9), aliphatic acids (C4-10), and gamma lactones (C5-9).

Methyl esters of C8 and C9 oxo fatty acids have also been found. Their formation can also be explained in terms of the ß-scission decomposition reactions illustrated above.

Figure 3.91 shows the chromatogram of the headspace vapours, collected at 105°C for 60 minutes, from a sample of oleic acid/CTMP that had been stored for 85 days at 40°C. The major volatiles collected were hexanal, heptanal, octanal, and nonanal. Lesser amounts of butanal, hexane, cyclopentane, pentanal, 1-heptene, heptane, 1-octene, octane, cyclohexanone, 2-pentyl furan, 2-nonanone, heptyl furan, and decanal were also found.

Figure 3.91 Chromatogram acquired from the headspace vapours of a one gram sample of CTMP/1%(w/w) oleic acid that had been stored, at 40°C, for 85 days.



The most obvious observation to be made about this chromatogram, compared to those for samples of linoleic acid, is the significantly smaller amounts of volatiles recovered. This is essentially due to the lower reactivity of oleic acid compared to linoleic acid.

The lack of 2-alkenals and oxo fatty acids indicates that these compounds, if formed, underwent oxidative decomposition in the vial during equilibration.

Octanal formation and nonanal formation can be explained by the decomposition of oleic acid 11-hydroperoxide and oleic acid 9- and 10-hydroperoxide, respectively (see Figures 3.87, 3.89, and 3.90).

Heptanal formation may be explained by the addition of oxygen to the heptyl radical, produced during the decomposition of oleic acid 11-hydroperoxide. It is likely that hexanal is produced primarily from the breakdown of the 2-alkenals and other unsaturated species present. Unlike observations made for linoleic acid, hexanal is not a primary product of autoxidation.

Grosch (1987) thinks it is unlikely that the reaction combination B and B2 takes place since the products; 1-decene, 2-nonene, methyl 8-nonenoate and methyl 9-decenoate were not found. In addition, none of the major products from the decomposition of oleic acid 8-hydroperoxide were found. However, small amounts of decanal, a major product of the decomposition of oleic acid 8-hydroperoxide, were found in the headspace vapours of samples of oleic acid/CTMP.

Figure 3.92 shows the amounts of nonanal recovered from samples of oleic acid/CTMP. The amounts of nonanal recovered are extremely low. At ambient temperatures, negligible amounts were found, even after 85 days storage. This is somewhat unexpected since nonanal is one of the major volatiles associated with the autoxidation of oleic acid.

Figure 3.93 shows the amounts of hexanal recovered from samples of oleic acid/CTMP. Hexanal formation is due primarily to the breakdown of unsaturated oxidation products. Therefore, hexanal accumulation can be indicative of the existence of a wider range of compounds, than in the case of nonanal and can give a better representation of the overall oxidation of the system.

Figure 3.92 Amounts of nonanal recovered (in nL) from samples of oleic acid/ CTMP stored at 20°C, 40°C and 50°C for up to 85 days (Headspace sampling temperature: 105°C).

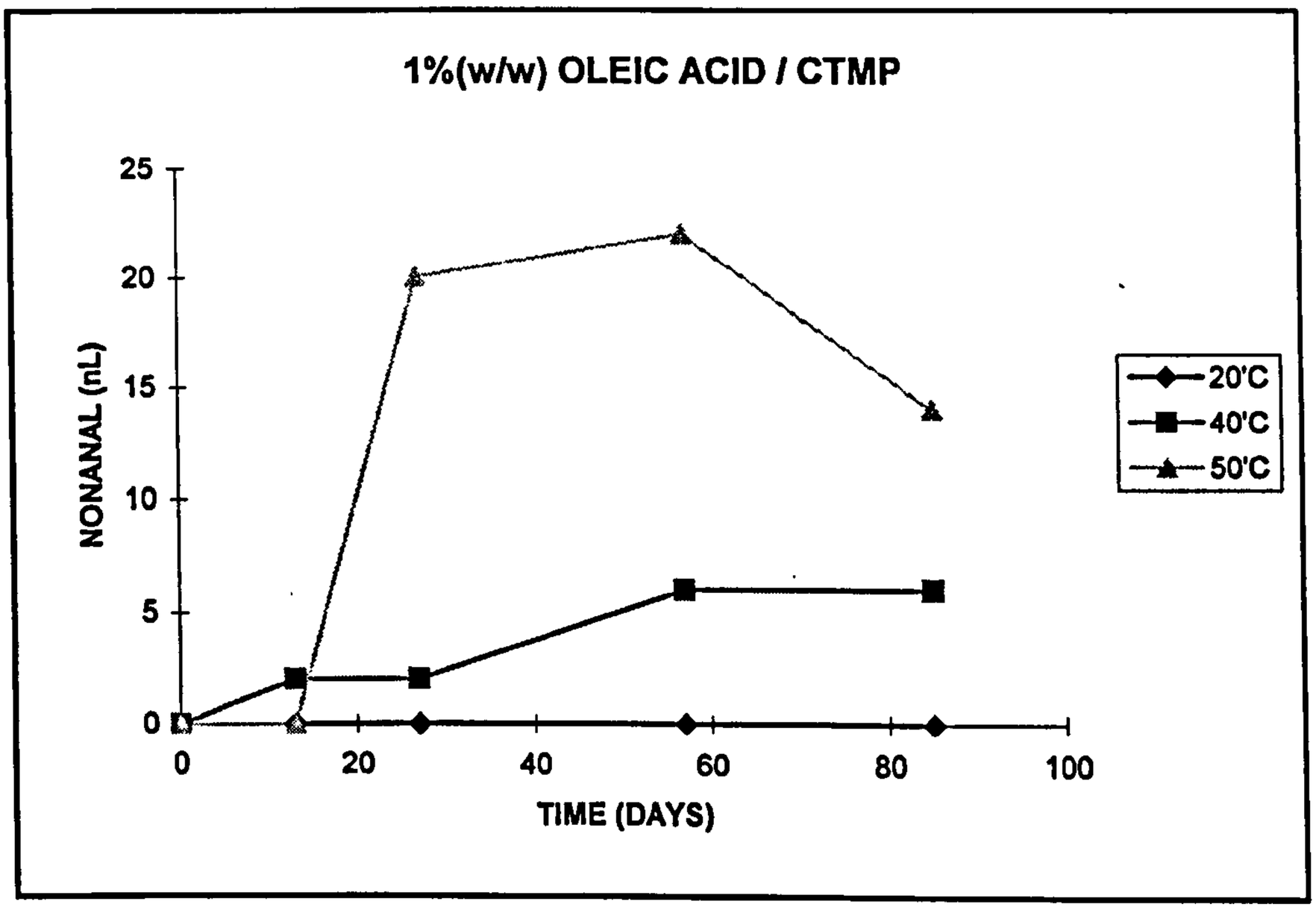
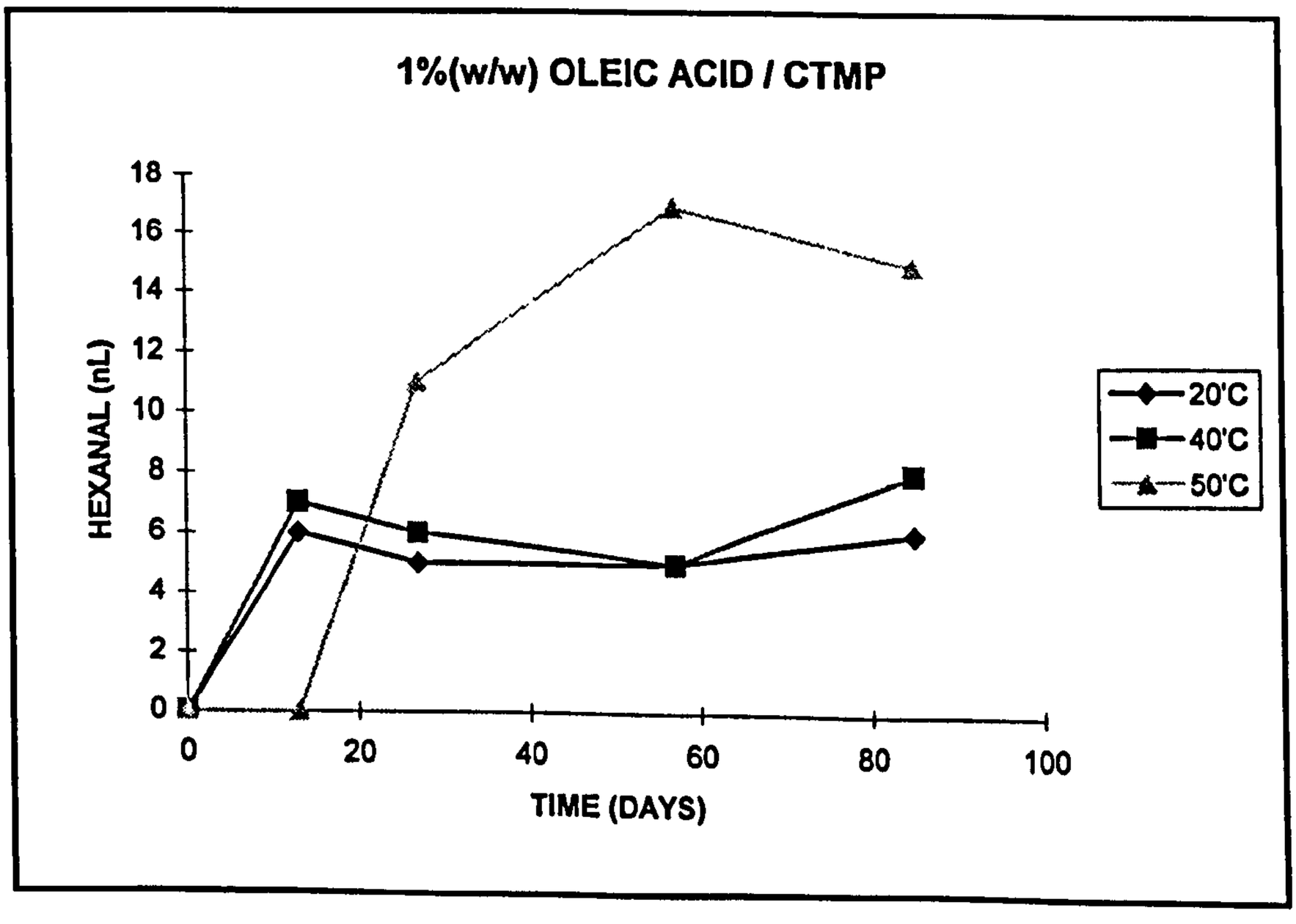


Figure 3.93 Amounts of hexanal recovered (in nL) from samples of oleic acid/ CTMP stored at 20°C, 40°C and 50°C for up to 85 days (Headspace sampling temperature: 105°C).

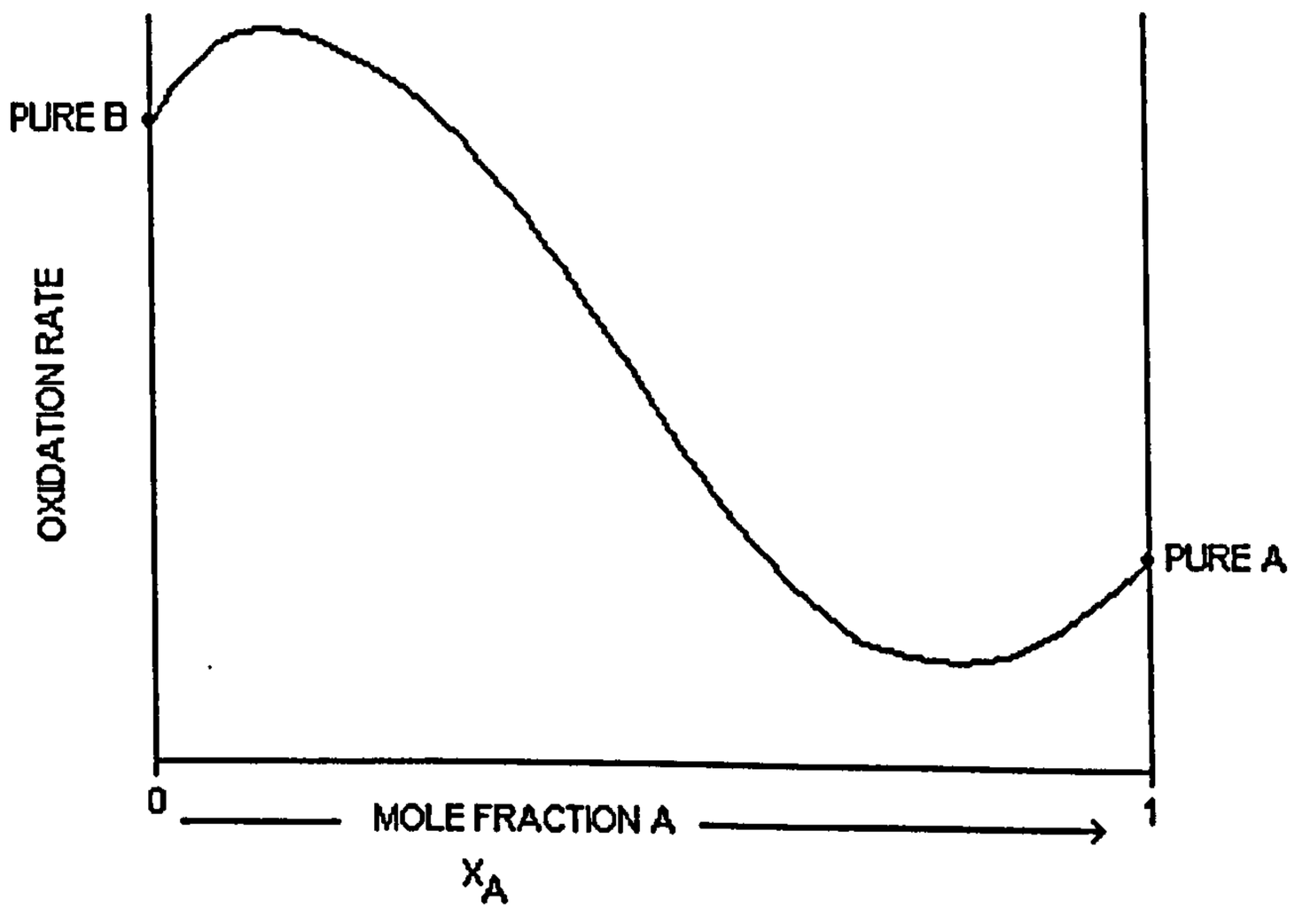


The amounts of hexanal recovered were very low, almost insignificant. The levels from samples stored at ambient temperature were slightly greater than those for nonanal, indicating that some oxidation of unsaturated species had taken place.

Determination of the effect of time and temperature on the presence of a combination of oleic and linoleic acid in CTMP

Labuza (1971) showed that on mixing a rapidly oxidising lipid with a slowly oxidising lipid, the expected dilution effect did not occur. In fact, both pro-oxidant and anti-oxidant phenomena were observed as shown in Figure 3.94.

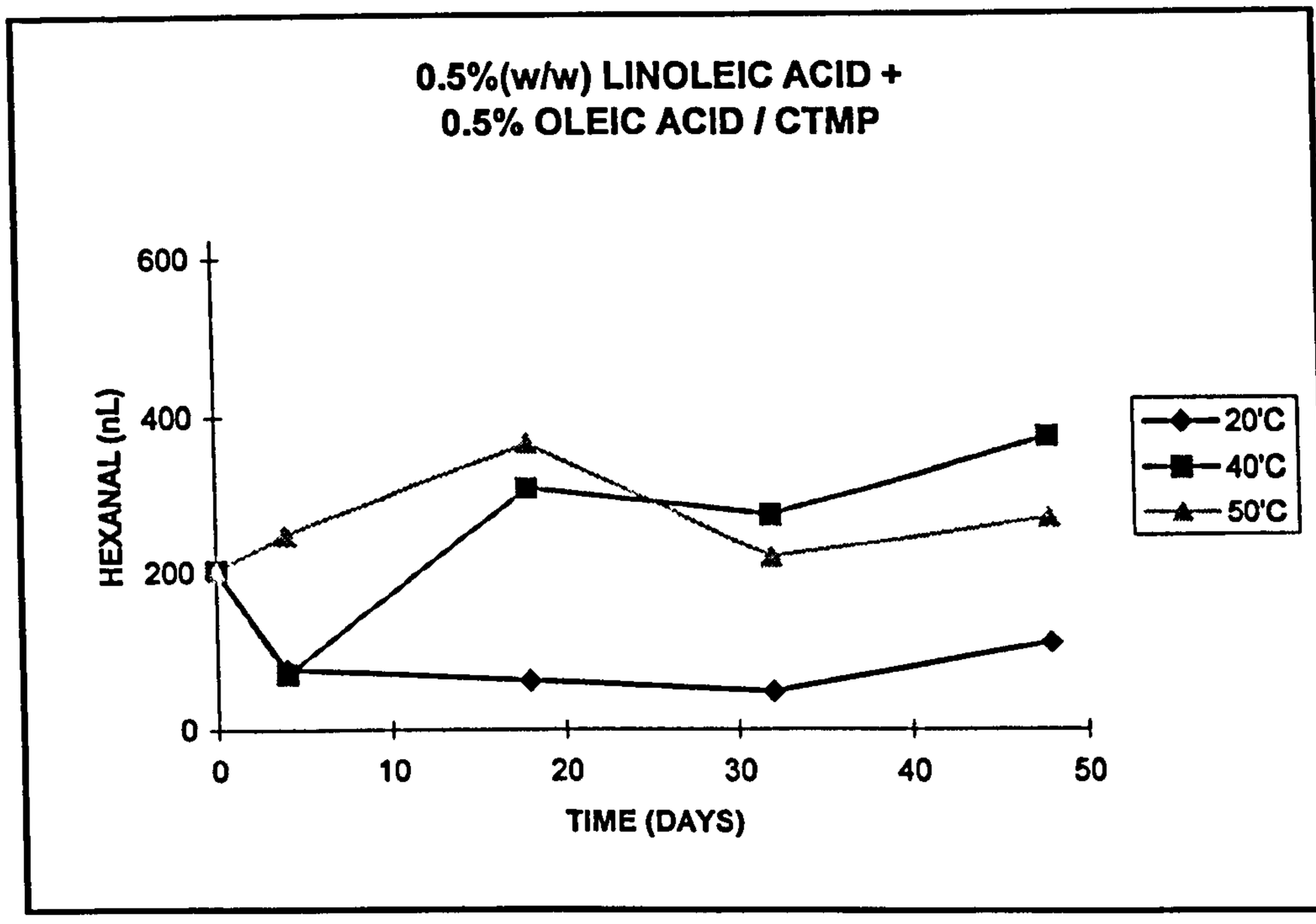
Figure 3.94 The effect of the addition of a competitive oxidation species on overall rate of oxidation.



From the above figure, it can be seen that the addition of a small amount of a highly reactive lipid to a less reactive lipid slows the oxidation rate and the addition of a small amount of less reactive lipid to a highly reactive lipid increases the oxidation rate. These findings have been verified for various lipids and attributed to the reactivity of the different peroxy radicals in the termination steps (Labuza, 1971).

Figure 3.95 shows the amounts of hexanal recovered from a sample of 0.5%(w/w) linoleic acid and 0.5%(w/w) oleic acid/CTMP that had undergone thermal degradation at 105°C for 60 minutes.

Figure 3.95 Amounts of hexanal recovered (in nL) from samples of 0.5%(w/w) linoleic acid + 0.5%(w/w) oleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 48 days (Headspace sampling temperature: 105°C).



To achieve a clearer comparison of the above data with those of pure linoleic acid (1%w/w)/CTMP, the quantitation values were multiplied by a factor of two. Figure 3.96 shows the graph for these values and Figure 3.97 shows the amounts of hexanal recovered from samples of 1%(w/w) linoleic acid/CTMP.

Comparing Figure 3.96 and Figure 3.97, it can be seen that at a storage temperature of 40°C the amount of hexanal recovered is greater for the mixture as time increases. At ambient temperatures, the amount of hexanal recovered is greater for the pure acid and increases significantly with time. At 50°C, there is no significant difference between the two systems.

The data suggests that no distinct competitive oxidation effect was apparent. However, differences at 20°C and 40°C could indicate some temperature dependence attached to this phenomenon.

Figure 3.96 Amounts of hexanal recovered (in nL), (multiplied by a factor of 2), from samples of 0.5%(w/w) linoleic acid + 0.5%(w/w) oleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 48 days (Headspace sampling temperature: 105°C).

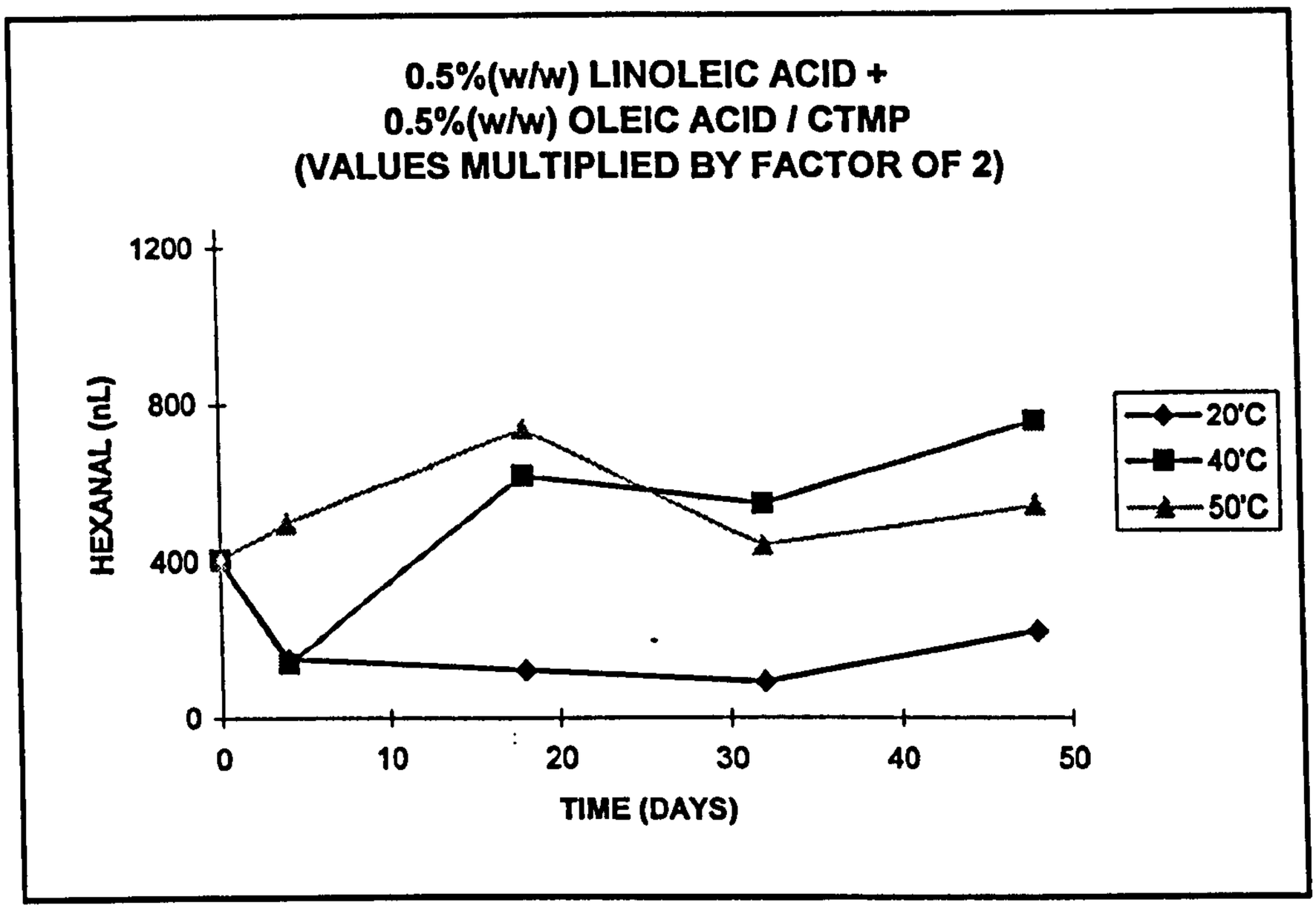
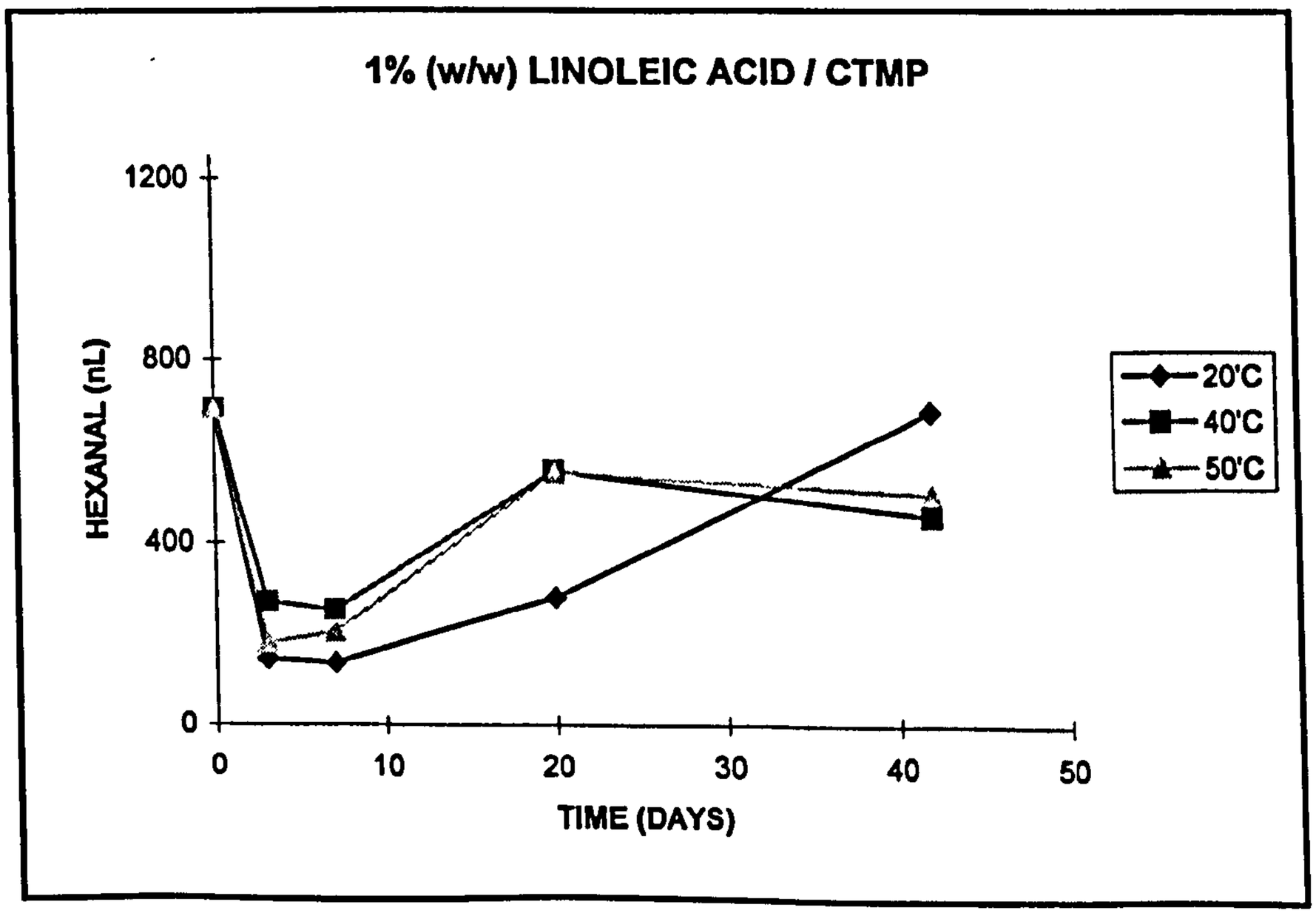


Figure 3.97 Amounts of hexanal recovered (in nL) from samples of (1%w/w) linoleic acid/CTMP stored at 20°C, 40°C and 50°C for up to 42 days (Headspace sampling temperature: 105°C).



Oxidation of unsaturated fatty acids within a carton-board matrix

Determination of the effect of time and temperature on the presence of linoleic acid in Koppargloss carton-board

The major volatiles produced from the oxidation of linoleic acid within the Koppargloss matrix were similar to those for linoleic acid within the CTMP matrix. In addition to these compounds, a number of other volatile compounds was found. These originated from components within the board material. Figure 3.98 shows the chromatogram acquired from the headspace vapours of a sample of linoleic acid/Koppargloss stored for 55 days at 20°C, using a headspace equilibration temperature of 105°C. In addition to the major oxidation volatile such as pentane, pentanal, 1-pentanol, hexanal, heptanal, *trans* 2-heptenal, 2-pentyl furan, and *trans* 2-octenal, the presence of dodecene and some branched alkenes, such as ethylmethyl heptene, were detected.

Figure 3.98 Chromatogram acquired from the headspace vapours of a one gram sample of Koppargloss/1%(w/w) linoleic acid that had been stored, at 20°C, for 55 days.

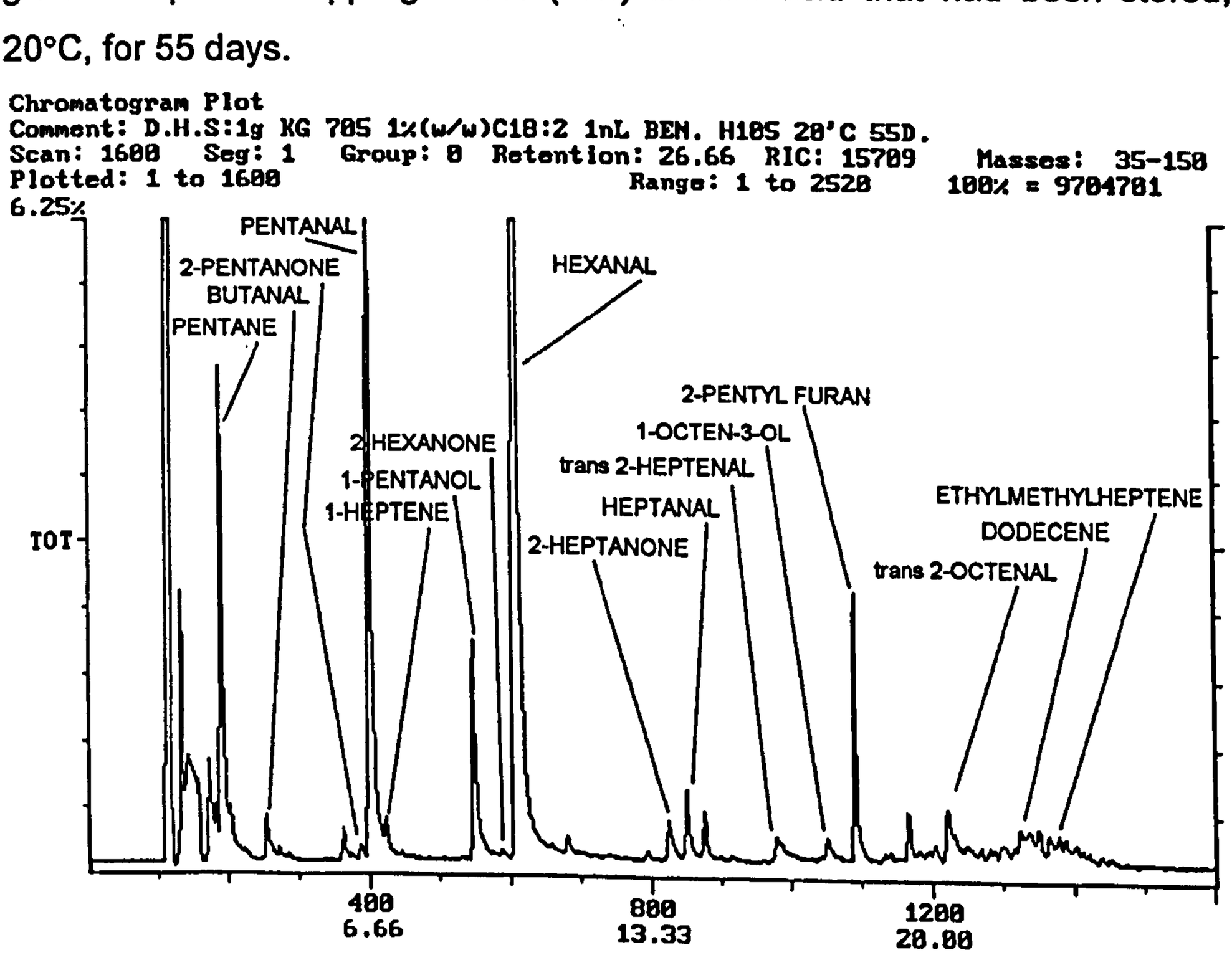


Table 3.4 shows the amounts of volatiles recovered from the sample of linoleic acid/Koppargloss stored for 55 days at 20°C, represented in the chromatogram shown in Figure 3.98.

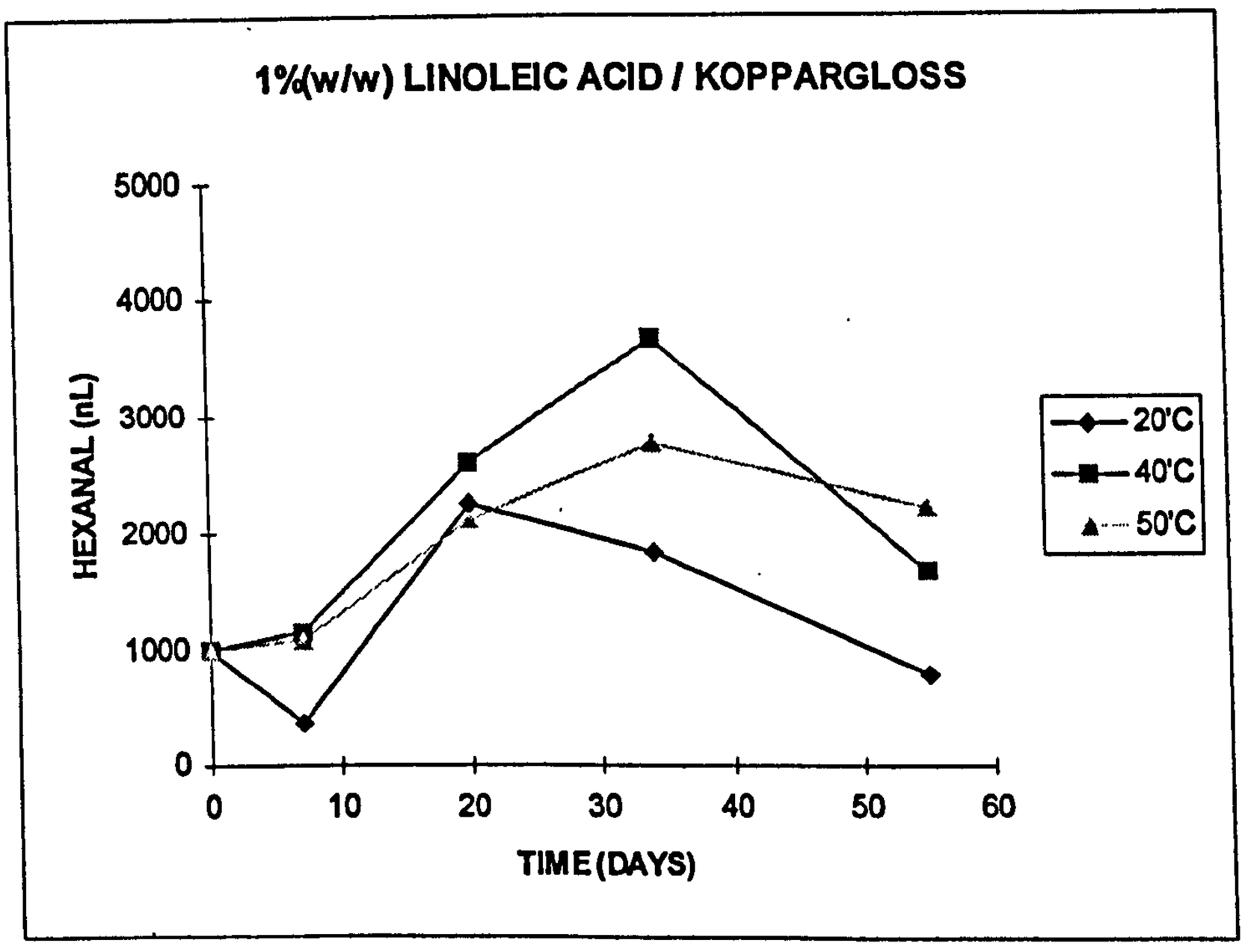
Table 3.4 The amounts of volatiles (in nL) recovered from a one gram sample of linoleic acid/Koppargloss stored for 55 days at 20°C.

NAME OF COMPOUND	RETENTION TIME (in minutes)	QUANTITATION ION (in m/z)	CALCULATED AMOUNT (in nL)
Pentanal	6.66	58	368
Hexanal	10.09	44	794
Heptanal	14.16	70	31
Octanal			
Nonanal			
trans 2-heptenal	16.33	83	77
trans 2-octenal	30.36	70	146
2-Pentanone	6.44	86	4
2-Hexanone	9.81	58	2
2-Heptanone	13.75	58	24
6-Undecanone		43	
1-Butanol	6.01	56	14
1-Pentanol	9.13	42	109
1-Octen-3-ol	17.53	57	51
1-Butoxy-2-propanol			
Methyl octanoate			
Pentyl pentanoate			
2-Pentyl furan	18.19	138	55
Pentane	3.16	41	125
Heptane	7.43	71	1
Nonane	14.96	57	4
Decane			
1-Hexene	4.51	56	4
1-Heptene	7.06	56	7
1-Undecene	22.28	55	8
1-Dodecene			
Toluene	9.39	91	7
Ethylbenzene			
1,2-Dimethylbenzene			
Styrene	14.16	104	3
a-Methyl styrene			
Propylbenzene			
1,2,3-Trimethylbenzene			
1,3,5-Trimethylbenzene	17.1	105	7

The low recovery of acyclic volatile compounds from the Koppargloss sample, as indicated in the table, suggests that vacuum purification pre-treatment of the board sample is effective in removing volatiles associated with the board surface binder.

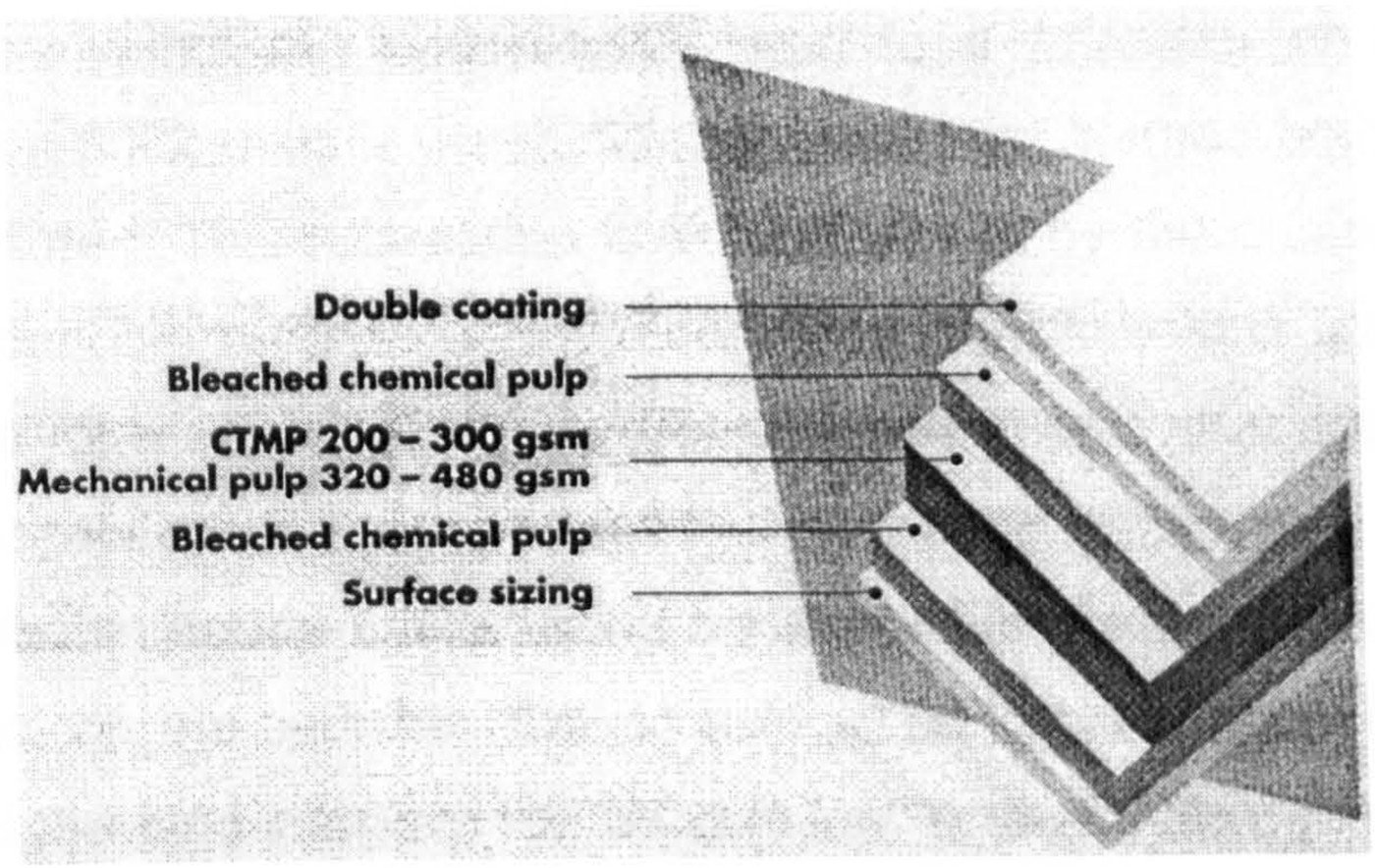
As hexanal was still the major volatile resulting from oxidation, the quantitation of levels of hexanal found within the headspace vapours of samples was thought to be an appropriate representation of oxidation extent within the system. Figure 3.99 shows the amounts of hexanal recovered from samples of linoleic acid/Koppargloss, sampled using an equilibration temperature of 105°C.

Figure 3.99 Amounts of hexanal recovered (in nL) from samples of linoleic acid/Koppargloss stored at 20°C, 40°C and 50°C for up to 55 days (Headspace sampling temperature: 105°C).



The amounts of hexanal recovered are significantly greater than those for the corresponding samples of linoleic acid/CTMP equilibrated at 105°C. This suggests that the Koppargloss matrix provides an environment that is more favourable to oxidation producing volatile products. To determine the reason for this increase in volatile formation, the structure and components of the carton-board need to be evaluated. Figure 3.100 shows the physical structure of Koppargloss (Stora, 1996).

Figure 3.100 The physical structure of Koppargloss carton-board.



The major component of the carton-board is CTMP. This CTMP is sandwiched between two layers of bleached chemical pulp. The pulp layers within this carton-board are mixed with fillers. Board materials tend to contain fillers at loading levels of less than 10% by weight of sheet and often less than 5% by weight of sheet.

Chalk and clay fillers are used in boards in addition to pigments such as titanium dioxide. Other fillers used may include synthetic alumino-silicates and precipitated silica. These fillers lower the density of the pulp fibre matrix in which the linoleic acid is impregnated, increasing the air volume and, thus, the availability of atmospheric oxygen within the matrix.

Carton-board contains retention aids such as aluminium sulphate and cationic starches that provide an increase in retention of material during manufacture. They will fix the resinous pitch and other sticky substances to fibres and fillers, thus preventing them from being deposited. Aluminium salts form a positively charged, colloidal system whose properties depend on both the pH and the nature of the anion in the aluminium salt. Aluminium salts are normally employed in concentrations of between 0.5 and 3%, in terms of the oven-dry stock.

Paper fibres, fillers, resinous particles and other components that make up the stock components are generally negatively charged. Retention aids interact with various different substances in the stock, with the aim of controlling the level of co-flocculation of dissolved constituents in water.

It is unlikely that retention aid compounds will have a marked influence on the rate and extent of oxidation of linoleic acid.

Sizing agents within the carton-board lead to a retarding of the rate of penetration of a fluid, usually water, through capillaries formed both within and between the fibres. This retardation is brought about by the creation of a low energy, hydrophobic surface at the fibre-water interface that increases the contact angle formed between a drop of liquid and the surface, thus, decreasing wettability.

The hydrophobic nature of the sizing agents within the board matrix has an opposite effect on the lipophilic linoleic acid. The contact angle between the fatty acid and the fibre interface will be very low (probably zero), leading to the spreading out of fatty acid over the surface of the fibres within the matrix. This increases the surface area available for the adsorption of oxygen in to linoleic acid, increasing the degree of oxidation.

The surface binder may be the most important ingredient in a pigmented coating. Its primary role is binding the pigment to the wood pulp. Greater-than-normal binder amounts are required to improve varnish or gloss ink holdout. Paper coating binders used in carton board packaging are commonly aqueous emulsions of synthetic polymers based on styrene-butadiene or styrene-acrylate copolymer latices.

This binder coating is porous, allowing the diffusion of oxygen and volatile compounds. Thus, its presence should not impede oxidation.

The greater amounts of hexanal found in samples of linoleic acid/Koppargloss equilibrated at 105°C may be due, (a) to the greater availability of oxygen within the board matrix as a result of the lower density created by the presence of fillers, and (b) to the greater surface area of fatty acid available for oxygen adsorption, introduced by the presence of sizing agents.

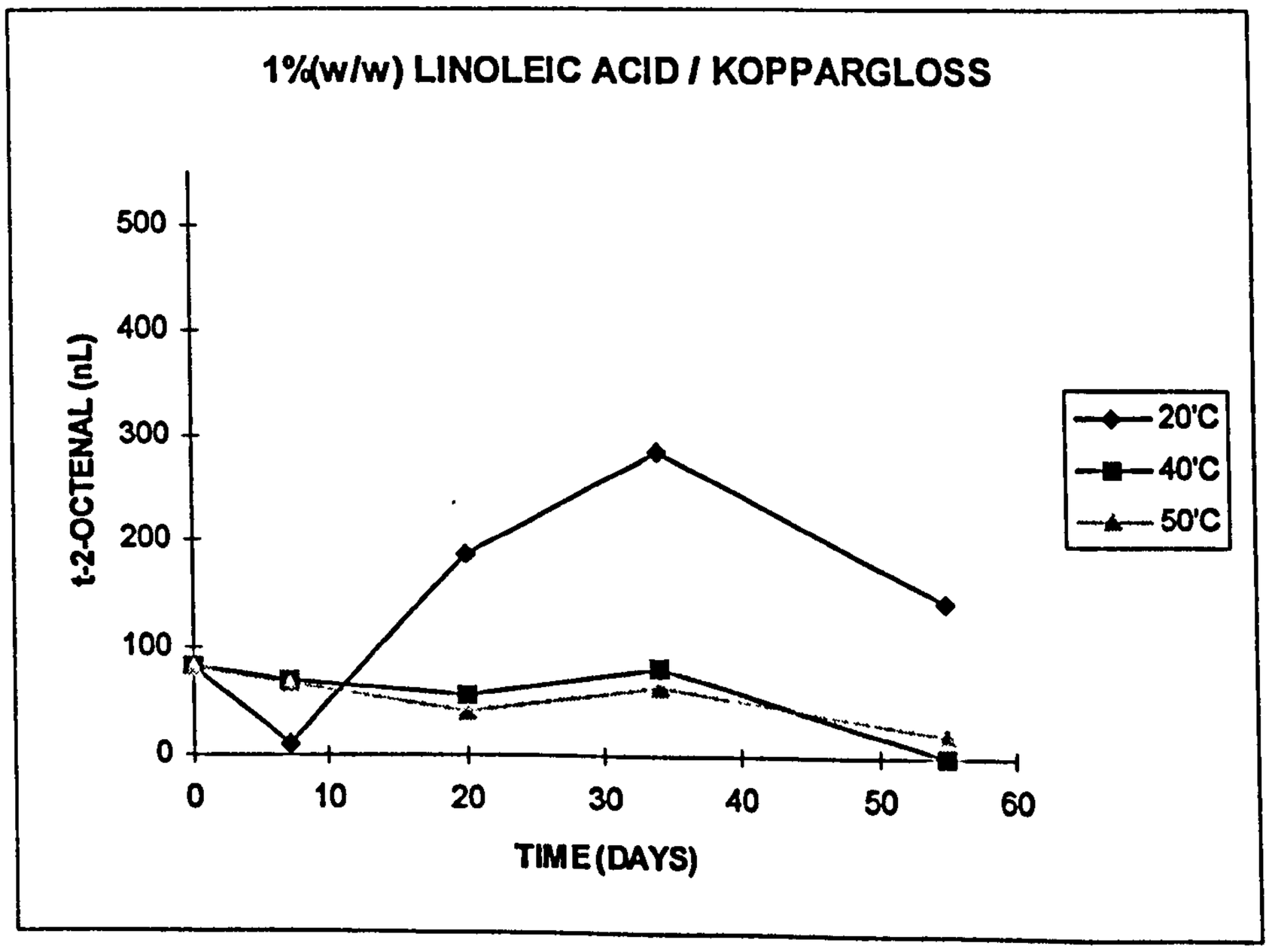
The amounts of hexanal recovered at ambient temperature were also very large. This suggests that a large number of hydroperoxide units will have formed during storage, a large proportion of which decompose during the headspace equilibration period to produce volatile products. After approximately three weeks, the amounts of hexanal start to decline, for samples stored at ambient temperature. This suggests that termination

reactions in the system become significant due to the decomposition of the large number of hydroperoxides. Higher molecular weight, non-volatile compounds may also have formed from the addition of free-radicals to growing active chains.

The hexanal levels decline after approximately four weeks at the higher storage temperatures. This indicates the thermal degradation of hexanal, the formation of non-volatile compounds and the increase in the number of termination reactions as the number of free radicals in the systems increase.

The amounts of *trans* 2-octenal were monitored to determine the formation of 2,4-decadienal from the decomposition of linoleic acid 9-hydroperoxide within the board system. Figure 3.101 shows the amounts of *trans* 2-octenal recovered.

Figure 3.101 Amounts of *trans* 2-octenal recovered (in nL) from samples of linoleic acid/Koppargloss stored at 20°C, 40°C and 50°C for up to 55 days (Headspace sampling temperature: 105°C).

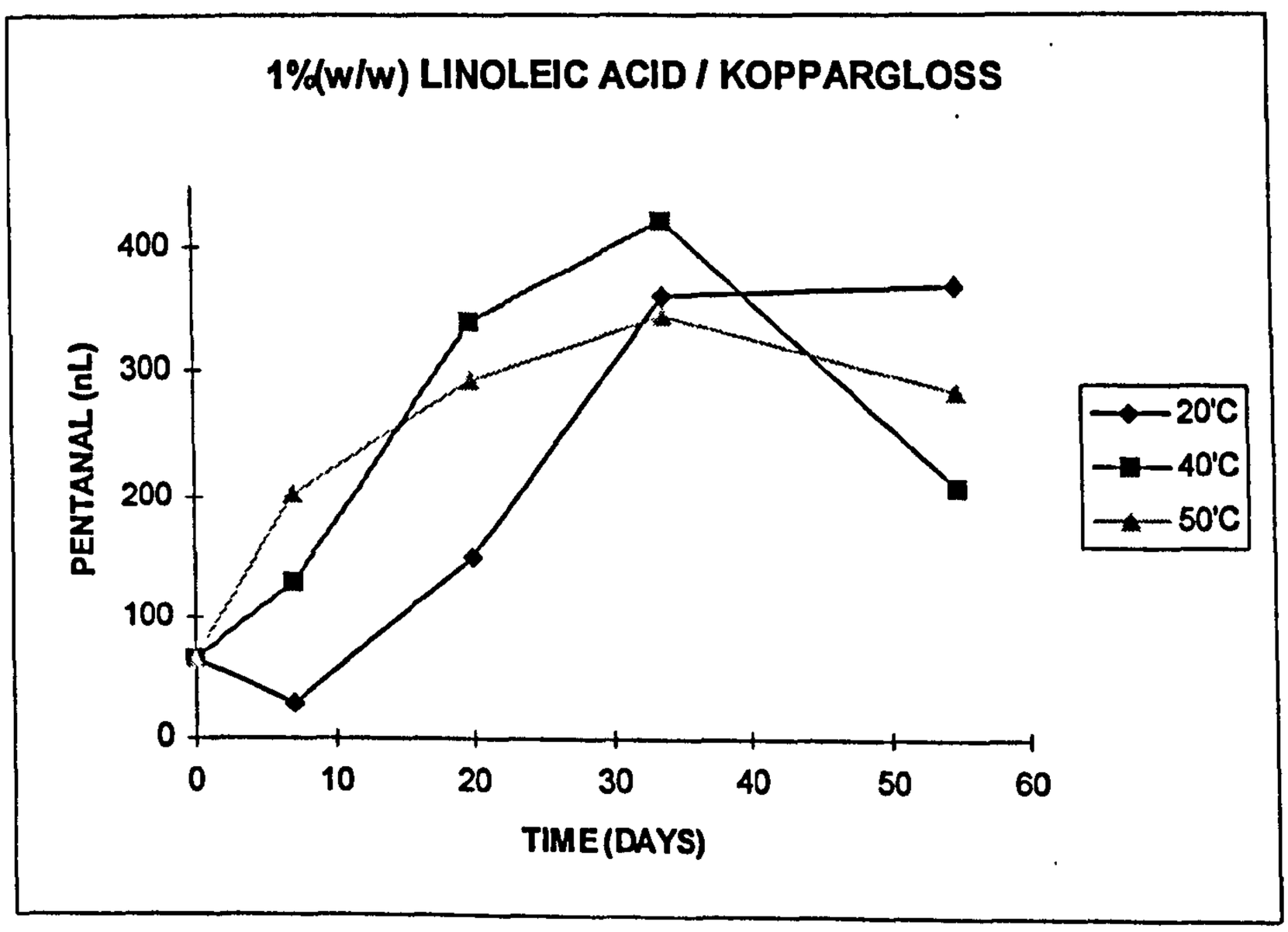


The amounts of octenal recovered at 40°C and 50°C are very similar to those for the linoleic acid/CTMP system. This suggests that 2,4-decadienal formation is no greater than it was in the CTMP system. This is hardly surprising since the Koppargloss system provides an environment which is more favourable to

oxidation. As discussed before, decomposition of 9- and 13-hydroperoxide isomers of linoleic acid in the presence of oxygen yields the same composition of aldehydes. The major product is hexanal and 2,4-Decadienal is absent, Grosch et al. (1981) and Schieberle and Grosch (1981).

Figure 3.102 shows the amounts of hexanal recovered from samples of linoleic acid/Koppargloss.

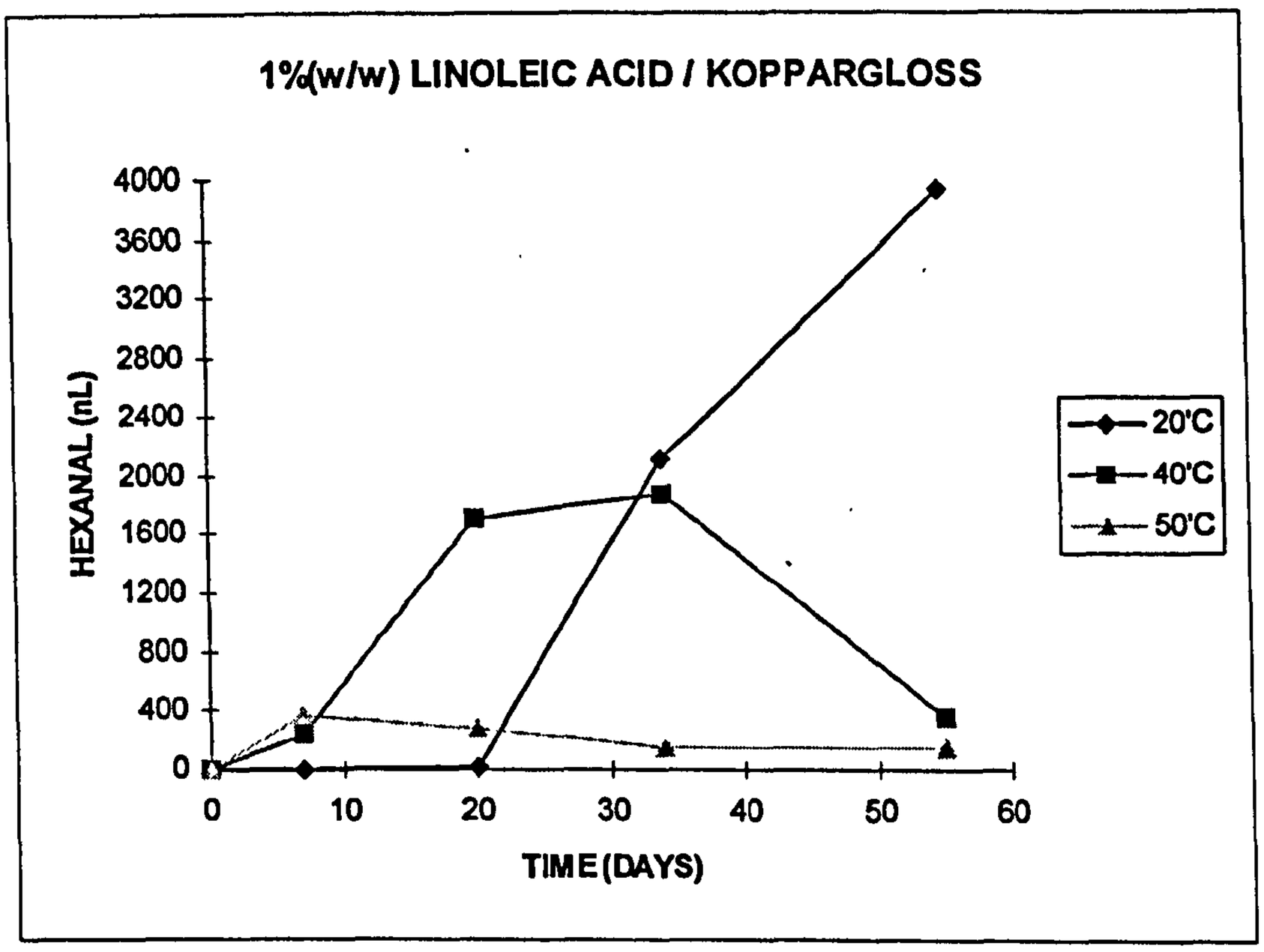
Figure 3.102 Amounts of pentanal recovered (in nL) from samples of linoleic acid/Koppargloss stored at 20°C, 40°C and 50°C for up to 55 days (Headspace sampling temperature: 105°C).



The amounts of pentanal are significantly greater than those of the corresponding CTMP samples. This finding suggests that pentanal is linked in some way to the formation of hexanal. Formation is likely to occur via the pentyl radical produced from the decomposition of linoleic acid 13-hydroperoxide (see Figure 3.19). If pentanal formation occurs by the addition of oxygen to the pentyl radical, produced during decomposition of linoleic acid 13-hydroperoxide, then oxygen must be readily available to interact with the radical before hydrogen abstraction or combination with a hydroxyl radical occurs.

The test was repeated using a headspace equilibration temperature of 50°C. Figure 3.103 shows the amounts of hexanal recovered from samples of linoleic acid/Koppargloss during the test.

Figure 3.103 Amounts of hexanal recovered (in nL) from samples of linoleic acid/Koppargloss stored at 20°C, 40°C and 50°C for up to 55 days (Headspace sampling temperature: 50°C).



The amounts of hexanal recovered from samples stored at 40°C and 50°C were smaller than those for the corresponding samples equilibrated at 105°C. Despite a slower build up, hexanal levels for samples stored at 40°C were greater than those of samples of linoleic acid/CTMP equilibrated at 50°C, supporting the theory that the board material provides a more favourable environment for fatty acid oxidation to take place. The slower build up of hexanal suggests that hydroperoxide formation is slower within the Koppargloss system.

The lesser amounts of hexanal, compared to samples equilibrated at 105°C, support the finding that hydroperoxide decomposition occurs more slowly at the lower headspace temperature.

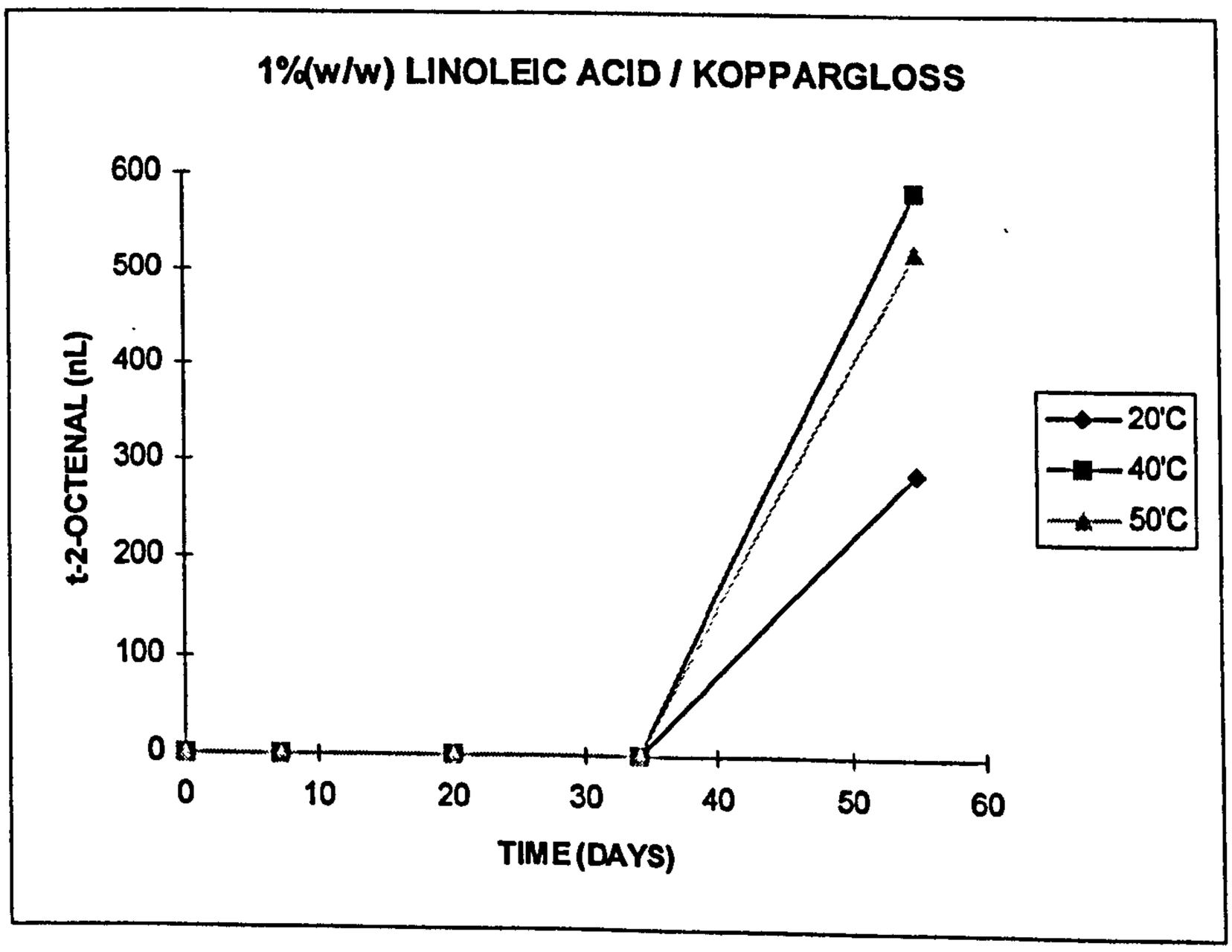
The small amounts of hexanal recovered from samples stored at 50°C suggest that extensive decomposition of hydroperoxides formed result in a

large number of termination reactions and in the formation of higher molecular weight non-volatile compounds.

The hexanal at ambient temperature was slow to build up. However, after approximately three weeks, the amounts of hexanal found rose sharply. At 55 days the amounts were greater than those at the higher storage temperatures. This suggests that hydroperoxide formation is initially slow at ambient temperature but, as time passes the number of radicals in the system builds up, increasing the number of hydroperoxides formed. These hydroperoxides decompose to yield hexanal, among other compounds. The number of termination reactions and the thermal degradation of hexanal are less significant at these storage and equilibration temperatures. Therefore hexanal levels continue to rise.

Figure 3.104 shows the amounts of *trans* 2-octenal recovered from samples during the test.

Figure 3.104 Amounts of *trans* 2-octenal recovered (in nL) from samples of linoleic acid/Koppargloss stored at 20°C, 40°C and 50°C for up to 55 days (Headspace sampling temperature: 50°C).



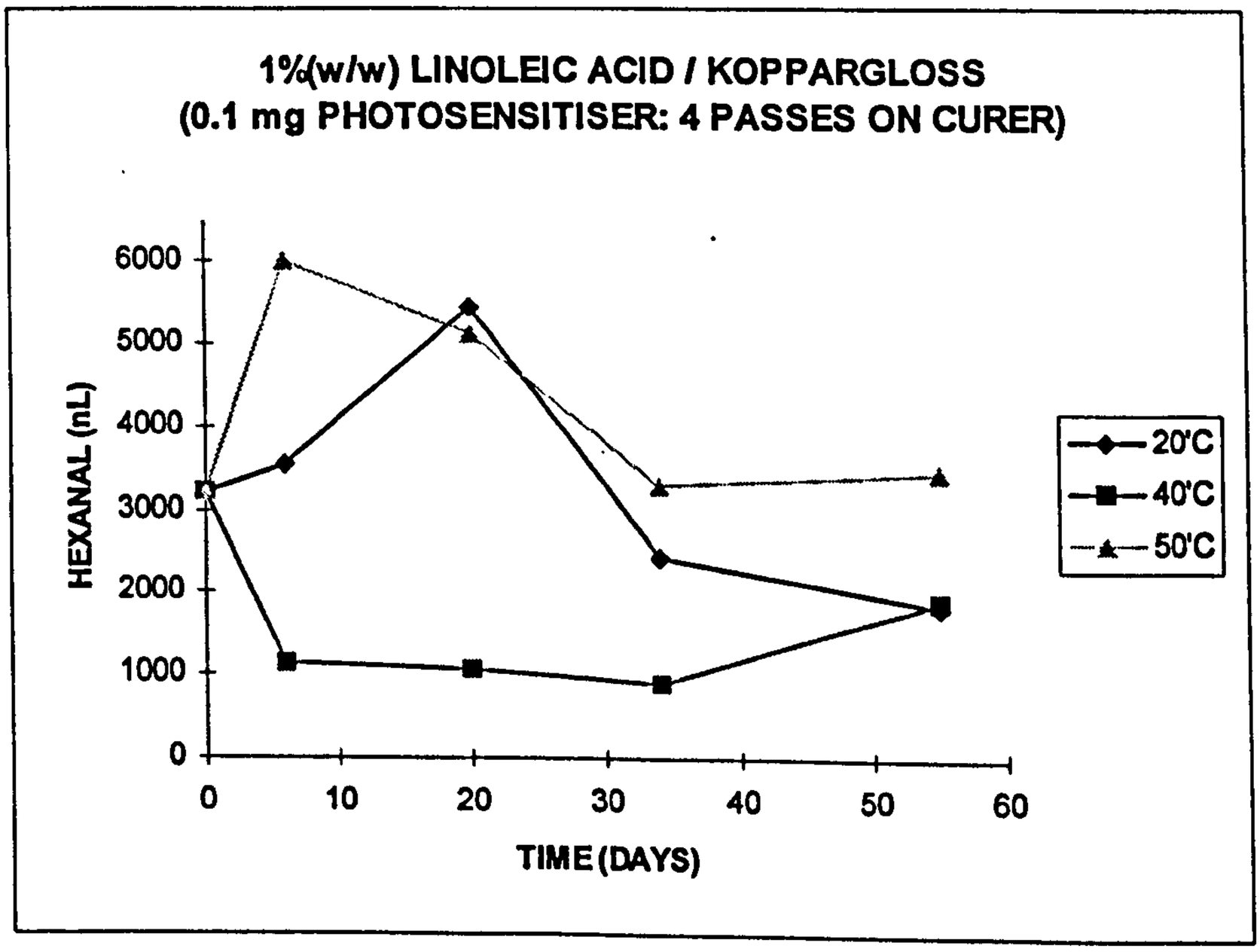
Unusually, *trans* 2-octenal was found in significant amounts in samples that were stored for 55 days. This suggests that decomposition of linoleic acid 9-

hydroperoxide to 2,4-decadienal occurs. It is possible that, at the longer storage periods, the amount of available oxygen within the test vials may have diminished somewhat favouring the formation of the relatively unstable 2,4-decadienal.

Determination of the effect of singlet oxygen on the oxidation of linoleic acid in Koppargloss carton-board

The amounts of hexanal recovered from samples of linoleic acid/Koppargloss impregnated with TSZnPh. are shown in Figure 3.105.

Figure 3.105 Amounts of hexanal recovered (in nL) from samples of linoleic acid/ Koppargloss + 0.1mg TSZnPh photosensitiser stored at 20°C, 40°C and 50°C for up to 55 days (Headspace sampling temperature: 105°C).



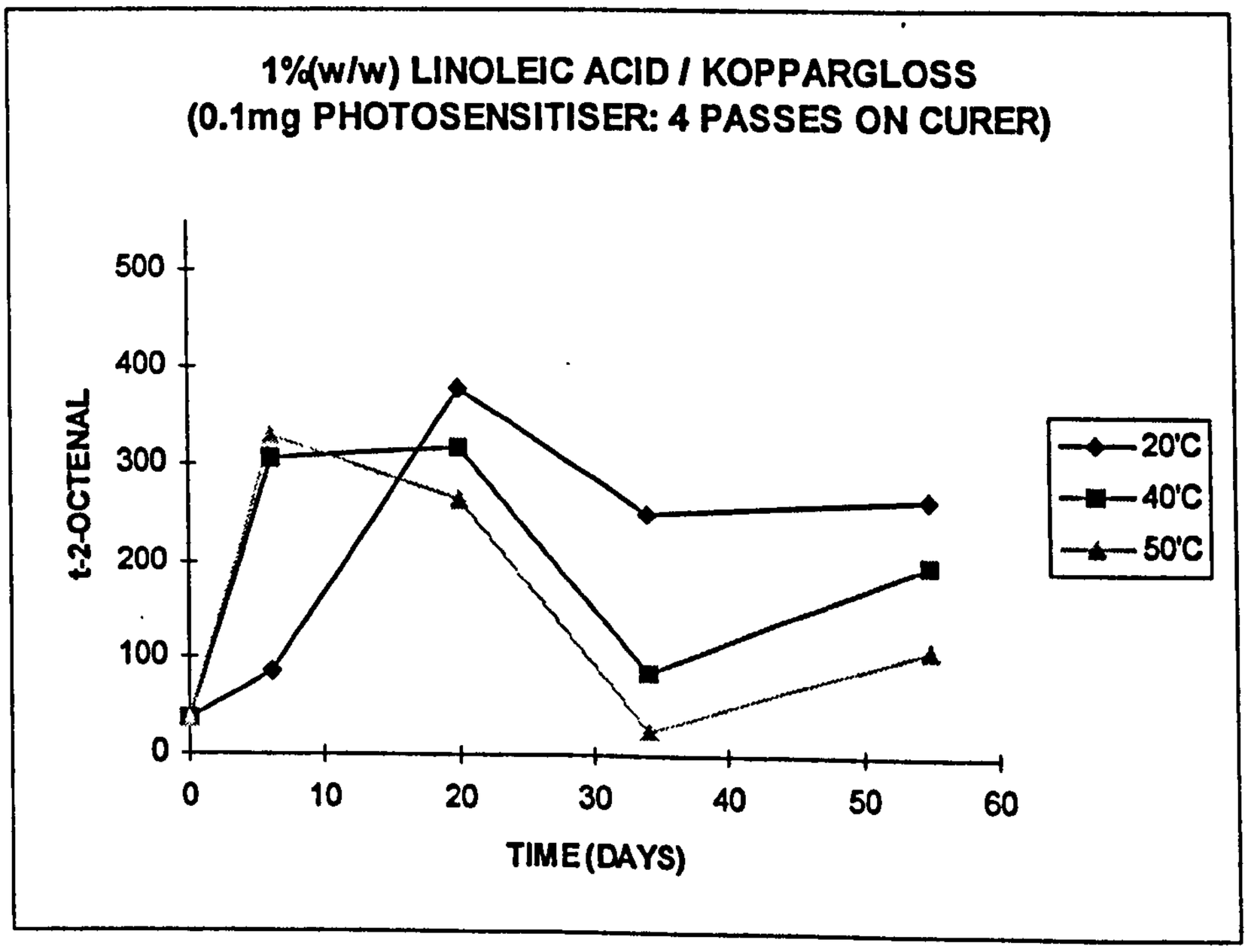
The amounts of hexanal are markedly greater than those for the corresponding Koppargloss samples, in the absence of photosensitiser. The greatest amounts were for samples stored at 20°C and 50°C. Interestingly, the smallest amounts were for samples stored at 40°C. This suggests that decomposition of hydroperoxides to form volatile compounds occurs so extensively at 50°C that formation of volatile compounds, such as hexanal, out-competes radical

termination reactions. At 40°C, hydroperoxide decomposition will be rapid, but termination may still reduce the formation of volatile compounds.

Hexanal levels from samples stored at ambient temperature built up rapidly over time. This further confirms that initial hydroperoxide formation resulting from photoxidation is extensive. Due to the large number of hydroperoxides, even their slow decomposition at 20°C results in the significant production of volatile compounds. The levels of hexanal declined after a time, probably as a result of termination reactions and the formation of non-volatile products.

Figure 3.106 shows the amounts of *trans* 2-octenal recovered from samples of linoleic acid/Koppargloss impregnated with photosensitiser.

Figure 3.106 Amounts of *trans* 2-octenal recovered (in nL) from samples of linoleic acid/Koppargloss + 0.1mg TSZnPh photosensitiser stored at 20°C, 40°C and 50°C for up to 55 days (Headspace sampling temperature: 105°C).



The levels of *trans* 2-octenal recovered were greater than those for the corresponding samples without photosensitiser and exposure to UV light, equilibrated at 105°C. This suggests that decomposition of linoleic acid 9-hydroperoxide does occur, producing 2,4-decadienal. This, in turn, breaks down to yield 2-octenal. The reason for the greater formation of *trans* 2-octenal is unclear. In previous tests, photoxidation had little effect on the formation of

trans 2-octenal. The findings of this test conflict with the findings of Tressl *et al.* (1981). They observed that the amounts of 2,4-decadienal, formed from photoxidation of methyl linoleate were much reduced relative to those found from autoxidation.

One explanation for the increased formation of *trans* 2-octenal lies in the large number of hydroperoxides that is initially produced through photoxidation. These hydroperoxides decompose at such a rate, as indicated by the rapid build up of the aldehyde at all storage temperatures, that decomposition of linoleic acid 9-hydroperoxide occurs before isomerisation can occur to the 13-isomer.

Tressl *et al.* (1981) reported a significant increase in the amount of *trans* 2-heptenal recovered from photoxidised methyl linoleate. In tests involving the impregnation of photosensitiser into linoleic acid/CTMP samples, the amounts of 2-heptenal recovered from samples were no greater than those recovered from samples in the absence of photosensitiser. This indicates that photosensitisation does not bring about a significant increase in the formation of hydroperoxy epidioxides, which are said to be precursors of 2-heptenal.

Evidence for the existence of hydroperoxy epidioxides within the linoleic acid/ CTMP system, during autoxidation, was indicated by the presence of 3-octen-2-one.

The amounts of *trans* 2-heptenal recovered from samples of linoleic acid/Koppargloss are shown in Figure 3.107, and the amounts recovered from samples of linoleic acid/Koppargloss impregnated with photosensitiser and irradiated are shown in Figure 3.108.

The levels of *trans* 2-heptenal in the Koppargloss system are generally greater for the photoxidised samples. This suggests that photoxidation favours the formation of hydroperoxy epidioxides from linoleic acid within the Koppargloss system, unlike CTMP.

Figure 3.107 Amounts of *trans* 2-heptenal recovered (in nL) from samples of linoleic acid/Koppargloss stored at 20°C, 40°C and 50°C for up to 55 days (Headspace sampling temperature: 105°C).

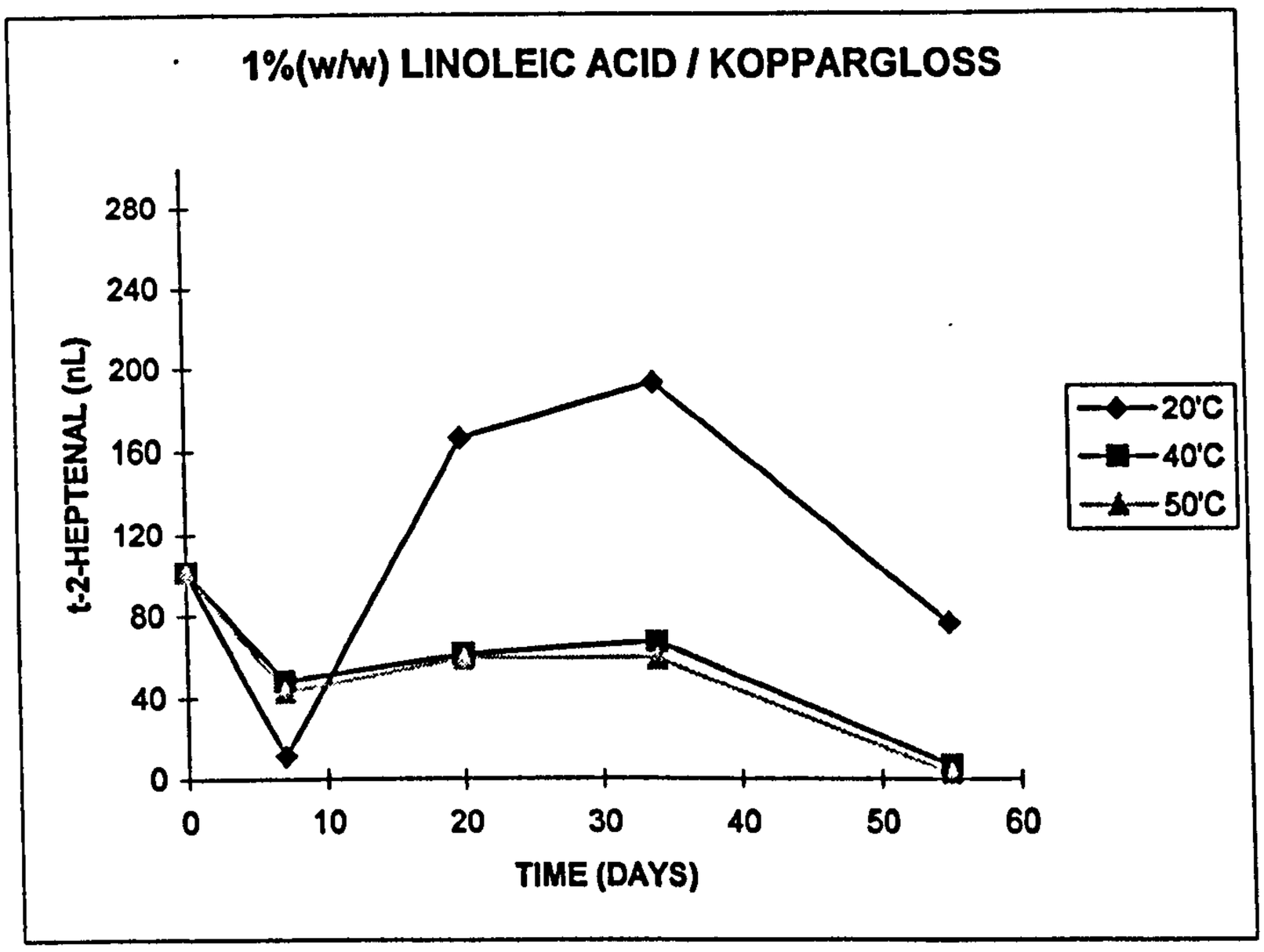
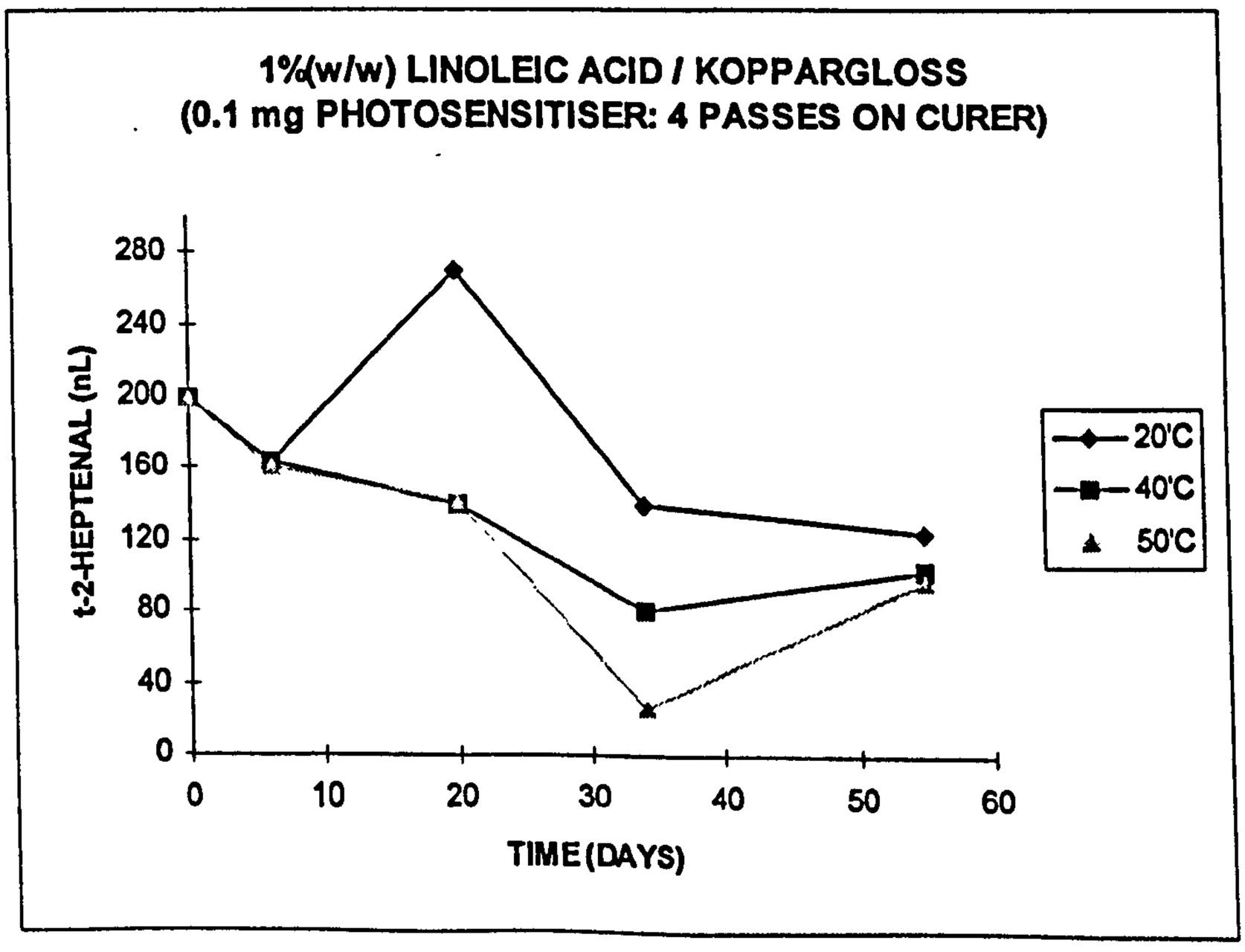


Figure 3.108 Amounts of *trans* 2-heptenal recovered (in nL) from samples of linoleic acid/Koppargloss + 0.1mg TSZnPh photosensitiser stored at 20°C, 40°C and 50°C for up to 55 days (Headspace sampling temperature: 105°C).



Determination of the effect of UV-cured ink on the presence of linoleic acid in Koppargloss carton-board

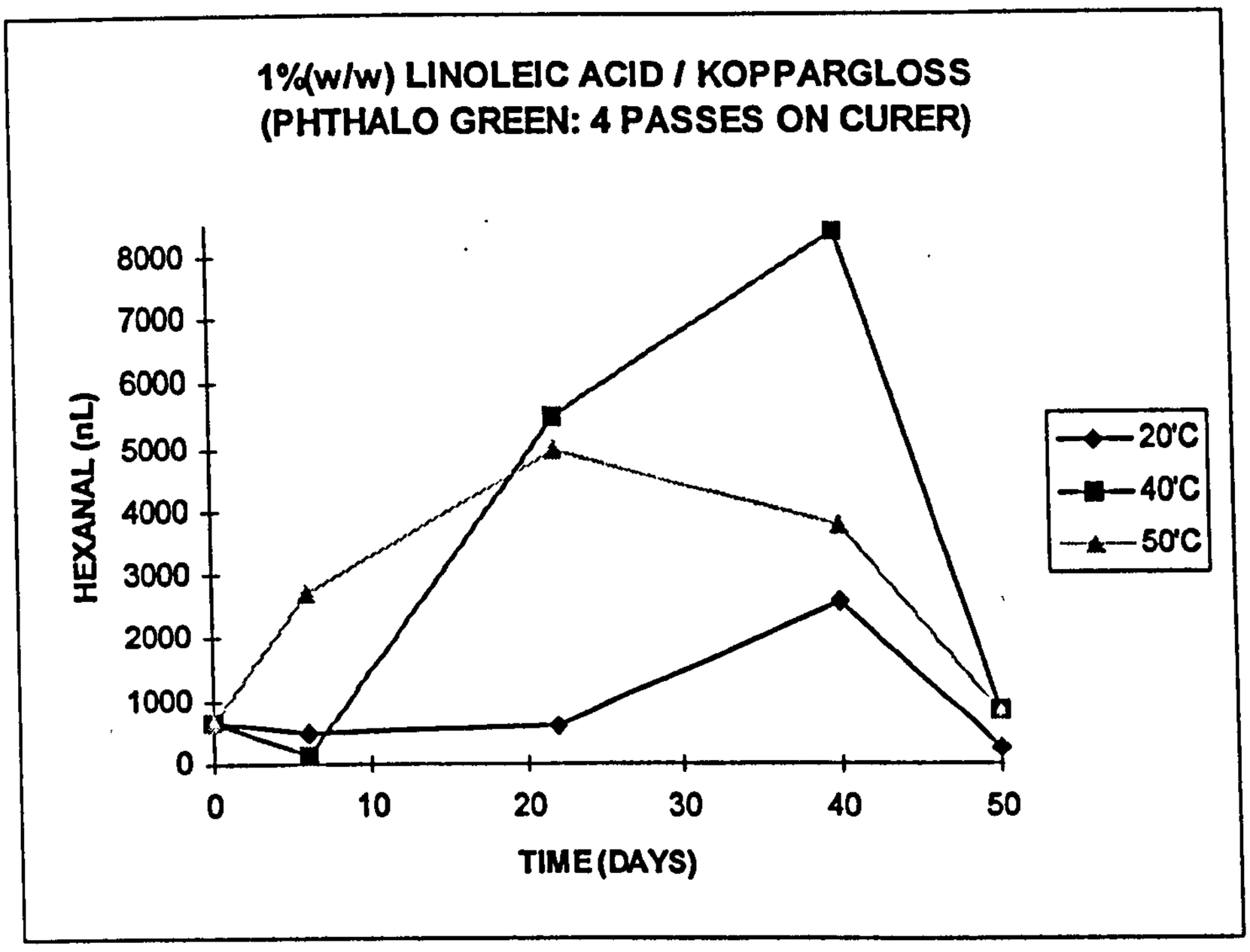
In comparison to the tests involving the print proofing of ink on to CTMP, the print proofing of ink on to Koppargloss is far more representative of the real packaging system in which carton-board is printed with UV-curable inks, via a lithographic process, on a production line. Koppargloss contains all the surface sizing agents, fillers, pigments and binders of the real system. It should, therefore, expected that the penetration of the ink into the matrix of board will be substantially less than that which occurred during the print proofing of the porous, absorbent, surface of the CTMP sheet. Taking this in to consideration, it might be expected that the effect of the ink, and its subsequent irradiation, on the formation of volatile compounds from the oxidation of linoleic acid would be less than that for the CTMP system.

Figure 3.109 shows the amounts of hexanal recovered from samples of linoleic acid/Koppargloss print proofed with Phthalo Green, using a headspace equilibration temperature of 105°C.

With the exception of samples stored at 20°C, the amounts of hexanal recovered are substantially greater than those for the corresponding Koppargloss samples without Phthalo Green ink, and irradiation. This discounts the idea that the reduced porosity and, thus, penetration of ink into the substrate matrix has an effect on reducing the degree of photoxidation, resulting from the presence of the ink.

The number of hydroperoxide groups formed from photo-sensitised oxidation, during irradiation of samples, is considerable. As a consequence, the formation of volatile compounds from the decomposition of hydroperoxides appears to have out-competed free radical termination. The hexanal amounts started to decline for samples that were stored at 50°C, after three weeks, probably as a result of the rate of termination and volatile product decomposition increasing as the number of radicals in the system rose. This decline in hexanal occurred from approximately six weeks, for samples stored at 40°C, as a result of the slower build up of free radicals in the system.

Figure 3.109 Amounts of hexanal recovered (in nL) from samples of linoleic acid/ Koppargloss print proofed with Phthalo Green ink, irradiated, and stored at 20°C, 40°C and 50°C for up to 50 days (Headspace sampling temperature: 105°C).



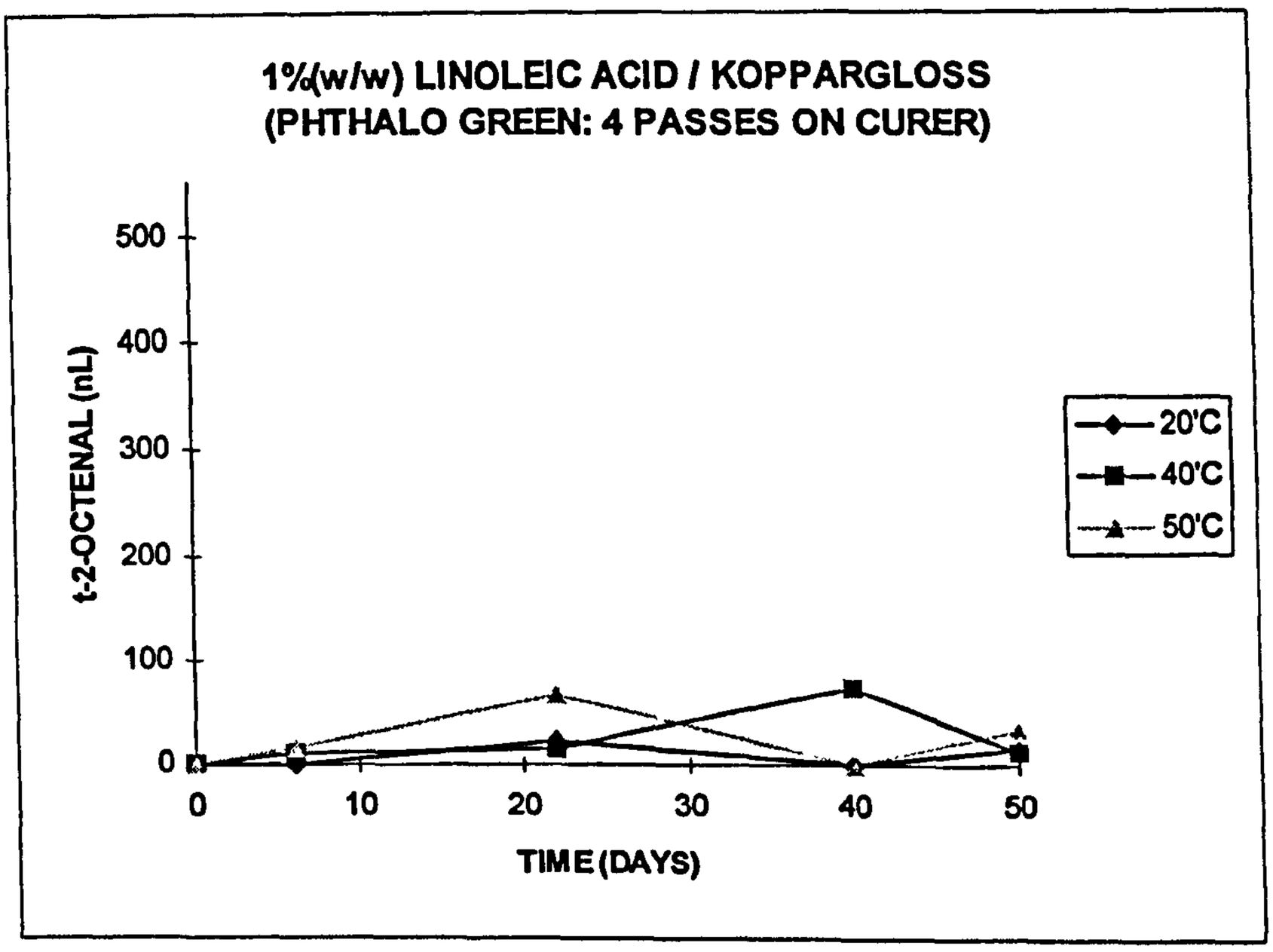
At an ambient storage temperature, the amounts of hexanal recovered were much less than from samples stored at the higher temperatures. As with the CTMP system, this was probably due to the lower rate of decomposition of hydroperoxides at ambient temperature and the radical quenching effect of the ink resin. The amounts of hexanal found, however, were several orders of magnitude greater than those for the corresponding inked CTMP system. This confirms the initial formation of large amounts of hydroperoxides during photoxidation.

The greater extent of photoxidation within the Koppargloss system, arises from the ability of the linoleic acid to adsorb more singlet oxygen. This could be a result of the increased surface area of the linoleic acid within the board matrix, caused by the presence of the sizing agents and fillers within the system. Based on this assumption, singlet oxygen appears to have a little penetration into the fatty acid medium.

The amounts of *trans*-2-octenal recovered from the CTMP system were very small, indicating a low degree of 2,4-decadienal formation. Figure 3.110 shows

the amounts of *trans* 2-octenal recovered from Koppargloss samples print proofed with the Phthalo Green ink. These amounts should determine if photoxidation within the Koppargloss system had an effect on the formation of 2,4-decadienal from the decomposition of linoleic acid 9-hydroperoxide.

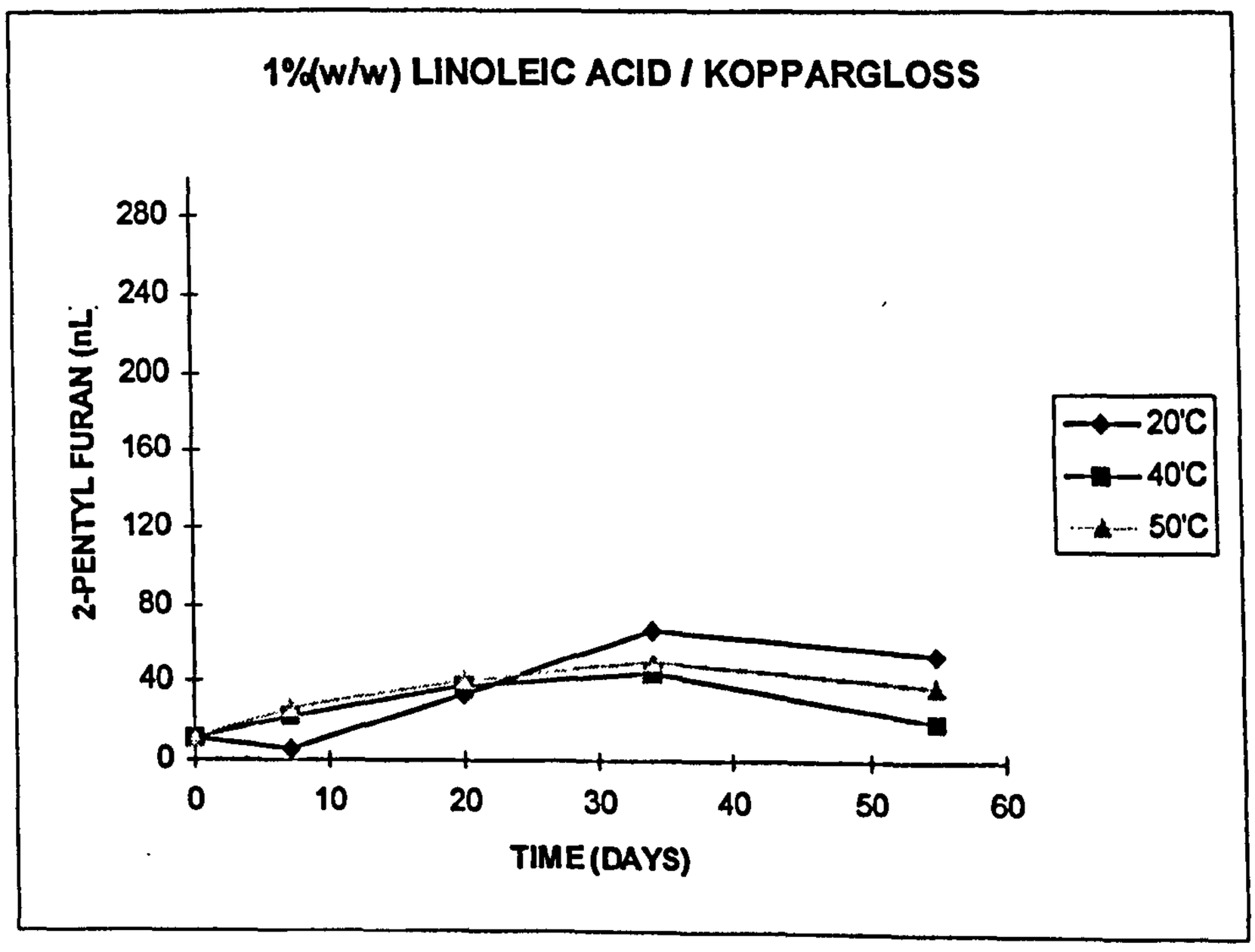
Figure 3.110 Amounts of *trans* 2-octenal recovered (in nL) from samples of linoleic acid/Koppargloss proofed with Phthalo Green ink, irradiated, and stored at 20°C, 40°C and 50°C for up to 50 days (Headspace sampling temperature: 105°C).



The amounts of octenal are smaller than those for the corresponding system in the absence of the ink and irradiation. This supports the idea that photoxidation results in reduced formation of 2,4-decadienal from the decomposition of linoleic acid 9-hydroperoxide. This conflicts with the findings involving systems impregnated with photosensitiser, in which levels of *trans* 2-octenal were found in similar or greater amounts. It is possible that the smaller levels of octenal in the inked systems may be due to the unsaturated aldehyde, and its di-unsaturated aldehyde precursor, interacting with active sites within the ink resin, subsequently being bound into the ink matrix. This concept is supported by the low recoveries of *trans* 2-heptenal for inked samples of Koppargloss which, too, could interact with active sites within the ink film.

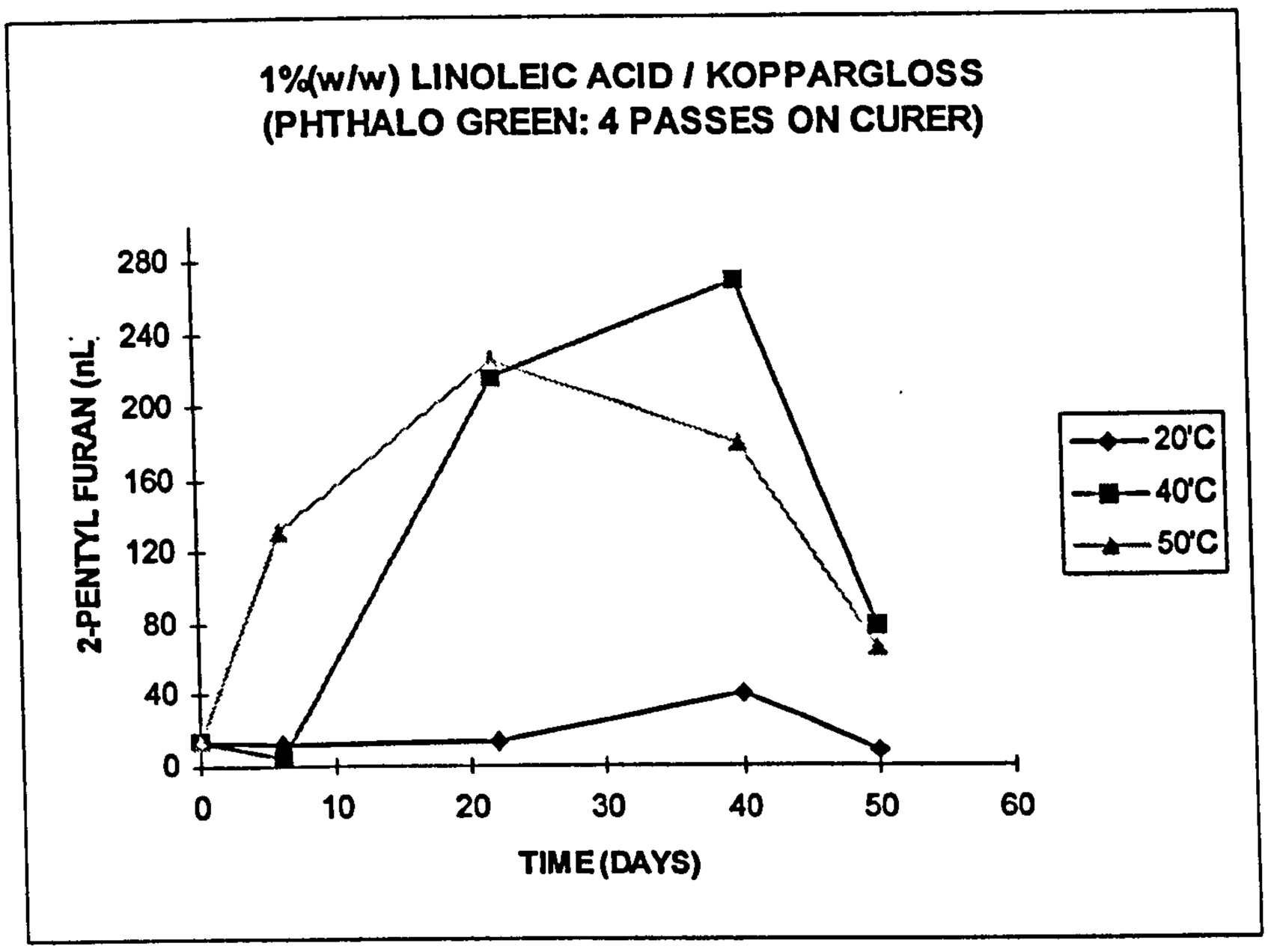
The amounts of 2-pentyl furan recovered from inked samples of linoleic acid/CTMP stored at 40°C and 50°C were significantly greater than those amounts for the corresponding uninked samples. To determine whether this was the case for the Koppargloss system, the amounts of 2-pentyl furan recovered from uninked samples of linoleic acid/Koppargloss and inked samples of linoleic acid/Koppargloss were plotted (Figure 3.111 and Figure 3.112, respectively).

Figure 3.111 Amounts of 2-pentyl furan recovered (in nL) from samples of linoleic acid/Koppargloss stored at 20°C, 40°C and 50°C for up to 55 days (Headspace sampling temperature: 105°C).



The amounts of 2-pentyl furan are significantly greater for inked samples stored at 40°C and 50°C. This confirms that photoxidation favours the formation of 2-pentyl furan, either via decomposition of a cyclic peroxide intermediate of linoleic acid 9-hydroperoxide or via decomposition of linoleic acid 10-hydroperoxide.

Figure 3.112 Amounts of 2-pentyl furan recovered (in nL) from samples of linoleic acid/Koppargloss proofed with Phthalo Green ink, irradiated, and stored at 20°C, 40°C and 50°C for up to 50 days (Headspace sampling temperature: 105°C).

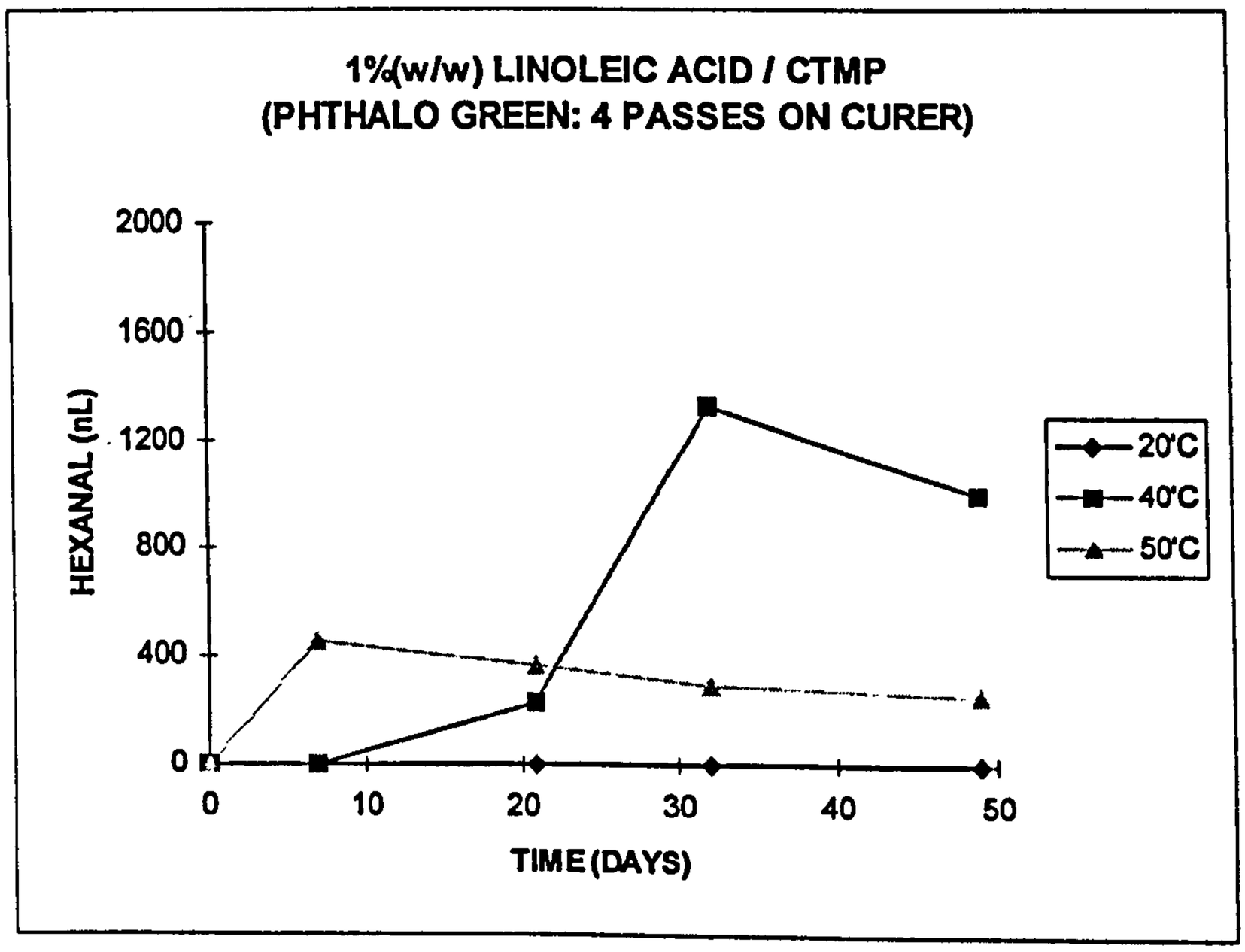


The test involving linoleic acid/Koppargloss print proofed with Phthalo Green ink was repeated using a headspace equilibration temperature of 50°C. Figure 3.113 shows the amounts of hexanal recovered during the test. The amounts of hexanal recovered are significantly less than those of samples equilibrated at 105°C and the corresponding uninked samples, equilibrated at 50°C. This suggests that, despite rapid formation of hydroperoxides during the initial photoxidation, the slow decomposition of hydroperoxides and, thus, the low concentration of free radicals may be quenched by the active sites within the ink film. This results in lower yields of volatile products. During the equilibration period at the higher temperature, hydroperoxide decomposition is rapid and the relative effect of radical quenching becomes somewhat less.

In addition, the ink film provides an impervious barrier against the escape of volatile compounds through one surface of the board samples. At an equilibration temperature of 50°C, this will reduce the recovery levels of volatile compounds because of the lack of mobility. At 105°C, the mobility of formed

volatiles will be considerably greater. Thus, a larger proportion of compounds escape from the board system into the vapour phase.

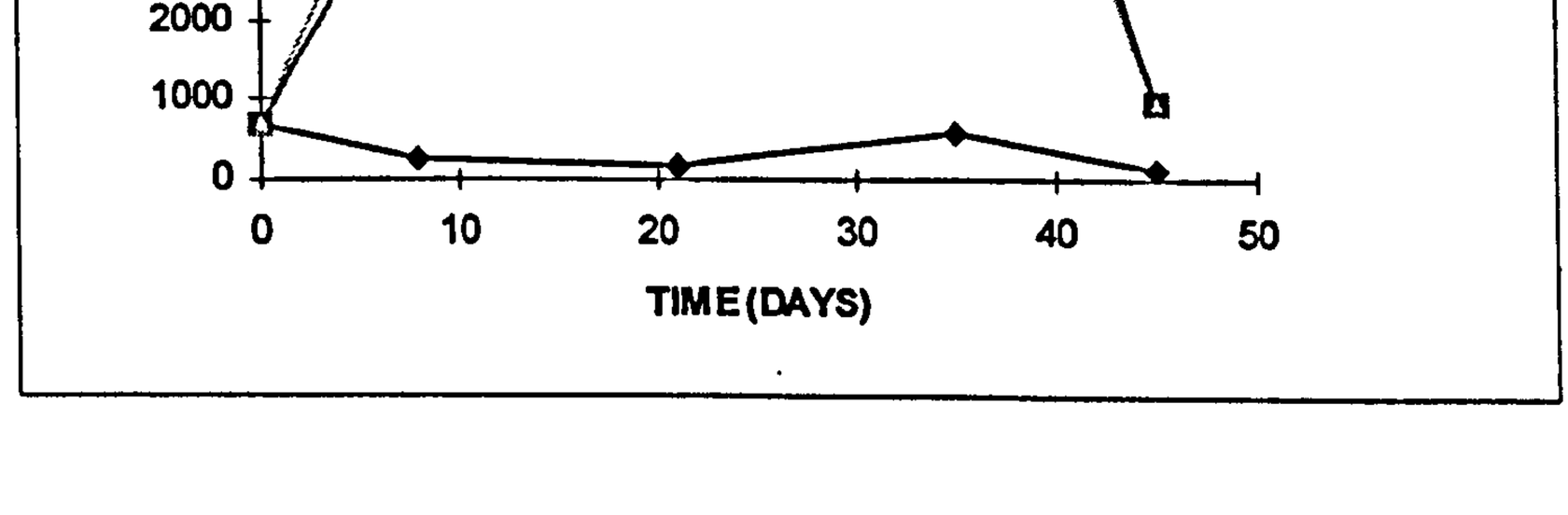
Figure 3.113 Amounts of hexanal recovered (in nL) from samples of linoleic acid/ Koppargloss print proofed with Phthalo Green ink, irradiated, and stored at 20°C, 40°C and 50°C for up to 49 days (Headspace sampling temperature: 50°C).



During the lithographic printing of UV-curable inks on to the carton-board substrate, mixing of the ink with small amounts of fountain (fount) solution occurs. To determine the effect of fount solution on autoxidation and on the photoxidation of linoleic acid within Koppargloss, Phthalo Green ink was mixed with fount/IPA and proofed onto samples of linoleic acid/Koppargloss. The fountain solution used was Dupont Fountsol F-5. This contains a citric acid, sodium citrate buffer, buffered to pH 5 and tetrasodium EDTA. Both these components, in addition to IPA could have an influence on the oxidation process. The increased humidity brought about by the introduction of water could also be significant.

Figure 3.114 shows the amounts of hexanal recovered from Koppargloss samples proofed with the ink/fount/IPA mix.

Figure 3.114 Amounts of hexanal recovered (in nL) from samples of linoleic acid/ Koppargloss proofed with Phthalo Green ink + fount/IPA, irradiated, and stored at 20°C, 40°C and 50°C for up to 45 days (Headspace sampling temperature: 105°C).



The amounts of hexanal recovered at 40°C and 50°C are slightly higher than those of the corresponding samples in which no fount/IPA was used. The increase in amounts is more pronounced for samples stored at 50°C. This suggests that the fount/IPA has an influence on the termination reactions of free radicals. Termination reactions are particularly significant for samples stored at 50°C due to the more rapid decomposition of hydroperoxides. Alternatively, hydroperoxide decomposition may be slightly less rapid at the lower pH imparted by the fount buffer system. A lower rate of decomposition results in a lower build up of free radicals and, thus, termination reactions and possibly a higher yield of volatile products.

The amounts of hexanal recovered from samples, at ambient temperatures, are very similar to those for inked samples with no fount/IPA, up to approximately six weeks. After six weeks storage at ambient temperature, a significant increase in the amount of hexanal was witnessed, before the amount declined again.

It appears from the test that the slight increase in humidity of the system, brought about by addition of water with the fount solution, had no effect on the rate of hydroperoxide decomposition. As mentioned earlier, increased solvent polarity within a system can slow down the bimolecular decomposition of hydroperoxides.

The levels of 2-pentyl furan found in samples conditioned with fount/IPA are very similar to those recovered from samples with no fount/IPA.

Benzophenone is still widely used in UV-ink formulations. This is because it is relatively inexpensive, but effective. Problems with respect to taint and odour arise from its volatility and its deposition on to UV lamps on the printing press. This deposition can seriously reduce the output efficiency of the lamps to the point where the lamps have to be replaced. Thioxanthone initiators are less volatile, have very low odour, and are more effective initiators. However, they are more expensive.

Figures 3.115 and 3.116 show the amounts of hexanal recovered from samples of linoleic acid/Koppargloss proofed with a benzophenone containing ink (UCL882) and with a thioxanthone containing ink (UB903), respectively using an equilibration temperature of 105°C. Both inks were manufactured by Coates Lorilleux International.

The hexanal from samples print proofed with the two inks is very similar, implying that the nature of the hydrogen abstraction initiator has little effect on the extent of photoxidation of linoleic acid in this case.

The amounts of hexanal recovered are significantly less than those for the corresponding samples printed with Phthalo Green, or for Koppargloss samples which were not print proofed. This suggests that the free-radical quenching effect on these two inks was somewhat greater than that of the Phthalo Green ink.

Relatively large amounts of hexanal were recovered from the samples stored at 50°C for nearly a week. This suggests that initial photoxidation occurs to a high degree, within both ink systems, resulting in a high degree of hydroperoxide formation. At 50°C, these hydroperoxides break down more quickly to produce a rapid build up of oxidation products, such as hexanal.

Figure 3.115 Amounts of hexanal recovered (in nL) from samples of linoleic acid/ Koppargloss proofed with UCL882 ink, irradiated, and stored at 20°C, 40°C and 50°C for up to 45 days (Headspace sampling temperature: 105°C).

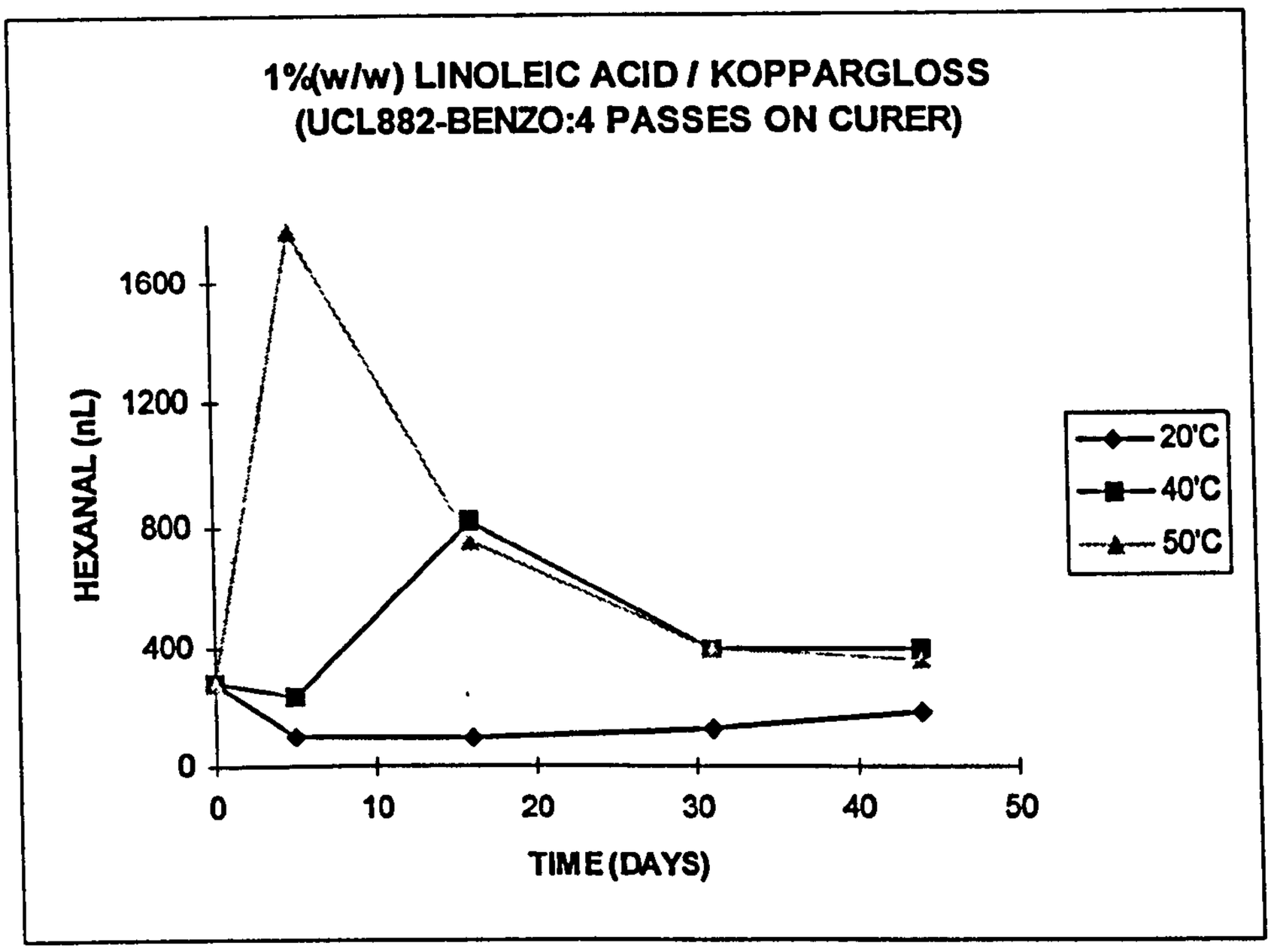
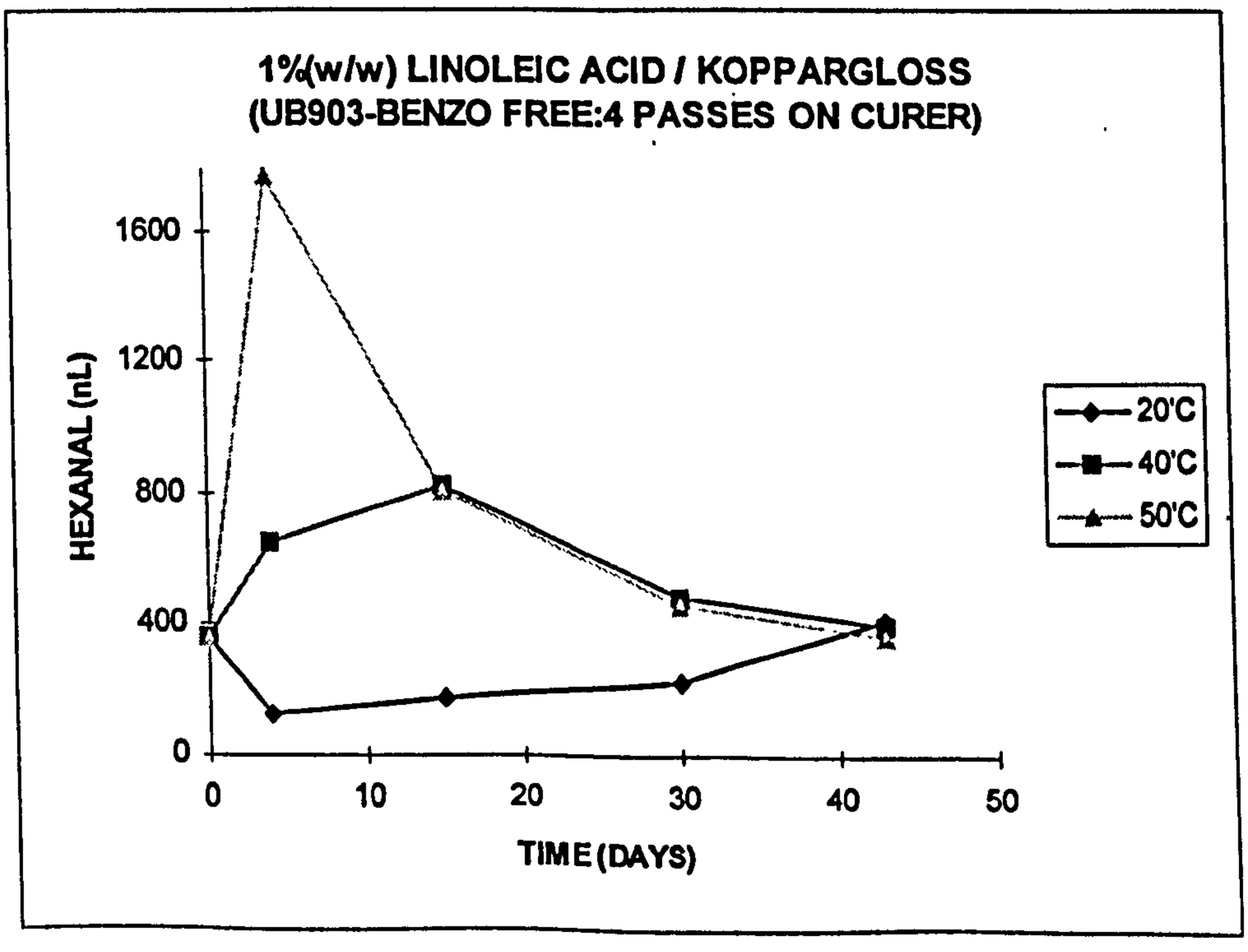
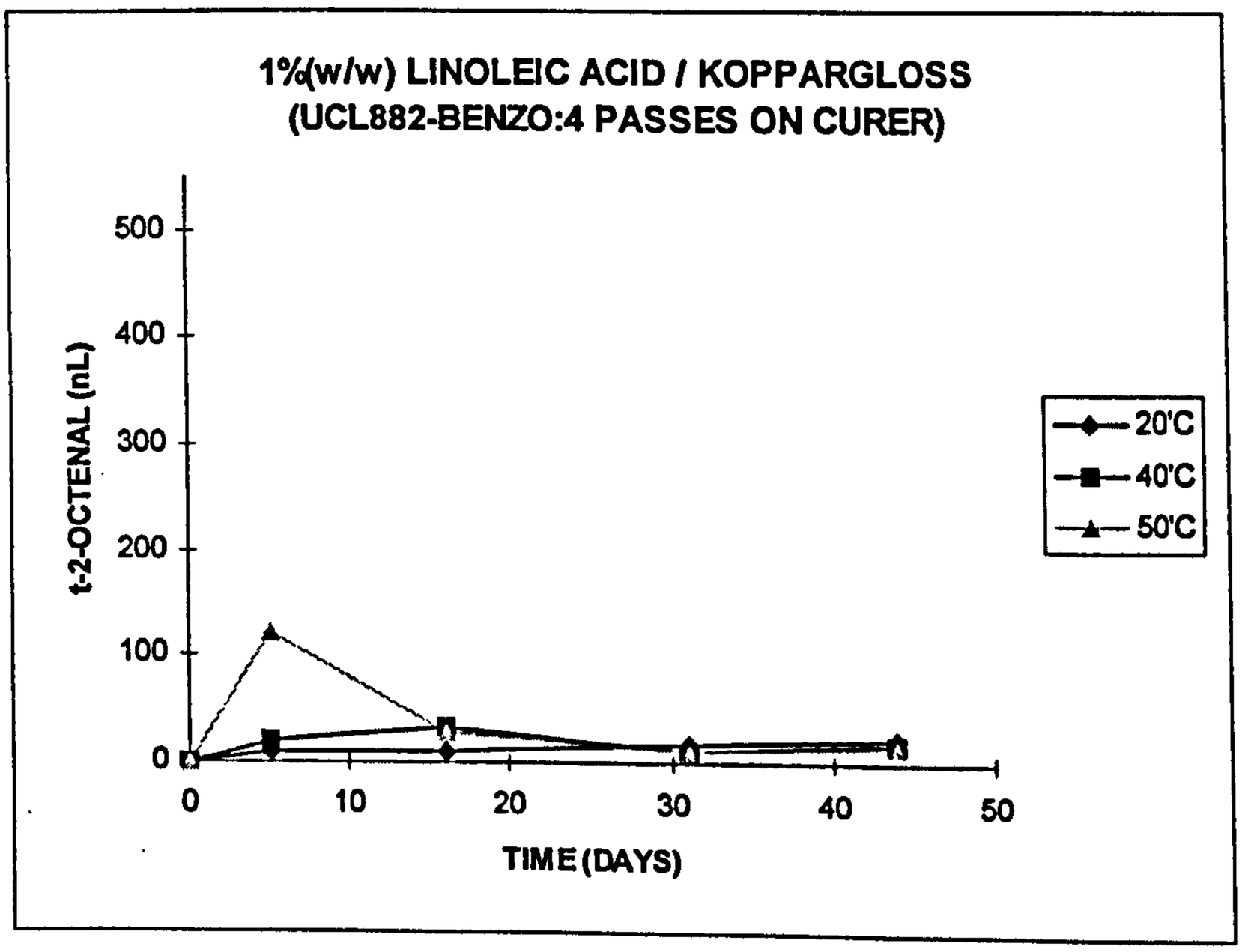


Figure 3.116 Amounts of hexanal recovered (in nL) from samples of linoleic acid/ Koppargloss proofed with UB903 ink, irradiated, and stored at 20°C, 40°C and 50°C for up to 44 days (Headspace sampling temperature: 105°C).



The effect of photoinitiator type on oxidation, with respect to hexanal formation, is insignificant. However, the initiator type may effect the formation of other volatiles. Figures 3.117 and 3.118, respectively, show the amounts of *trans* 2-octenal recovered from samples of linoleic acid/Koppargloss print proofed with the two inks.

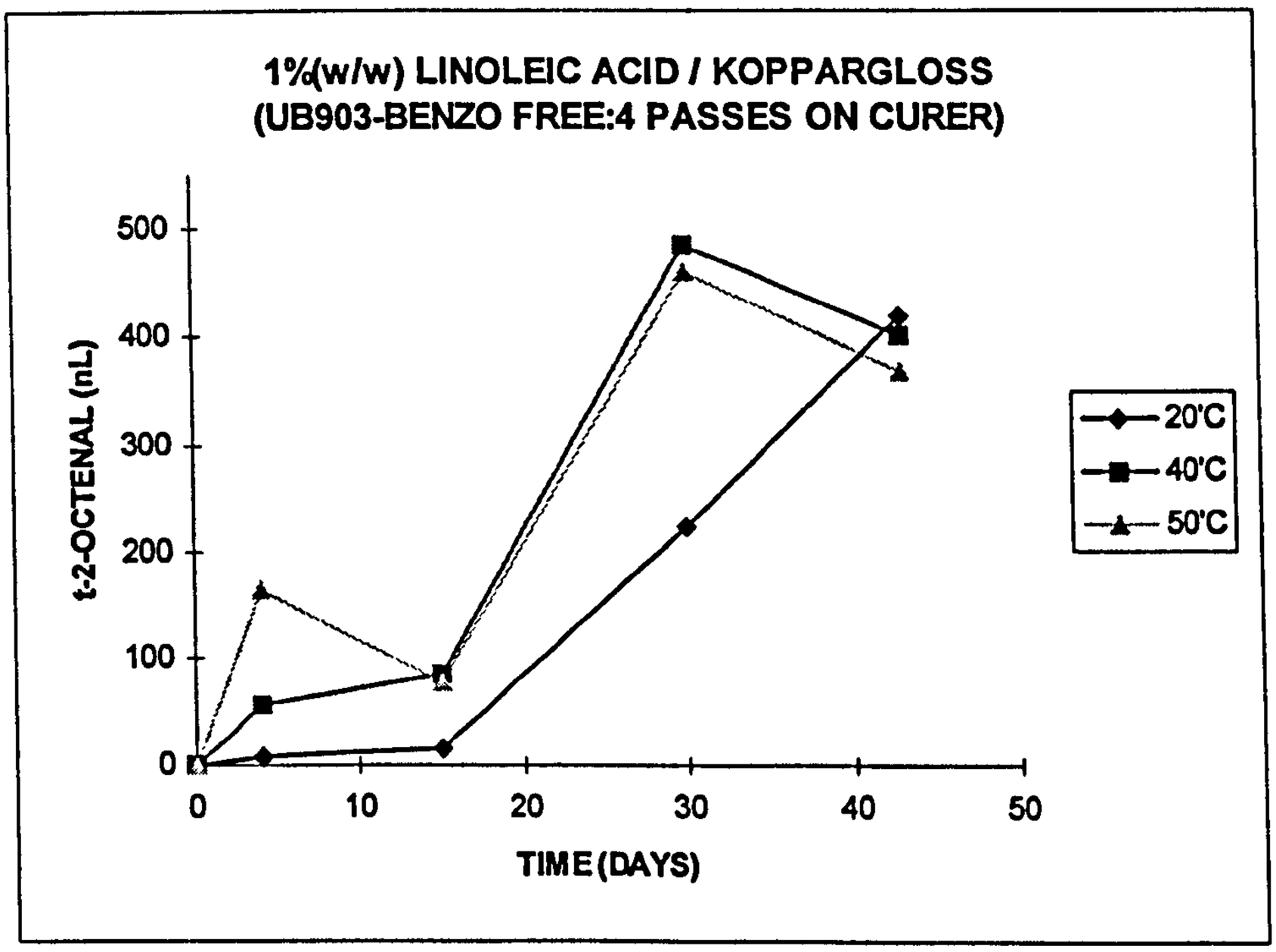
Figure 3.117 Amounts of *trans* 2-octenal recovered (in nL) from samples of linoleic acid/Koppargloss print proofed with UCL882 ink, irradiated, and stored at 20°C, 40°C and 50°C for up to 45 days (Headspace sampling temperature: 105°C).



The amounts of *trans* 2-octenal that were recovered from samples print proofed with the thioxanthone (UB903)-containing ink are significantly greater than those for the benzophenone ink. This suggests that the thioxanthone ink formulation favours the formation of linoleic acid 9-hydroperoxide, and its decomposition to 2,4-decadienal.

In addition, the amounts of octenal were greater than those for linoleic acid/Koppargloss samples print proofed with Phthalo Green ink and samples not treated with ink. This further conflicts with the findings of Tressl *et al.* (1981) in that 2,4-decadienal formation does occur during photoxidation.

Figure 3.118 Amounts of *trans* 2-octenal recovered (in nL) from samples of linoleic acid/Koppargloss print proofed with UB903 ink, irradiated, and stored at 20°C, 40°C and 50°C for up to 44 days (Headspace sampling temperature: 105°C).



The amounts of pentanal recovered from samples proofed with the two inks were very similar. The formation of pentanal over time for each ink system showed a trend that was comparable with that of hexanal, indicating that pentanal formation is in some way linked to the formation of hexanal.

The amounts of 2-pentyl furan levels recovered from samples print proofed with the two inks (UCL882 and UB903) were less than those samples proof printed with the Phthalo Green ink. Slightly less 2-pentyl furan was detected for samples printed with the thioxanthone ink.

A further two inks were tested, one was a benzophenone containing ink and the other contained no benzophenone. These two inks were supplied by Swale Process. A headspace equilibration temperature of 105°C was used for the analysis.

Figures 3.119 and 3.120 show the amounts of hexanal recovered from samples of linoleic acid/Koppargloss print proofed with the benzophenone containing ink and the benzophenone free ink, respectively.

Figure 3.119 Amounts of hexanal recovered (in nL) from samples of linoleic acid/ Koppargloss print proofed with Swale benzophenone containing ink, irradiated, and stored at 20°C, 40°C and 50°C for up to 46 days (Headspace sampling temperature: 105°C).

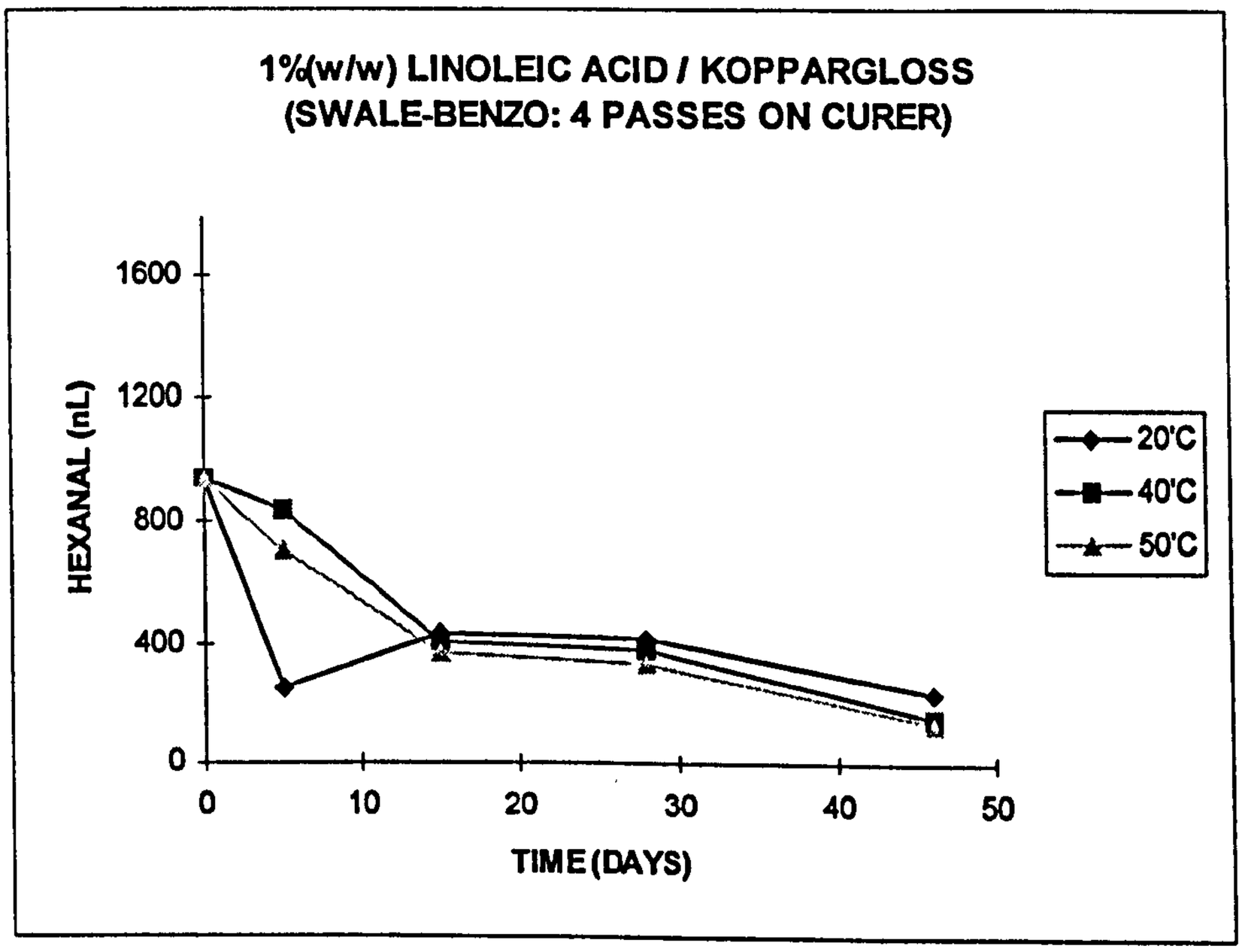
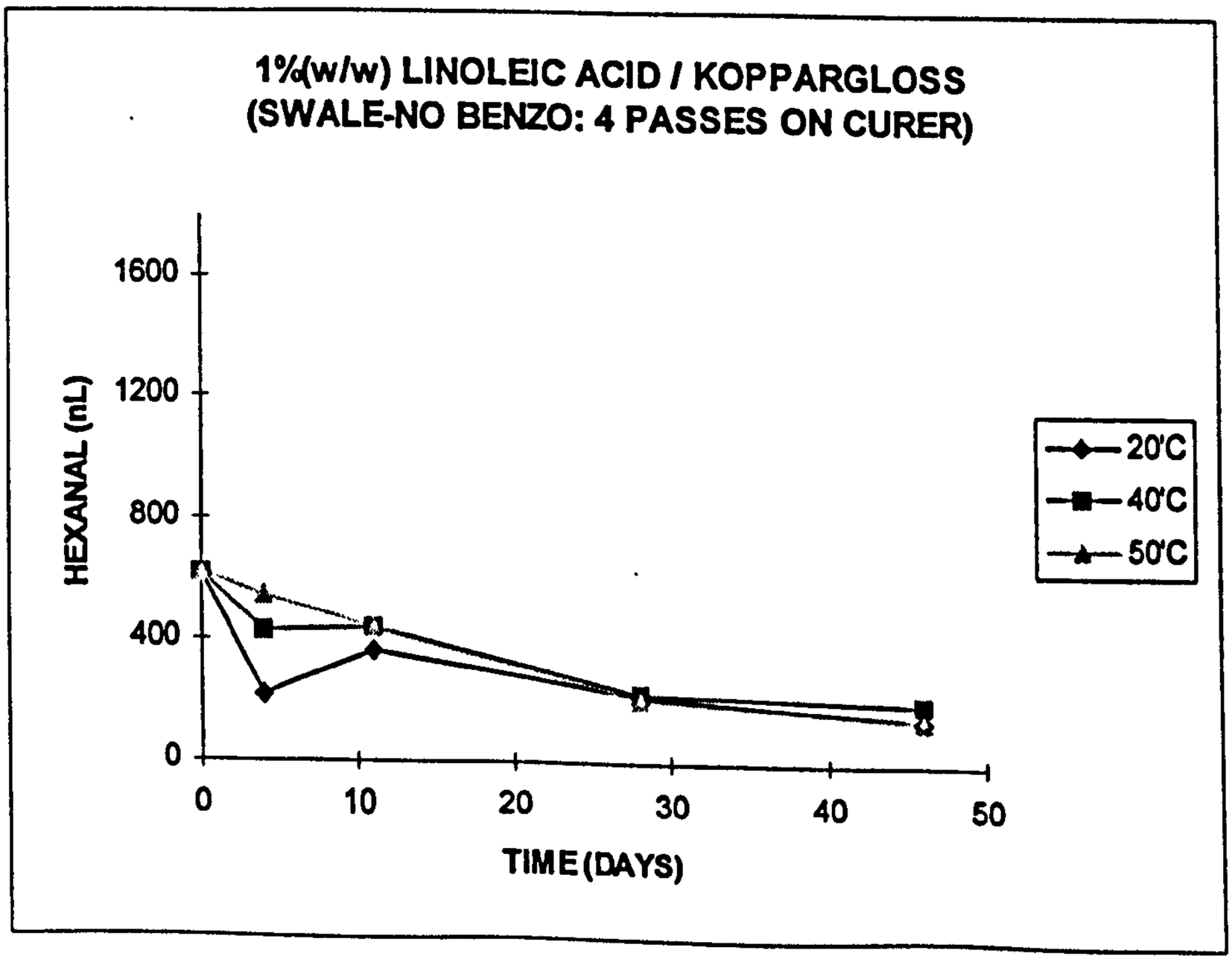


Figure 3.120 Amounts of hexanal recovered (in nL) from samples of linoleic acid/ Koppargloss print proofed with Swale ink containing no benzophenone, irradiated, and stored at 20°C, 40°C and 50°C for up to 46 days (Headspace sampling temperature: 105°C).



Hexanal amounts were generally less than the those obtained using the Coates Lorilleux inks. The highest levels of hexanal were found for the unstored samples which underwent thermal decomposition. Levels, thereafter, appeared to decline with time. Slightly smaller amounts were obtained with samples print proofed with the benzophenone free ink.

No rapid formation of hexanal, from samples proofed with either ink occurs. This suggests that initial rapid hydroperoxide formation did not occur and, therefore, that photoxidation was not so significant. The ink resin film produced from UV-curing may be more impermeable to the diffusion of oxygen. This reduces the amount of oxygen available for oxidation within the Koppargloss matrix. The permeability of the ink film is affected by the wax additives used in the formulation.

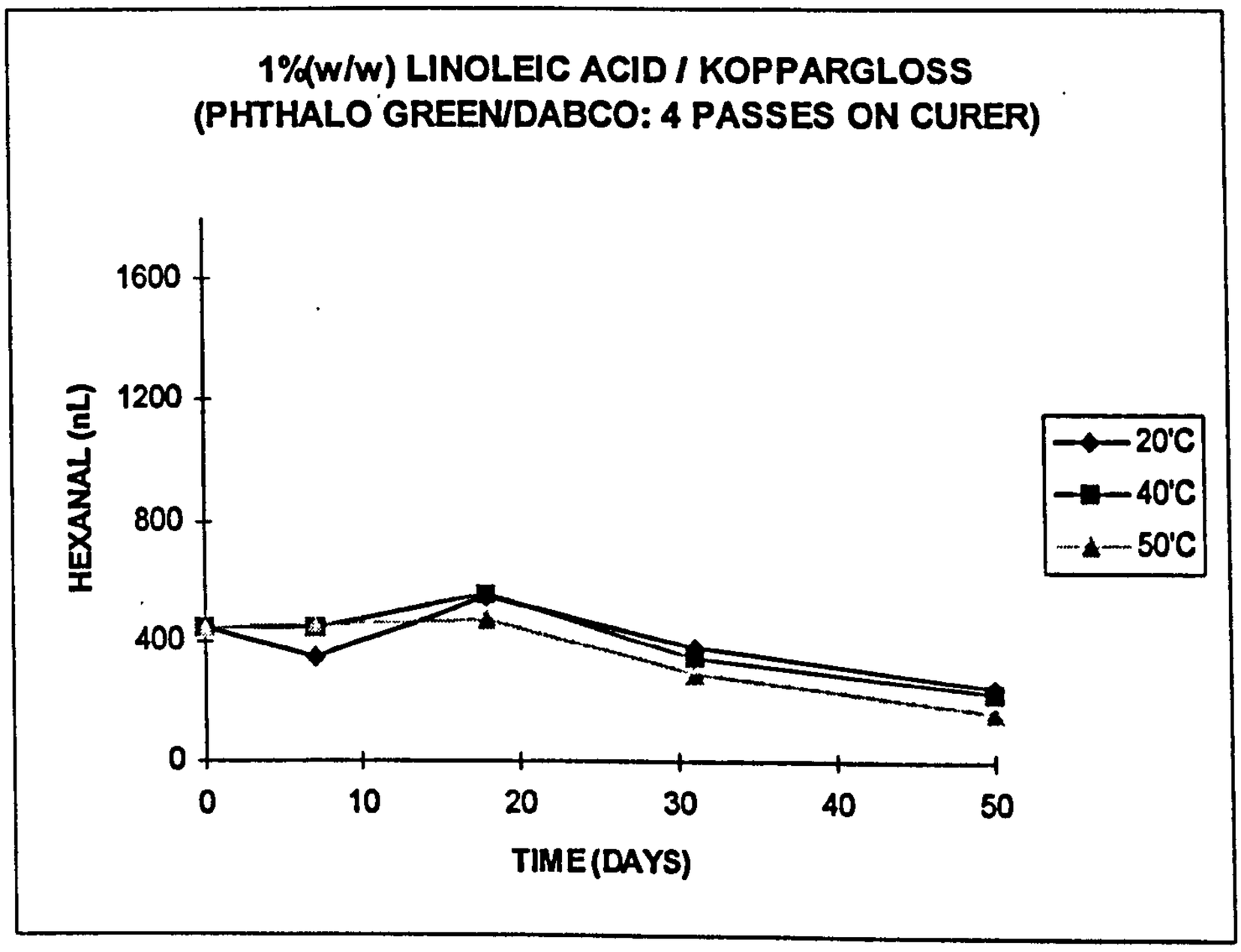
Determination of the extent of photoxidation in linoleic acid/Koppargloss systems print proofed with Phthalo Green ink and irradiated.

Experiments involving the print proofing of some UV-curable inks on to linoleic acid impregnated substrates have shown that photoxidation occurs at a significant level in the UV irradiated systems. To confirm the involvement of singlet oxygen in photoxidation reactions, a test was carried out in which a known singlet oxygen quencher was introduced into an ink-proofed linoleic acid/Koppargloss system that was irradiated. The quencher deactivates the singlet oxygen to the triplet ground state and, thus, removes the possibility of it being involved in photoxidation. Any volatile oxidation products formed can then be attributed to autoxidation alone.

The singlet oxygen quencher used was 1,4-diazabicyclo[1,1,1]octane (DABCO). DABCO quenches singlet oxygen by a charge-transfer mechanism. This reaction involves the interaction of the very electron deficient singlet oxygen molecule with electron donors to give a charge transfer complex. Since singlet oxygen acts as an electron accepting component, easily oxidised compounds, such as DABCO, are effective quenchers.

Figure 3.121 shows the amounts of hexanal, recovered during the test from samples of the linoleic acid/Koppargloss proofed with Phthalo Green ink and irradiated. These samples were applied with DABCO to their upper surface prior to print proofing with the ink.

Figure 3.121 Amounts of hexanal recovered (in nL) from samples of linoleic acid/ Koppargloss applied with DABCO to the upper surface, print proofed with Phthalo Green ink, irradiated, and stored at 20°C, 40°C and 50°C for up to 50 days (Headspace sampling temperature: 105°C).



Compared to the hexanal levels obtained for samples of linoleic acid/Koppargloss proofed with Phthalo Green, in the absence of DABCO, the levels are significantly less. This indicates that DABCO has a marked effect on the formation of volatile compounds from oxidation.

The amounts of hexanal recovered from samples stored at the different storage temperatures were similar. This could indicate that the more rapid production of free radicals, at the higher storage temperatures, resulting from increased hydroperoxide decomposition that occur in other systems not containing DABCO, is being counter-acted by an additional process. This process is considered to be a free radical quenching effect resulting from the DABCO, involving the deactivation of free radical precursors of hexanal and of other volatile compounds.

The free radical quenching effect of DABCO could be supported by the fact that the amounts of hexanal recovered from samples of linoleic

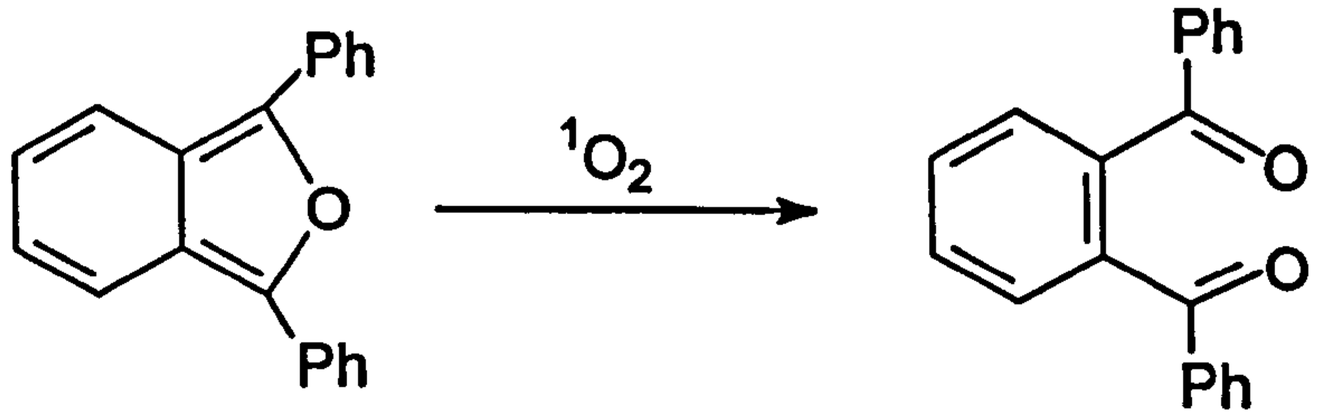
acid/Koppargloss, that were not proofed with ink and where no photoxidation had taken place, were much greater than those samples coated with DABCO.

The amounts of pentanal recovered from samples treated with DABCO were less than those of samples prepared in its absence. This supports the concept that DABCO has an additional effect on decreasing the formation of oxidation volatiles, other than quenching singlet oxygen.

The test involving the use of 1,3-diphenylisobenzofuran (DPBF) as a singlet oxygen indicator confirmed the involvement of singlet oxygen within ink proofed linoleic acid/Koppargloss systems.

Singlet oxygen reacts chemically with DPBF to produce *o*-dibenzoylbenzene, that is colourless. The reaction is shown in Figure 3.122 (Wasserman and Lipshutz, 1979).

Figure 3.122 Reaction of singlet oxygen with DPBF.



1,3-Diphenylisobenzofuran
(BRIGHT YELLOW)

o-Dibenzoylbenzene
(COLOURLESS)

The DPBF impregnated samples of linoleic acid/Koppargloss print proofed with Phthalo Green ink, with a bright yellow colouration, were bleached significantly, to a paler yellow, during UV irradiation on the belt-curer.

The print proofed Koppargloss samples were also visually assessed for the intensity of the yellow colouration prior to headspace analysis and just after headspace analysis. It was found a pale yellow colouration was evident in stored samples, prior to headspace analysis, up to 33 days at 20°C, up to 18 days at 40°C, and up to 5 days at 50°C. At longer storage periods, the DPBF was bleached colourless.

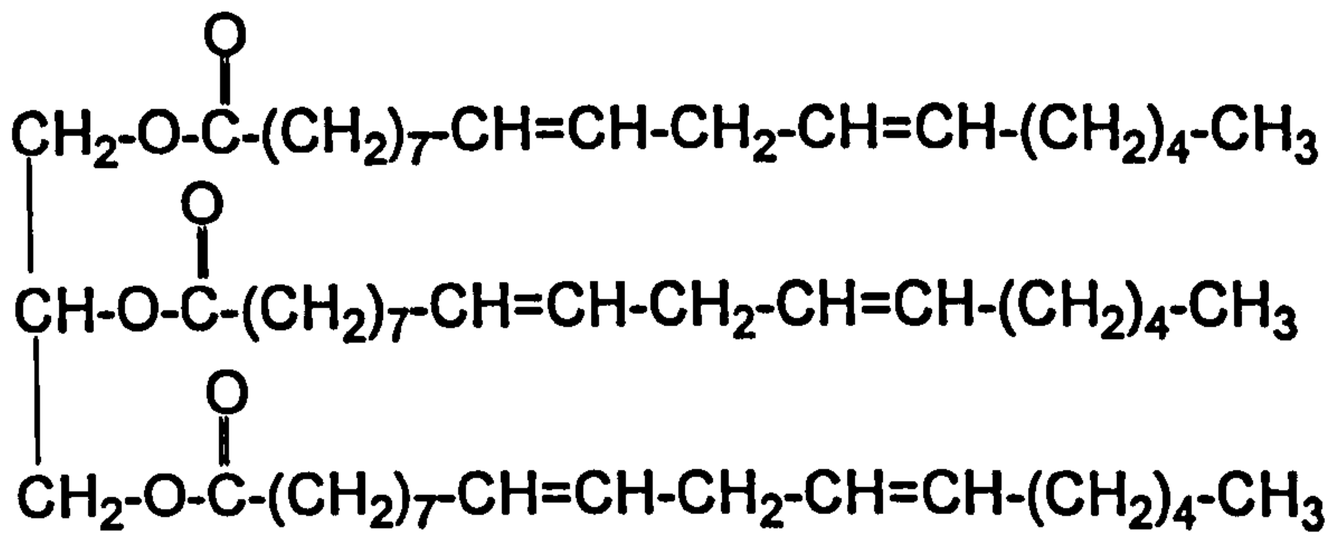
These findings suggest that some singlet oxygen activity occurs after the UV irradiation stage when samples are stored in the dark. The activity of singlet

oxygen is temperature dependent, taking five days to bleach DPBF colourless at 50°C and 33 days to bleach DPBF colourless at 20°C.

After headspace analysis, the remaining yellow colouration was bleached colourless for all samples, regardless of the storage temperature, further indicating the action of singlet oxygen under dark conditions.

Determination of the effect of time and temperature on the presence of trilinolein in Koppargloss carton-board

Softwoods contain about 0.3-0.4% of fats by weight (Fengel and Wegener, 1984). A large proportion of this fat component is present in the form of triglycerides. The triglyceride form of linoleic acid is trilinolein. Trilinolein has the following molecular structure:

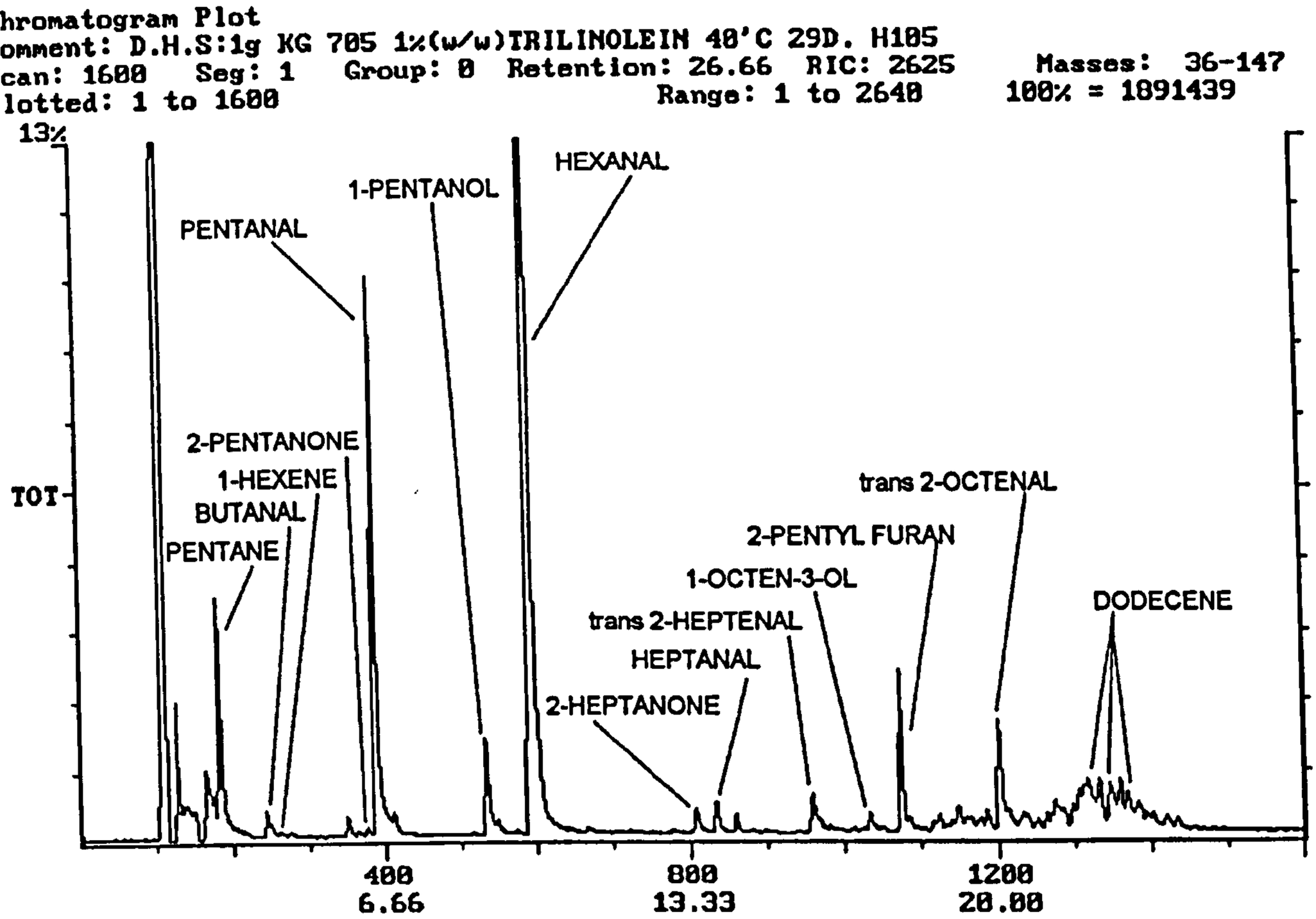


The molecule consists of three linoleate chains joined to a glycerol backbone by means of ester linkages. Unsaturated triglycerides, such as trilinolein, tend to be oils at room temperature.

Trilinolein oxidises to produce a similar yield of volatile compounds as do linoleic acid and its ester derivatives (Ellis *et al.*, 1968, Selke *et al.*, 1980, and Grosch, 1987). To determine whether this is true for trilinolein, within the carton-board system, samples of Koppargloss were impregnated with trilinolein and stored, as described earlier.

Figure 3.123 shows the chromatogram acquired from the headspace vapours of a sample of trilinolein/Koppargloss stored for 29 days at 40°C. The major volatile oxidation products from trilinolein within the Koppargloss system are similar to those for the linoleic acid system. This concurs with the findings of previous workers.

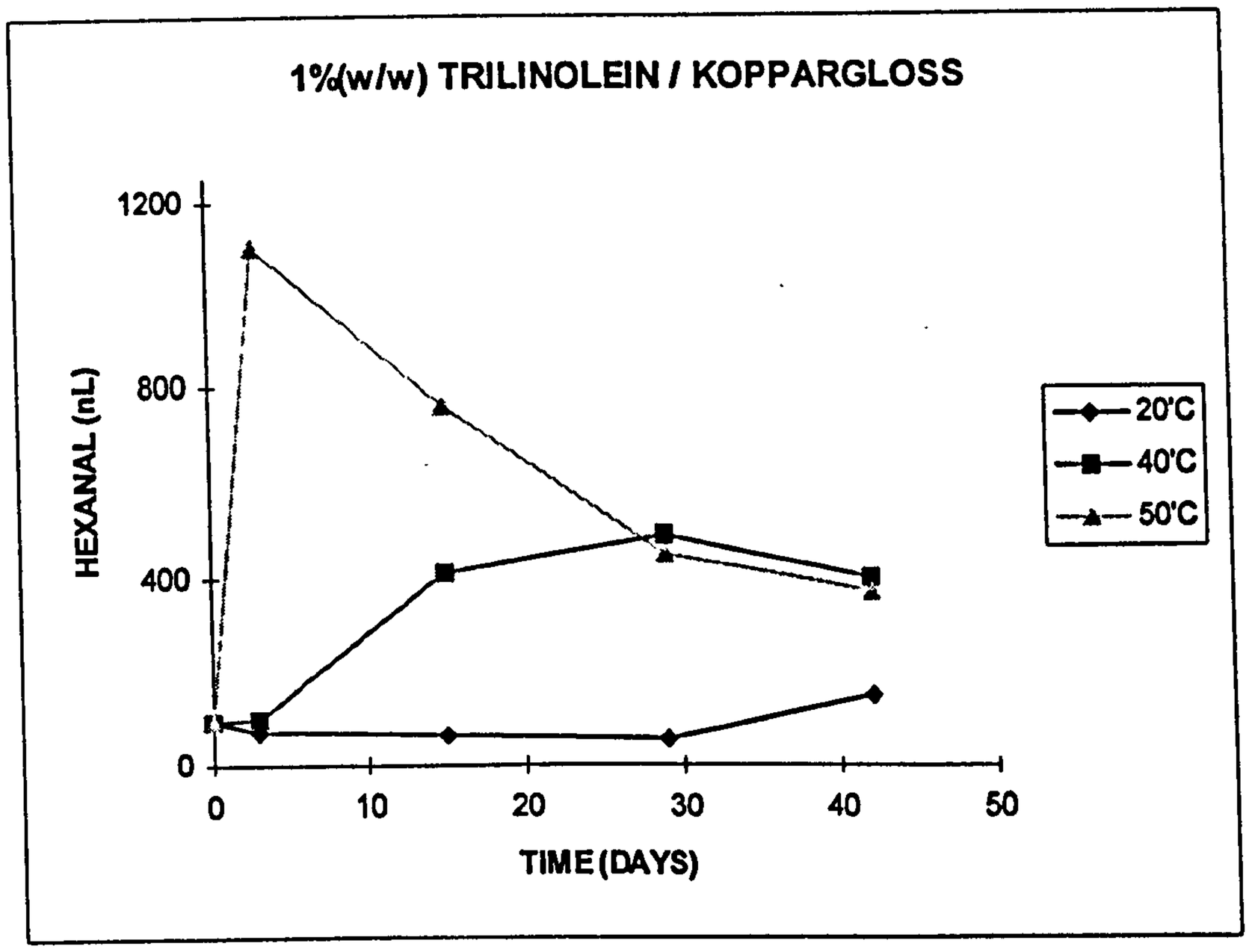
Figure 3.123 Chromatogram acquired from the headspace vapours of a one gram sample of Koppargloss/1%(w/w) trilinolein that had been stored, at 40°C, for 29 days.



As hexanal is still the major volatile product of oxidation, its formation against time is a good indicator of the general oxidation extent of the system. Figure 3.124 shows the amounts of hexanal recovered from samples of trilinolein/Koppargloss during the test, using a headspace equilibration temperature of 105°C.

The amounts of hexanal recovered are significantly smaller than those for linoleic acid/Koppargloss. This suggests that the triglyceride is more stable to oxidation than is the free acid. A rapid build up of hexanal is witnessed from the sample stored for 3 days at 50°C. This suggests that hydroperoxide formation within the system is reasonably rapid at this temperature. The small amounts of hexanal recovered from samples stored at ambient temperature suggest that hydroperoxide formation is markedly slower for trilinolein at ambient temperature.

Figure 3.124 Amounts of hexanal recovered (in nL) from samples of trilinolein/Koppargloss stored at 20°C, 40°C and 50°C for up to 42 days (Headspace sampling temperature: 105°C).



The small amount of hexanal recovered from the sample analysed immediately after preparation, compared to the corresponding linoleic acid/Koppargloss sample, confirms that trilinolein is more stable to thermal decomposition.

The amounts of *trans* 2-octenal and pentanal recovered from trilinolein samples were less than those for the corresponding linoleic acid samples.

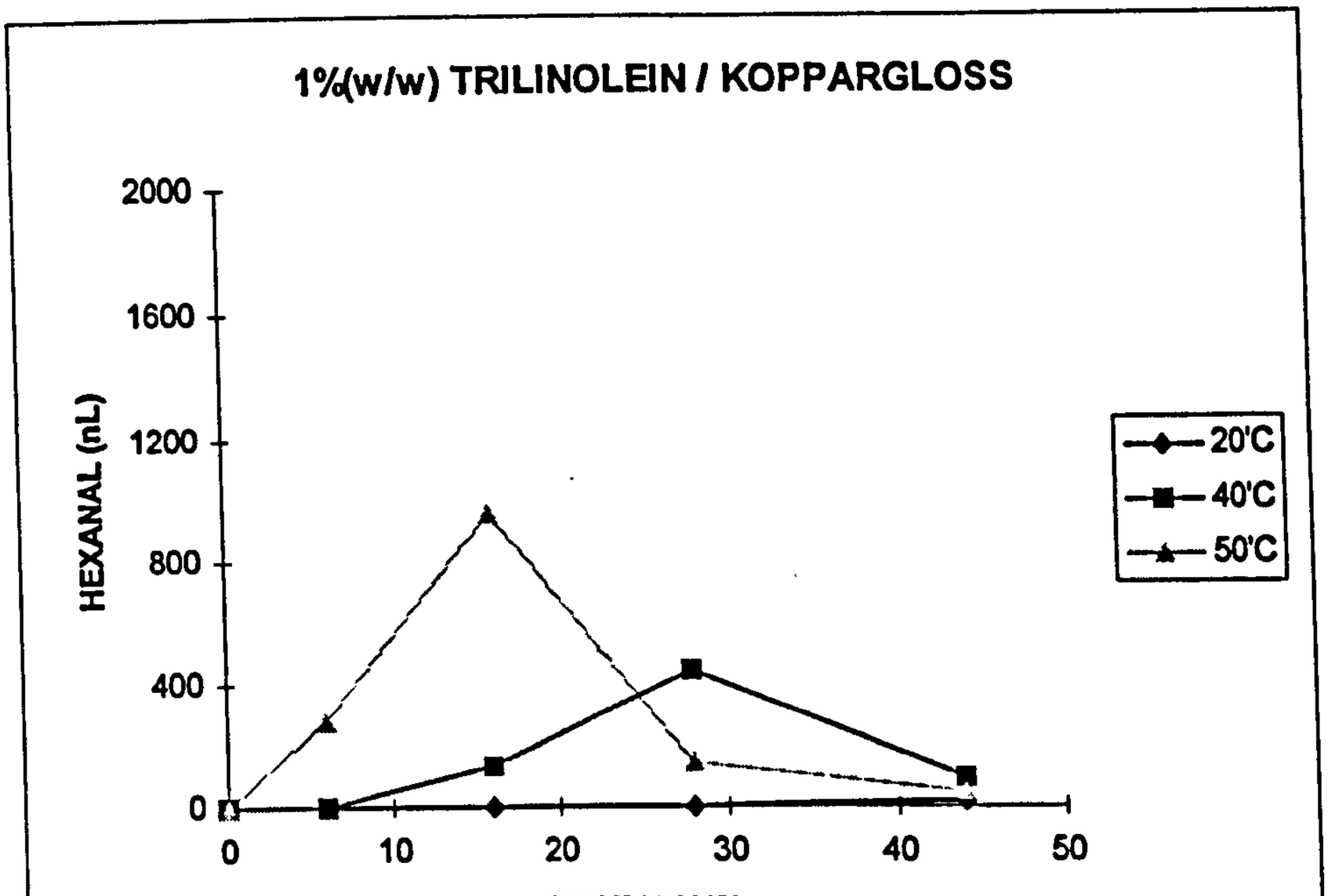
During the headspace equilibration period at 105°C, decomposition of the triglyceride to the free acid form occurs. To determine the effect this decomposition had on oxidation rate and extent of trilinolein, the test was repeated using a headspace equilibration temperature of 50°C.

Figure 3.125 shows the amounts of hexanal recovered from samples of trilinolein/Koppargloss during the test using an equilibration temperature of 50°C.

The amounts of hexanal for these samples are substantially less than for trilinolein samples equilibrated at 105°C, and much less than that of linoleic acid/Koppargloss samples equilibrated at 50°C. This indicates that oxidation occurs at a significantly slower rate for the triglyceride than for the free fatty acid, particularly when the triglyceride is not broken down to the free acid form

at the elevated equilibration temperature. The amounts of pentanal formed from samples were also very small.

Figure 3.125 Amounts of hexanal recovered (in nL) from samples of trilinolein/Koppargloss stored at 20°C, 40°C and 50°C for up to 42 days (Headspace sampling temperature: 105°C).



3.3.3 DETERMINATION OF THE EFFECT OF TIME AND TEMPERATURE ON THE FORMATION OF ODOROUS COMPOUNDS FROM PHOTOINITIATORS IN UV-CURABLE INKS

3.3.3.1 EXPERIMENTAL

Photoinitiators are fairly reactive species by their nature. To determine whether the storage time and the storage temperature has an effect on the rate of breakdown of the photoinitiator, a series of tests was carried out in which samples of photoinitiator were stored in 22 cm^3 glass headspace vials for up to eight weeks, at storage temperatures of 20°C, 40°C and 50°C. The volatiles present in the vials at the end of the storage period were sampled by static headspace analysis, at a headspace equilibration temperature of 105°C.

The GC method was altered slightly to accommodate the longer retention times of the analytes. The column oven temperature programme is shown was:

INJECTOR TEMP.	150	°C
COLUMN OVEN INITIAL TEMP.	40	°C
COLUMN OVEN FINAL TEMP.	238	°C
COLUMN RUN TIME	44	min.
TEMP.RISE	4.5	°C/min.
GC/MS TRANSFER LINE TEMP.	240	°C

The mass spectrometer was set up to scan for ions in the range of 35m/z to 240m/z.

Three photoinitiators were analysed: benzophenone, isopropyl thioxanthone and 1-phenyl-1,2-propanedione-2-(o-ethoxy carbonyl)oxime (Quantacure PDO). The initial two initiators are hydrogen abstraction initiators and the last a cleavage initiator.

The following tests were carried out:

- 200 mg benzophenone stored alone for up to 48 days at 20°C, 40°C and 50°C.
- 200 mg isopropyl thioxanthone stored alone for up 48 days at 20°C, 40°C and 50°C.
- 200 mg benzophenone + 100 mg ethyl 4-(dimethylamino)benzoate (photoactivator) stored for up to 56 days at 20°C, 40°C and 50°C.
- 200 mg isopropyl thioxanthone + 100 mg ethyl 4-(dimethylamino)benzoate stored for up to 56 days at 20°C, 40°C and 50°C.
- 200mg Quantacure PDO stored for up to 48 days at 20°C, 40°C and 50°C.

3.3.3.2 RESULTS

The initial two tests involved storage of benzophenone and isopropyl thioxanthone for up to 48 days at 20°C, 40°C and 50°C.

The chromatograms acquired for the headspace vapours of these samples showed very little change over the test period. Samples were tested at 6, 17, 29 and 48 days. The chromatograms acquired for the benzophenone-containing samples showed only one component, benzophenone. It was noted that the peak area of the benzophenone recovered increased with increasing storage temperature. This indicates the lower vapour pressure of this compound at ambient temperatures. The chromatograms acquired for the isopropyl thioxanthone samples did not show up the thioxanthone. This indicates the low volatility of this compound. Only the one peak for toluene was evident for these samples. Toluene was probably a residual solvent from the synthesis of the isopropyl thioxanthone in a solvent system.

Both the above tests showed the stability of these two photoinitiators in an isolated system at storage temperatures up to 50°C. In addition, no degradation was evident during the headspace equilibration period at 105°C.

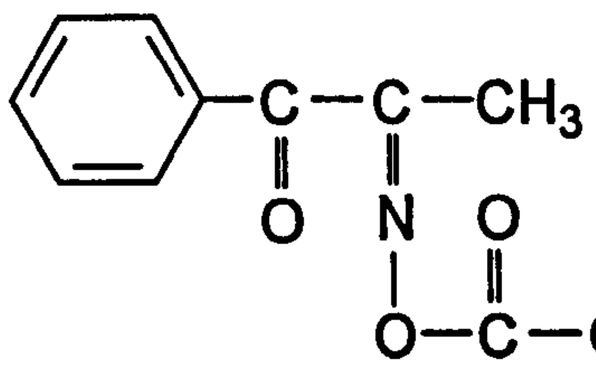
The above tests were repeated with the addition of the photoactivator, ethyl 4-(dimethylamino)benzoate. Samples were tested at 4, 16, 37 and 56 days.

From the chromatograms for the benzophenone-based samples, two peaks were evident initially, that of benzophenone and that of the amine compound. The peak for the amine was small and not well resolved, indicating the low volatility of this compound at the temperatures existing during the test. After 4 days storage at 50°C an additional peak was found. The compound was probably an amine. This compound was measured at 16 days for the samples stored at 40°C. However, it was not found in samples stored at 20°C. As the peak area of this compound increased, the peak area of the amine photoactivator decreased, indicating the breakdown of this photoactivator. At eight weeks storage, no ethyl 4-(dimethylamino)benzoate was recovered from samples stored at 40°C and 50°C.

The chromatograms acquired from samples of isopropyl thioxanthone and ethyl 4-(dimethylamino)benzoate showed the presence of only two components throughout the test, that of toluene and that of 4-(dimethylamino)benzoate. No additional products formed and recovery of the amine photoactivator did not decline with time. This indicates that the breakdown of the amine activator in the benzophenone system involved benzophenone since ethyl 4-

(dimethylamino)benzoate was found to be stable in the isopropyl thioxanthone system.

A final test was carried out using the cleavage initiator 1-phenyl-1,2-propanedione-2-(o-ethoxy carbonyl)oxime (Quantacure PDO), for which the molecular structure is illustrated:



Quantacure PDO

Samples were tested at 5, 17, 30 and 44 days. The chromatograms acquired from samples indicated the presence of a number of volatile compounds, including 2,2-dimethylbutane, 3-methylbutylhydroxylamine, 1-hexene, hexane, methyl cyclopentane and cyclohexane. After 5 days storage at 50°C, small amounts of methyl benzoate and ethyl benzoate were found. These two compounds were found in samples stored at 20°C and 40°C after 17 days. The photoinitiator itself has almost no odour. However, the taint implications of the presence of these two ester compounds would be significant.

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4 METHODS FOR THE ISOLATION AND CONCENTRATION OF VOLATILE COMPOUNDS FROM CARTON-BOARD MATERIALS

4.1 INTRODUCTION

Some odorous compounds need only be present at in very small amounts to cause a deterioration in the organoleptic quality of a carton package. Static headspace techniques provide a high amount of information regarding volatile components of a system, though the technique is not sensitive at very low levels. This is due to the nature of the system. Once equilibrium has been achieved within the headspace system, no net transfer of volatile compounds to the gaseous phase occurs. As a result, only a small proportion of the volatile compounds within the analyte are sampled. In addition, some very volatile components may be lost from the system during introduction of the headspace vapours into the GC. This occurs during the sample loop fill stage, when carrier gas purges the headspace vapours out of the vial into the open sample loop. Some volatiles may escape out of the open end of the sample loop to the atmosphere.

Another drawback of the static headspace system is the possibility of thermal degradation of analyte components during any thermal equilibration period prior to sampling.

In order to detect and characterise volatile compounds that are present at a very low level, techniques of sample concentration are needed. These techniques also offer conditions which are less favourable to thermal degradation occurring in the presence of oxygen.

A commonly used concentration technique for the study of odorous volatile compounds from packaging materials is the dynamic headspace method (Söderhjelm and Eskelinen, 1985). The dynamic headspace procedure, or purge and trap, involves the trapping of much larger volumes of headspace volatiles to obtain sufficient material for analysis. Methods by which volatiles are stripped from the sample by continuous flow of an inert gas can be prefixed by the term 'dynamic headspace'.

Vacuum distillation extraction procedures can be employed as a technique of concentrating and trapping larger volumes of headspace volatiles. Under the

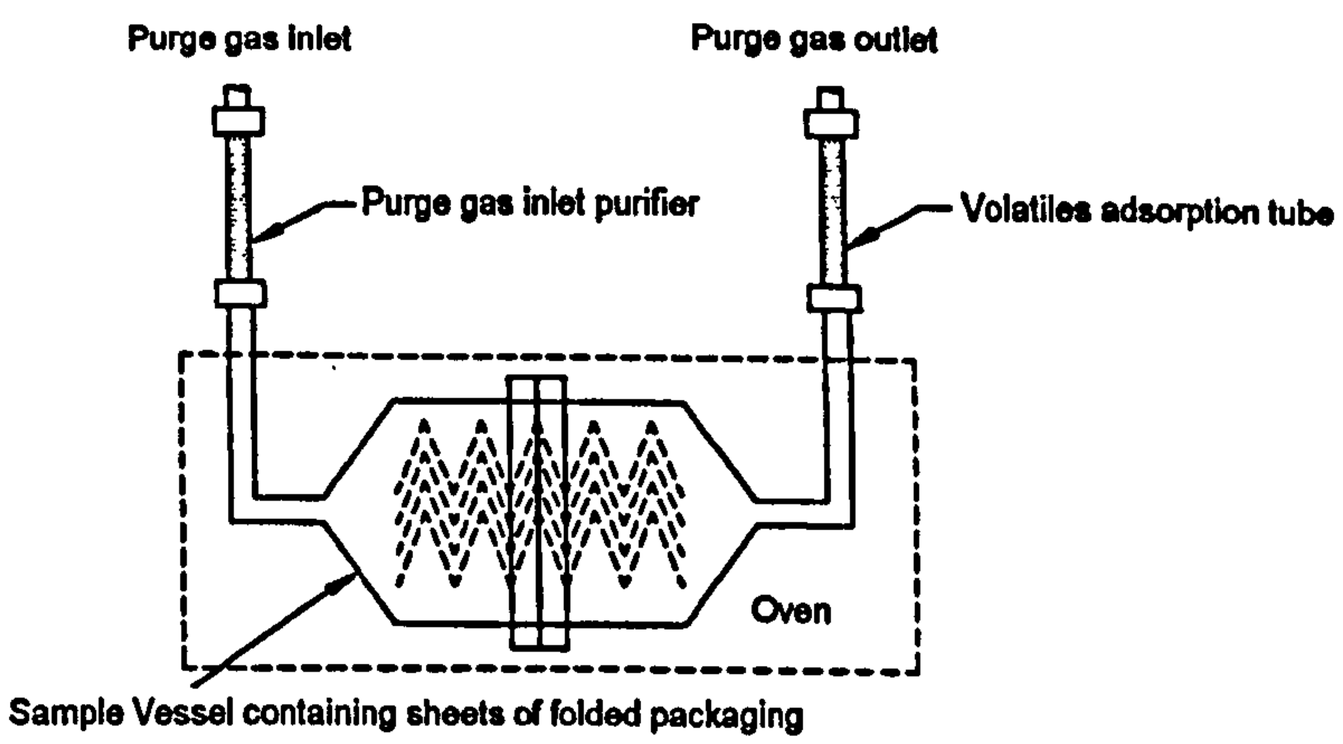
lower pressures involved in the technique, volatile compounds have a greater tendency to escape to the vapour phase. This characteristic of volatile compounds can be used to extract the volatiles from the packaging material in which they reside.

4.2 DYNAMIC HEADSPACE ANALYSIS

In dynamic headspace analysis, the volatile components within a sample matrix are removed by a purging gas. This purging gas passes through a solid adsorbent where the volatiles within the gas are adsorbed and trapped. After this purge cycle, the trap is rapidly heated and the volatiles within the trap are thermally desorbed. These desorbed volatiles are swept into the into the inlet of the GC.

Figure 4.1 shows a very simple purge and trap device. The apparatus consists of a purge gas inlet purifier, a heated sample chamber, and an adsorption tube (Tice, 1993).

Figure 4.1 Diagram of a simple purge and trap device.



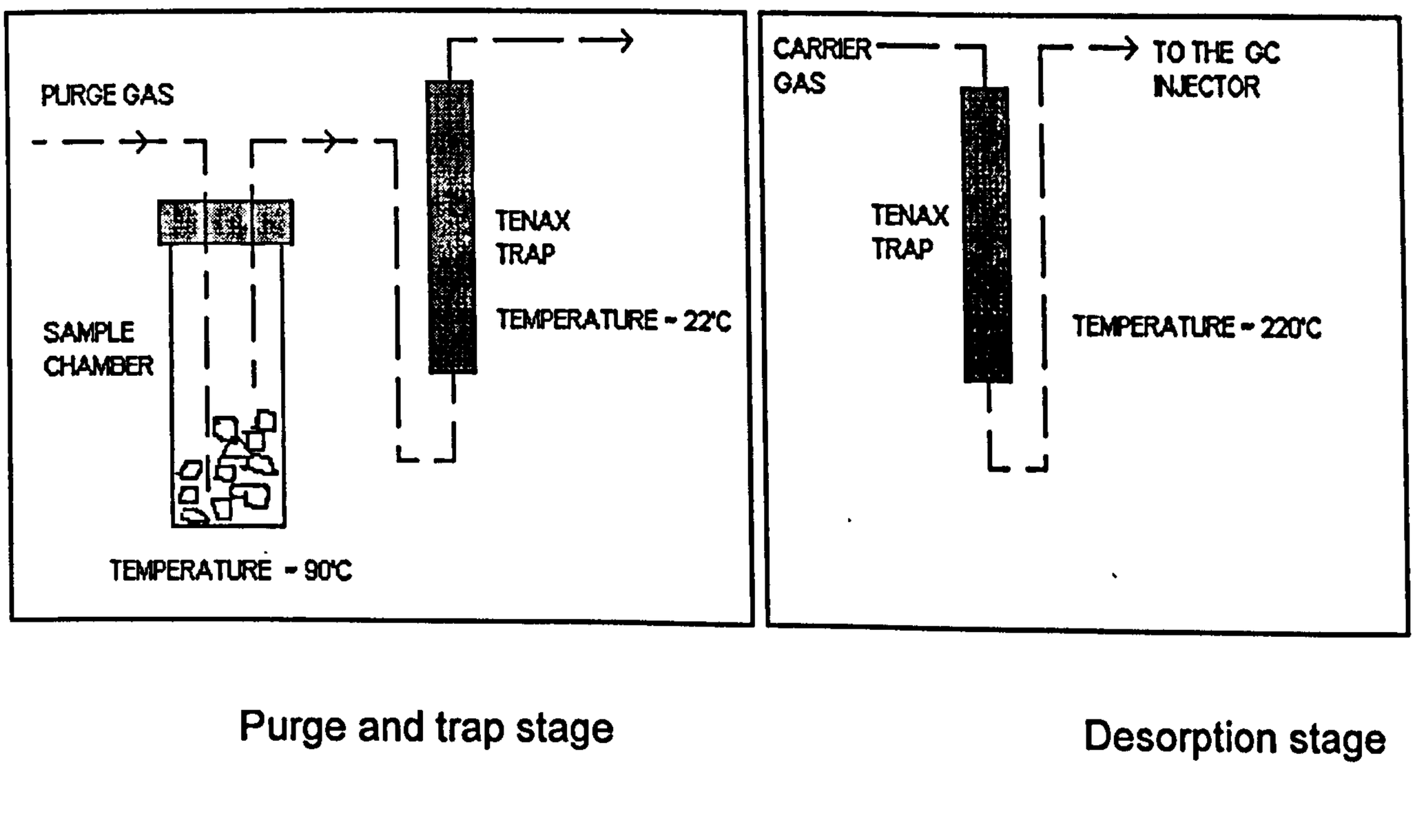
The sample chamber containing the packaging sample may be heated to increase the amounts of volatiles entering the vapour phase. Transfer of the trapped volatiles in the above illustrated system may be achieved by thermal desorption, or by solvent elution and injection as a solvent system.

Studies of volatile compounds have used Tenax GC as a polymer adsorbent for the trapping of volatiles (Boland *et al.*, 1984; Dirinck *et al.*, 1984; Söderhjelm

and Eskelinen, 1985), Chromosorb 105 (Maarse, 1993) and charcoal (Grob and Zürcher, 1975; Borén *et al.*, 1982 and 1985).

The principle of the purge and trap device used by Söderhjelm and Eskelinen (1985) is shown in Figure 4.2. About 2 grams of the material to be tested are kept in a 100 cm^3 serum bottle at 90°C for 10 minutes. The sample is then purged with helium gas, at a flow rate of 50 cm^3/min., and the volatiles collected on the trap consisting of Tenax resin and silica gel. The trap is then rapidly heated to about 220°C and the volatiles thermally desorbed for 4 minutes into the inlet of the GC. Before reuse, the trap is baked at 250°C for 7 minutes to remove all contaminants and reduce carry-over effects from one analysis to the next. If further concentration of volatiles is needed, a cold trap, cooled with liquid nitrogen, can be positioned after the Tenax trap in the purge line.

Figure 4.2 The purge and trap principle.



Wyatt (1986) reports the use of two solid adsorbent traps within the purge and trap system. The first trap, after the sample chamber, is a large bore trap consisting of a series of adsorbent materials. After the purge stage, the flow through the large bore trap is reversed and heat is applied to the trap. The volatiles are desorbed from the trap and proceed to a small bore trap (trap to trap transfer). After the transfer to the small bore trap is complete, thermal desorption is repeated and volatiles are flushed into the inlet of the GC.

The purge and trap technique is a continuous gas extraction in which an exhaustive purge of the sample transfers the analytes from the sample to the adsorbent trap. Recovery of analytes is highly dependent upon the purge efficiency of the analytes. Purge efficiency does not take into account the desorption and transfer efficiencies. These are taken into account by the percentage recovery which also takes into account analyte solubility and vapour pressure. The percentage recovery can be expressed as follows:

% Recovery = A/B x 100

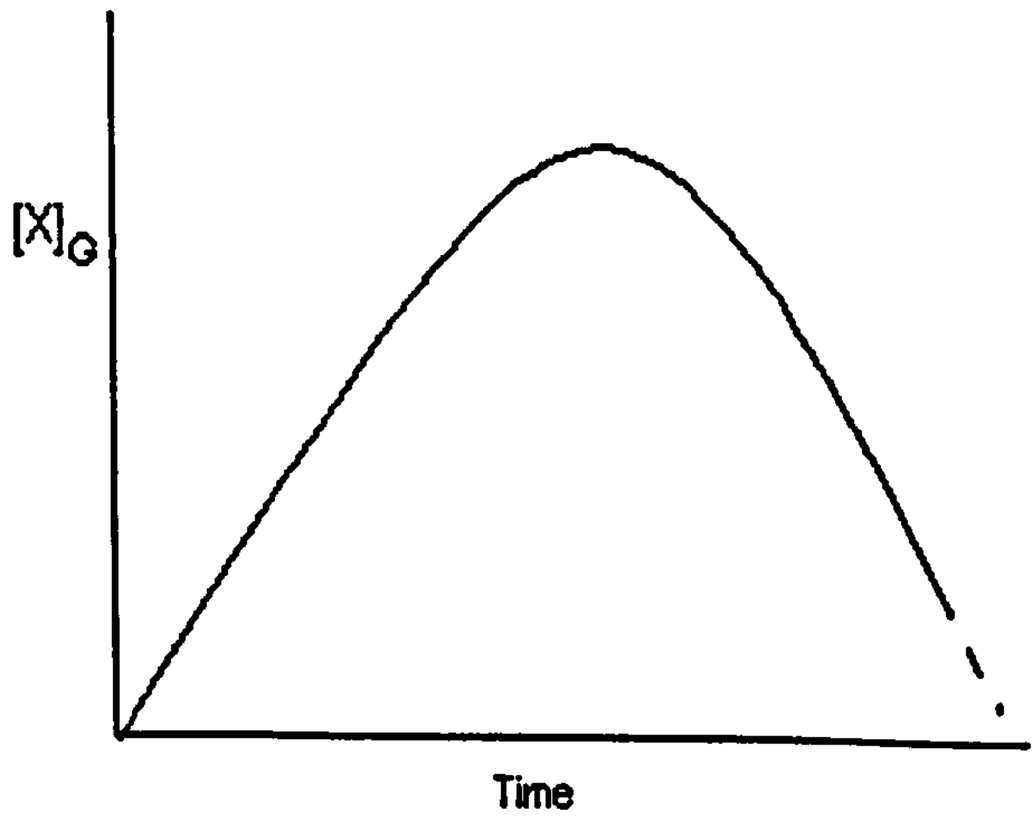
where:

A = the peak area of the analyte after purge and trap

B = the peak area of the analyte after direct injection

The total amount of volatile component removed from the sample is proportional to the purge volume and to the purge efficiency. The purge volume is a function of purge flow rate and the purge time. Figure 4.3 shows a typical plot of the concentration of analyte in the gas phase ($[x]_G$) against the purge time. The plot shows that, as the purge time and purge volume increase, ($[x]_G$) increases to a maximum before declining. When $[X]_G = 0$, 100% recovery of the volatile analyte has been achieved.

Figure 4.3 A typical plot of $[x]_G$ against time for the purge cycle.



The static headspace procedure never permits 100% recovery of volatile analytes. Figure 4.4 shows the plot of the concentration of analyte in the gas

phase ($[x]_G$) against time for the static headspace equilibration cycle. The plot indicates that once equilibrium has been achieved, further net recovery of volatiles cannot be achieved. In fact, $[x]_G$ may decline after a time, due to thermal degradation of the analyte. The sensitivity of static headspace analysis is limited by the sample matrix, the injection volume and the partition coefficient (K) of the analyte.

Raoult's law states that the vapour pressure of a dissolved solute (i.e. its partial pressure p_i) over its solution is directly proportional to its mole fraction in the solution $x_{s(i)}$ and the proportionality constant is the vapour pressure p°_i of the pure analyte (i.e. when $x_{s(i)} = 1$):

$$p_i = p^\circ_i \cdot x_{s(i)}$$

Raoult's law is only valid for ideal mixtures and, in most cases, there is a deviation from this law. To compensate for this deviation, another factor is introduced in to the above equation:

$$p_i = p^\circ_i \cdot \gamma_i \cdot x_{s(i)}$$

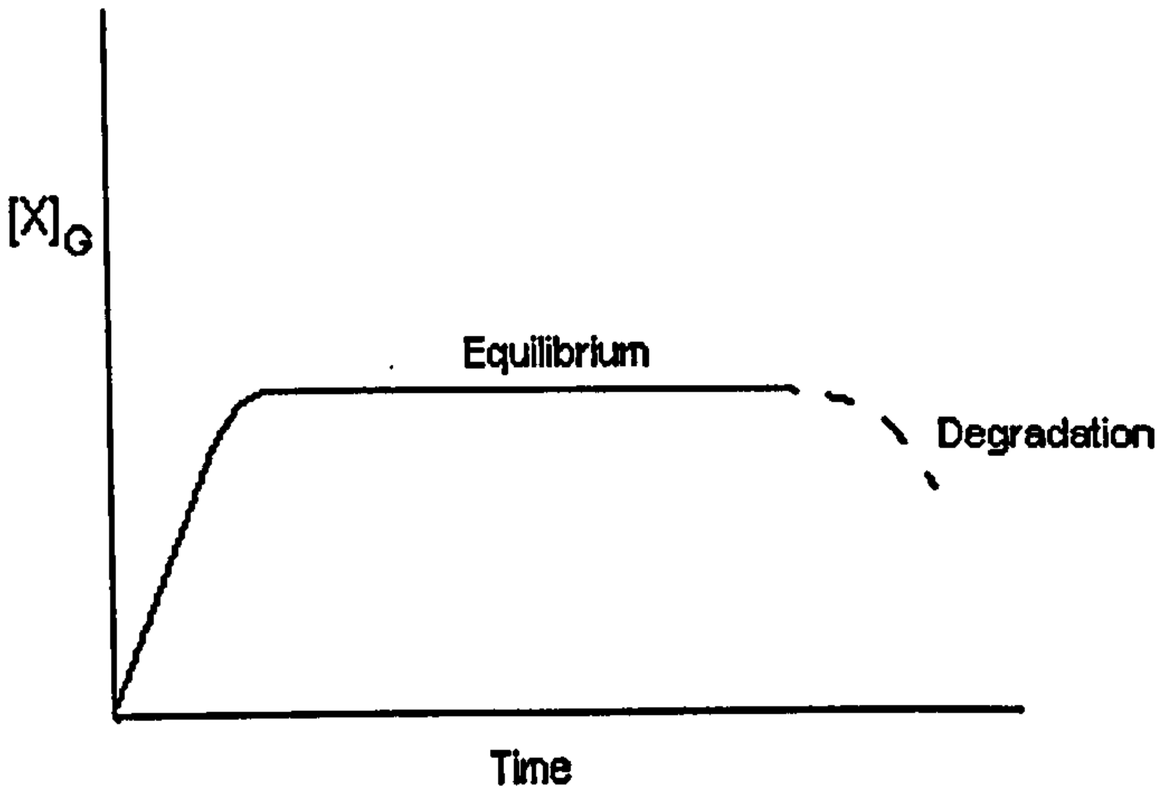
This parameter is the activity coefficient (γ_i) of compound i. The activity coefficient is considered as a correction factor to concentration (molality), which modifies it to true 'active concentration'.

The value of the activity coefficient depends on the nature of component i and reflects the intermolecular interaction between analyte and the other sample components, particularly the sample matrix. This is the so-called matrix effect (Kolb and Ettre, 1997). The ideal dilute system can be represented by Henry's law. In this ideal system each dissolved analyte molecule is surrounded only by solvent molecules. The partition coefficient, K, can be expressed as follows:

$$K \propto 1/ p^\circ_i \cdot \gamma_i$$

The partition coefficient is proportional to the reciprocal of the vapour pressure and to the activity coefficient of the analyte. Increasing these values decreases the value of the partition coefficient and increases $[x]_G$.

Figure 4.4 A typical plot of $[x]_G$ against time for the static headspace equilibration cycle.



4.2.1 DEVELOPMENT OF THE DYNAMIC HEADSPACE SYSTEM

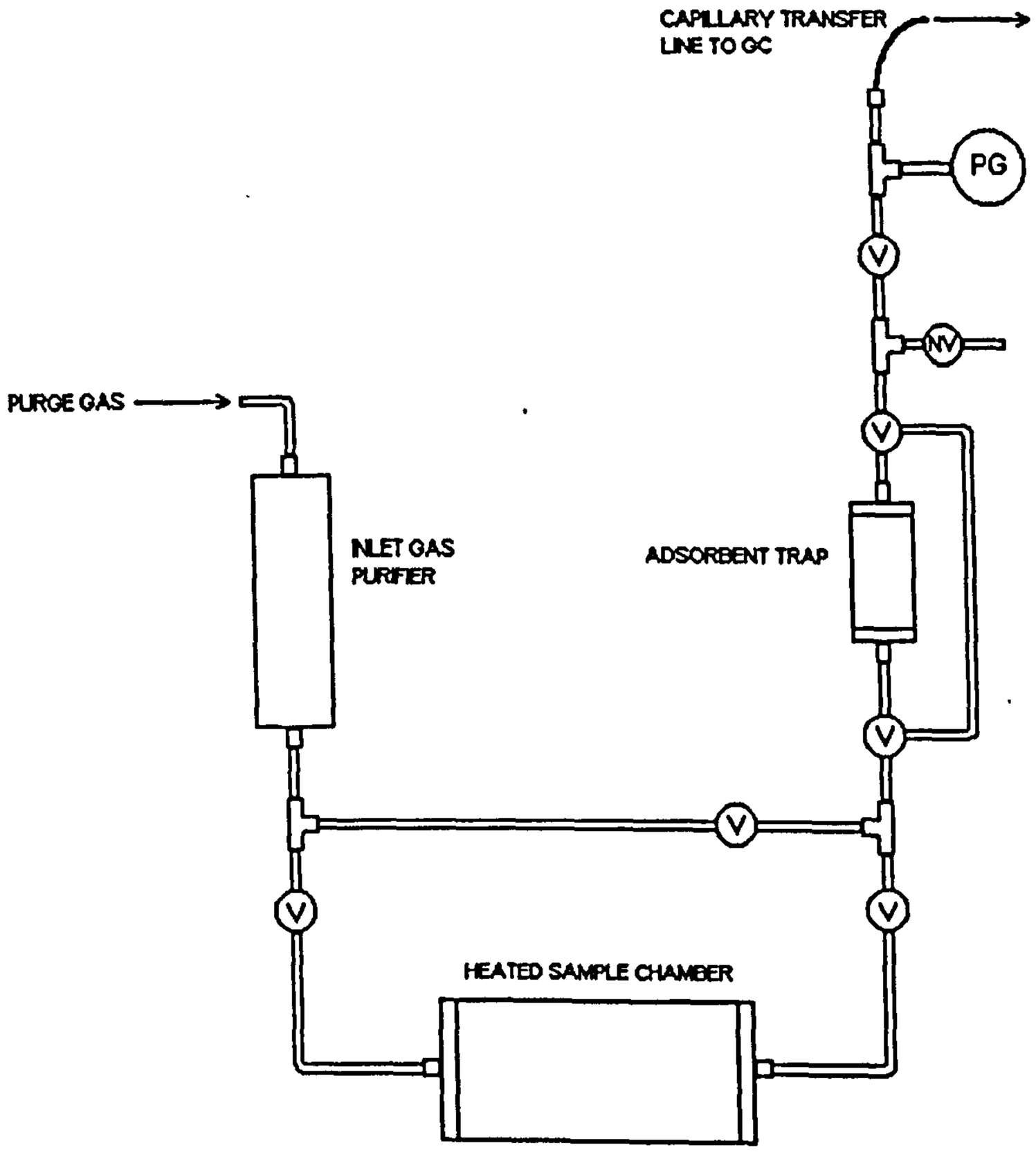
A dynamic headspace system was developed, based on literature covering existing dynamic headspace systems (Boland *et al.*, 1984; Dirinck *et al.*, 1984; Borén *et al.*, 1975, 1982 and 1985; Söderhjelm and Eskelinen, 1985). Figure 4.5 contains the design of the apparatus.

The system was constructed using ¼" stainless steel tubing. All tube joints were connected using ¼" Swagelok fittings. The gas valves were either two way on/off valves, with stainless steel internal surfaces, or three way on/off valves, with stainless steel internal surfaces. The sample chamber was constructed from stainless steel tubing, machined to provide a smooth unblemished internal surface. The tube was sealed at each end by stainless steel end caps that were bolted to the tube. These end caps sandwiched Viton rubber gaskets to provide an air tight seal. ¼" Swagelok fittings were placed in the end caps and stainless steel tubing used to connect the input gas flow and output gas flow.

A flexible heating element, sheathed in stainless steel, was wrapped around the exterior of the tube from end to end, in a coil that was perpendicular to the tube length, and set in heat conducting ceramic paste. A 100 volt a.c. power supply was connected to the heating element. Temperature control was obtained by a thermocouple sensor, placed within the wall of the sample

chamber. This thermocouple was connected to an electronic temperature controller that controlled the power to the heating element by means of a solid state relay. The heating element was insulated with a jacket of aluminium foil.

Figure 4.5 The laboratory developed dynamic headspace system.



where:

V = on/off gas valve

NV = needle metering valve

PG = pressure gauge (0-28 psi)

The adsorbent trap was of a stainless steel tube construction, containing activated charcoal pellets. Activated charcoal, or carbon, was chosen for the following reasons:

- Activated charcoal has a non-polar surface or very slightly polar surface resulting from the surface oxide groups and inorganic impurities.

- Activated charcoal is not so sensitive to deactivation by moisture. Due to its large internal surface, it adsorbs more non-polar and weakly polar organic molecules than other sorbents (Yang, 1997).

- The heat of adsorption, is generally lower on activated charcoal than other sorbents. Consequently, adsorbed molecules are desorbed more easily (Yang, 1987).

A flexible heating element was coiled around the exterior of the tube as with the sample chamber and connected to a 48 volt power supply. Stainless steel end caps were bolted to the tube sandwiching Viton rubber gaskets.

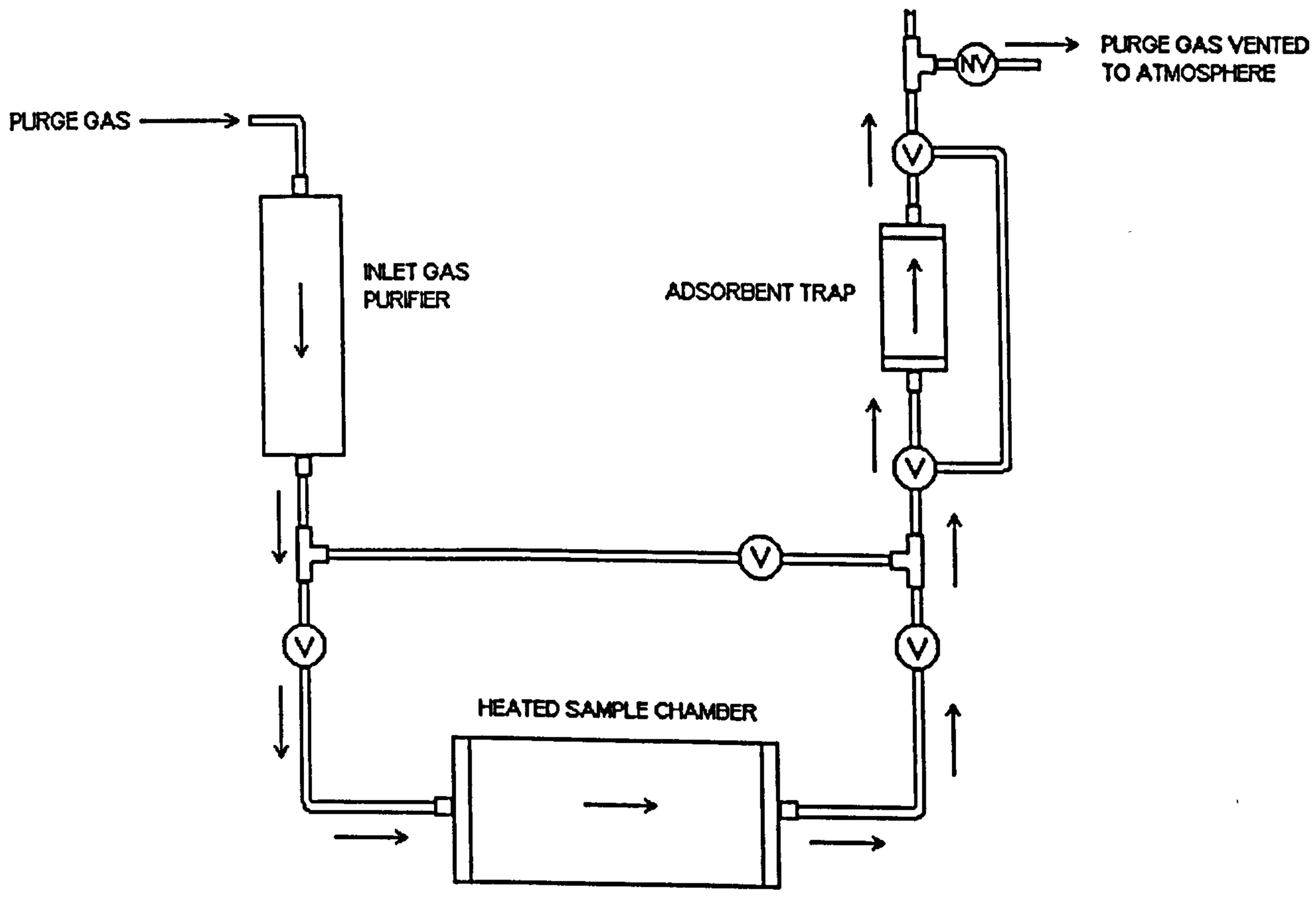
Swagelok fittings were mounted into the end caps and stainless steel tubing connected to carry the input and output gas flows.

Temperature control of the trap assembly was achieved using a thermocouple sensor within the wall of the trap. This thermocouple was connected to an electronic temperature controller that controlled the heating element by means of a solid state relay. In order to obtain efficient thermal desorption of the adsorbent trap, rapid heating the trap is needed during the desorption stage. This was achieved using an additional booster heater consisting of a piece of stainless steel (approximately 25 cm in length and 1.5 cm wide) wrapped around the outside of the trap and heating element assembly. This piece of steel was insulated from the trap body and the other heater coil using heat conducting ceramic paste. This piece of steel was connected via two copper heat insulators to a 6 volt power supply providing a current of 180 amps. The power to the heating element was controlled by an electronic timer for which a set time of heating was programmed into the device.

Figure 4.6 illustrates the flow of gas through the system during the sample purge cycle. The purge gas, helium (99.996% purity), passed through the inlet purifier, which removed any traces of impurities such as hydrocarbons, water, etc., and through the heated sample chamber which contained the material

being analysed. The purge gas passed over the sample and carried away any volatiles entering the vapour phase from the sample. The purge gas, containing the volatiles stripped from the sample, passed into the adsorbent trap which was held at approximately room temperature. Volatiles carried within the gas were adsorbed inside the trap on 5.0 grams of activated charcoal whilst the purge gas passed through the other end of the trap. The purge gas was then vented to the atmosphere. The flow of helium purge gas through the system was controlled by the needle metering valve situated at the atmospheric vent.

Figure 4.6 The flow of gas through the system during the purge cycle.



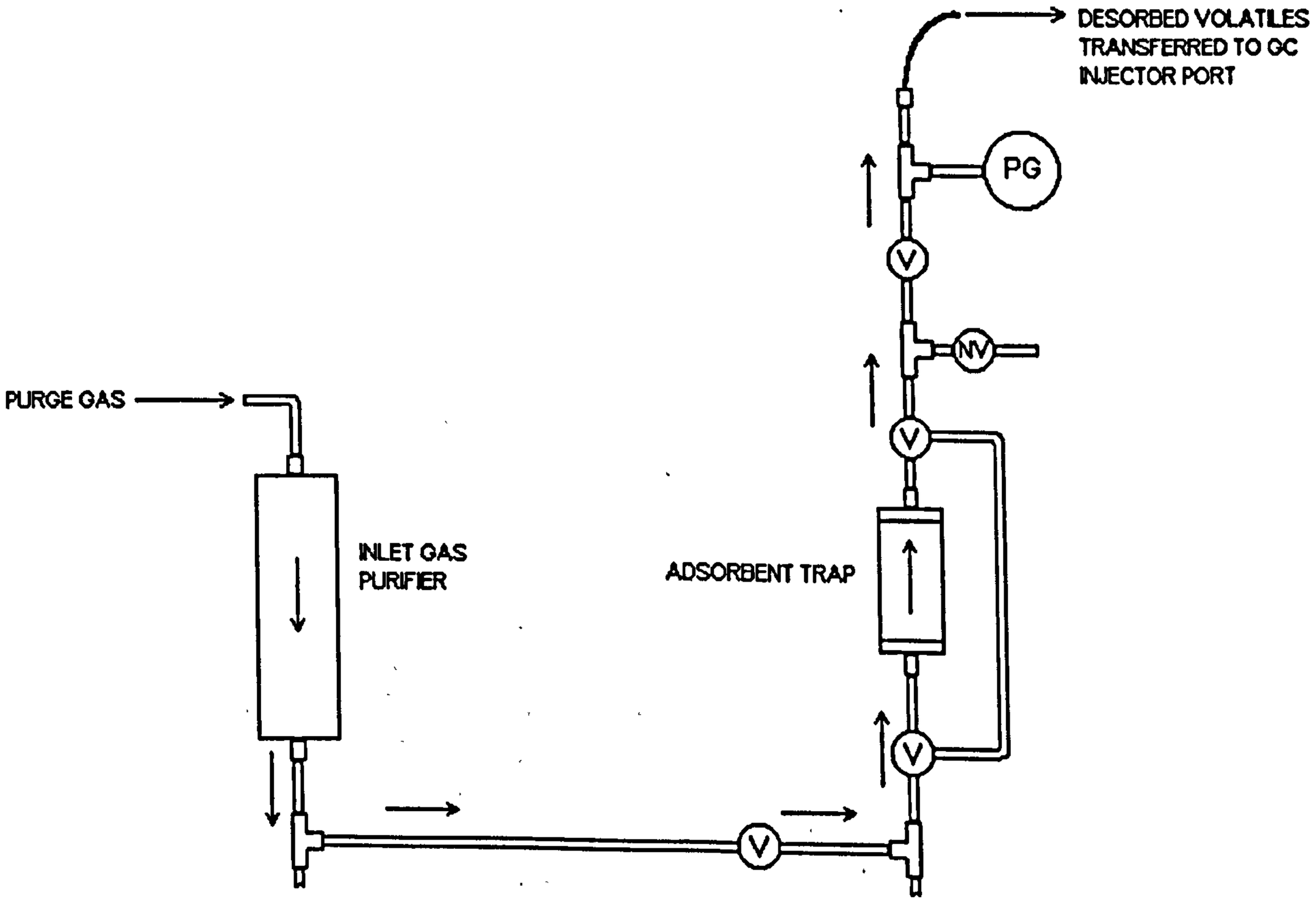
In the desorption stage, during which volatiles were desorbed from the trap, the valves connecting the sample chamber inlet and outlet gas pipes to the rest of the system were closed and the valve within the sample chamber bypass pipe was opened. Gas leaving the inlet purifier passed directly into the adsorbent trap. The flow of gas is illustrated in Figure 4.7. The trap was heated rapidly

during this desorption stage to assist in the desorption of volatiles which were carried from the trap by the helium carrier gas.

The atmospheric vent valve was closed during the desorption stage so that no gas could leave the system. The carrier gas containing the desorbed volatiles was allowed to pass, via another valve, to a capillary transfer line that connected the apparatus to the GC injector port. The pressure of carrier gas at the entry point to the capillary was indicated on a pressure gauge, situated at this point. The internal surfaces of the pressure gauge in contact with the carrier gas were of stainless steel construction to minimise adsorption of analyte molecules within the gauge.

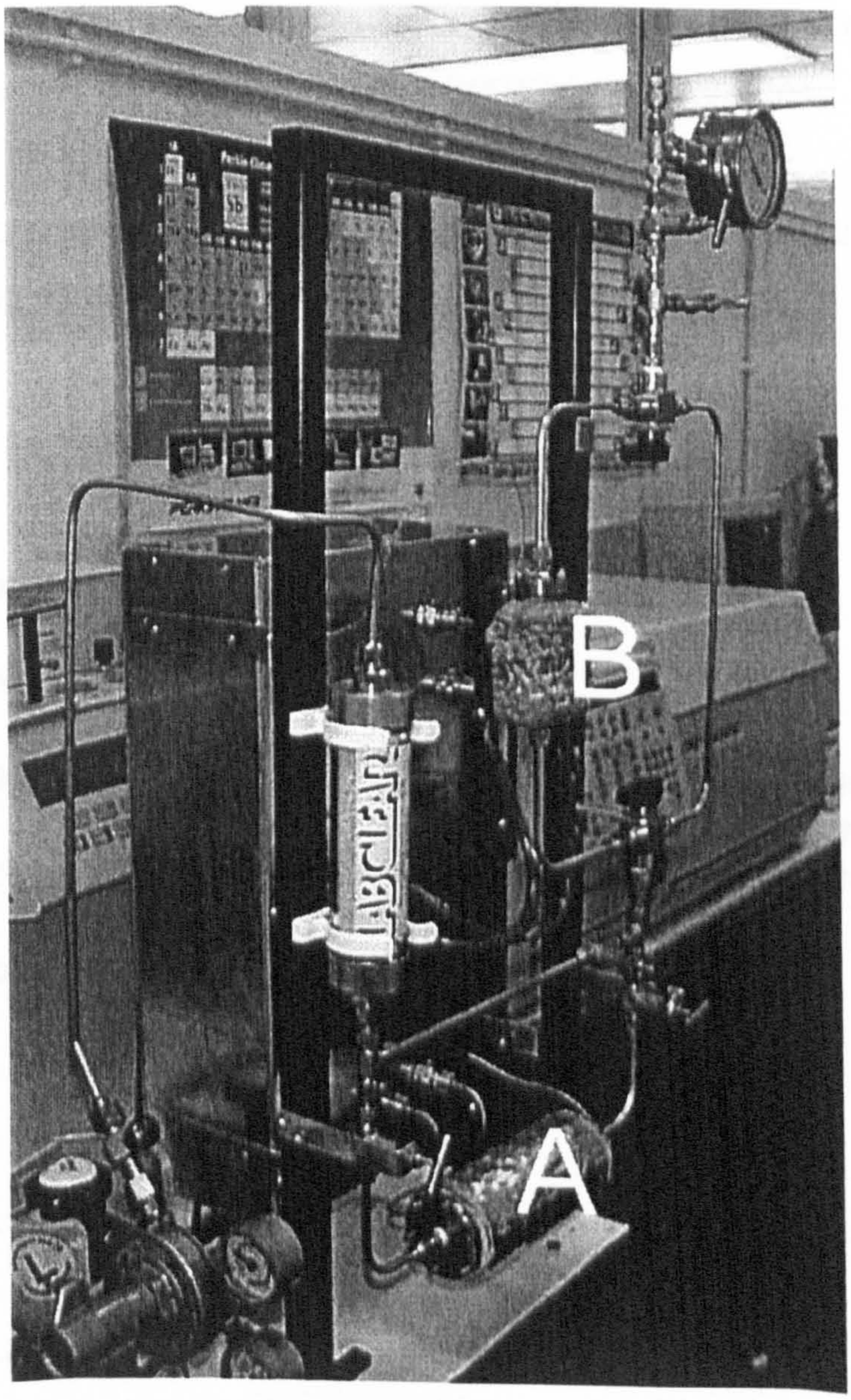
The capillary transfer line consisted of a 1.2 metre length of silica capillary with a deactivated internal surface. The internal diameter of the capillary bore was 320μm. Transfer of volatiles to the GC occurred through the capillary at room temperature.

Figure 4.7 The flow of gas through the system during the desorption stage.



During periods when the apparatus was in a standby mode, i.e. during periods in which the adsorbent trap was cooling down to room temperature after an analysis, gas flow through the system was continued to prevent adsorption of residual volatiles within the system and to reduce carry-over effects. During these standby periods, the two three way valves were adjusted so that gas flowed via the bypass pipe around the adsorbent trap. This was to prevent adsorption and condensation of residual volatiles within the trap during cooling down. Figure 4.8 shows a photograph of the dynamic headspace apparatus. The horizontal sample chamber is shown at the bottom of the photograph (A) and the smaller, vertically positioned, adsorbent trap (B) shown further up the apparatus.

Figure 4.8 Photograph of the dynamic headspace apparatus.

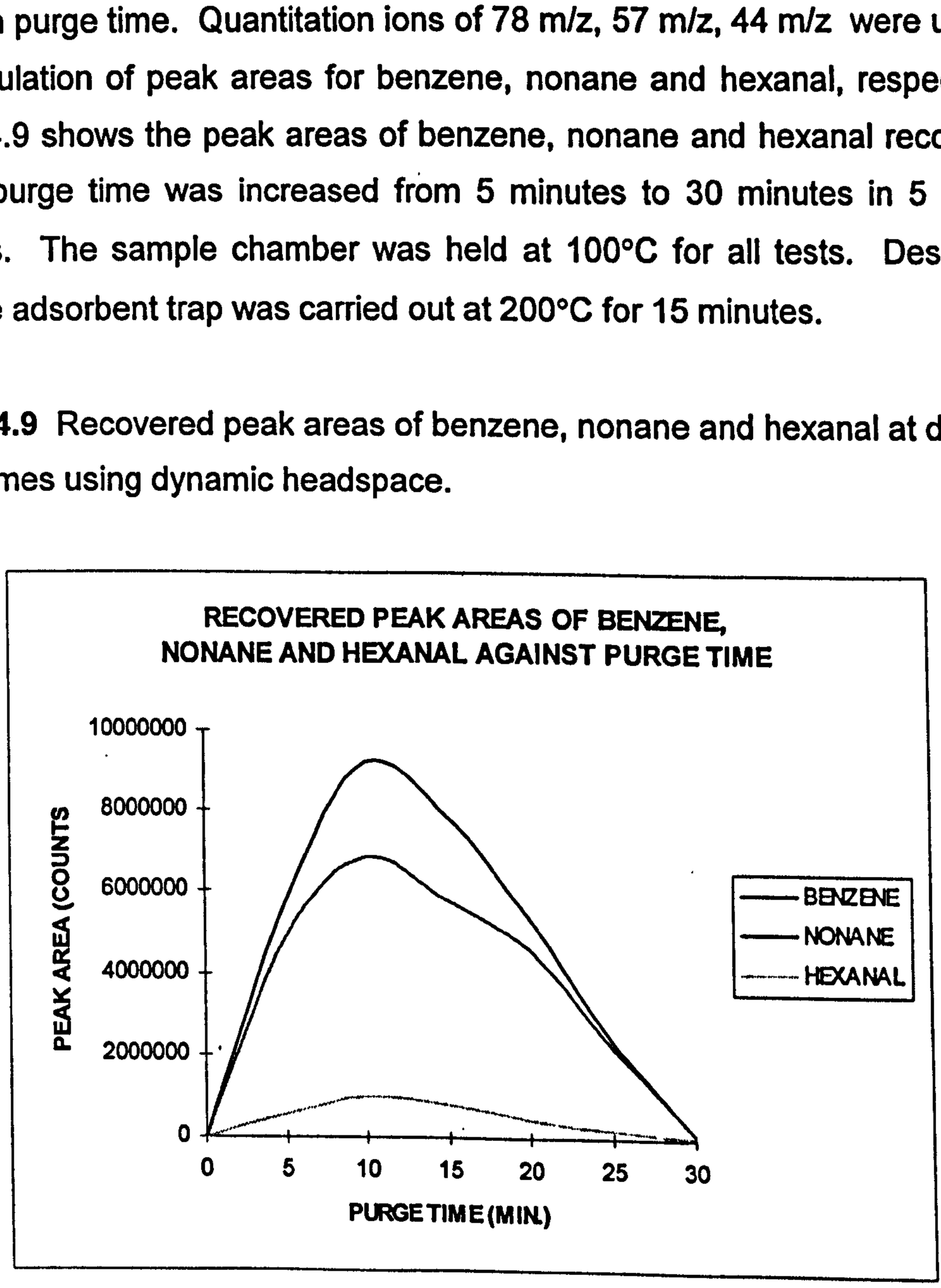


4.2.1.1 Experimental

Benzene, nonane and hexanal were used as test compounds to develop the dynamic headspace procedure. 1 nL of each of these compounds was impregnated into 10 gram samples of purified CTMP. A series of tests was carried out to determine the optimum purge time, the optimum purge gas flow rate and the optimum desorption conditions.

The optimum purge time is very dependent on the flow rate of the purging gas. A purge time of up to thirty minutes was considered convenient for analyses and an initial purge flow rate of 40 cm^3/min. was used to determine optimum purge time. Quantitation ions of 78 m/z, 57 m/z, 44 m/z were used in the calculation of peak areas for benzene, nonane and hexanal, respectively. Figure 4.9 shows the peak areas of benzene, nonane and hexanal recovered, as the purge time was increased from 5 minutes to 30 minutes in 5 minute intervals. The sample chamber was held at 100°C for all tests. Desorption from the adsorbent trap was carried out at 200°C for 15 minutes.

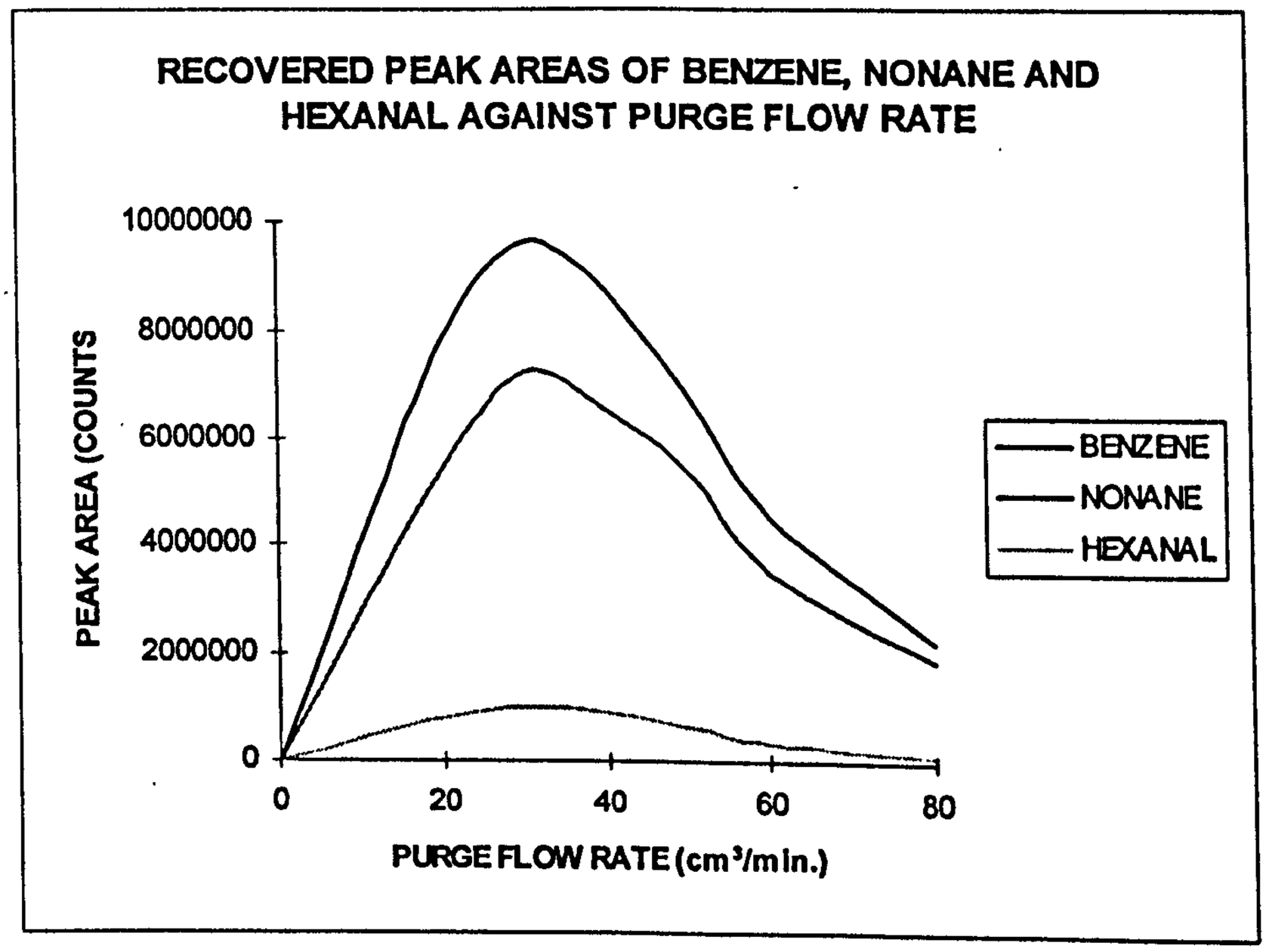
Figure 4.9 Recovered peak areas of benzene, nonane and hexanal at different purge times using dynamic headspace.



The maximum recoveries of all three compounds were experienced at a purge time of 10 minutes. The % recovery of each of these compounds at this time for benzene, nonane and hexanal were 72%, 67% and 76%, respectively, based on the expression for % recovery described earlier in this section.

The optimum purge gas flow rate was determined using the optimum purge time of 10 minutes. Figure 4.10 shows recovered peak areas of benzene, nonane and hexanal using purge flow rates of 20, 30, 40, 50, 60, 80 cm^3/min. The sample chamber was held at 100°C for all tests. Desorption from the adsorbent trap was carried out at 200°C for 15 minutes.

Figure 4.10 Recovered peak areas of benzene, nonane and hexanal at different purge flow rates using dynamic headspace.



A purge gas flow rate of 30cm^3/min. yielded the highest recoveries from all three volatiles. The % recovery at this flow rate, was 75%, 71% and 75% for benzene, nonane and hexanal, respectively.

A number of tests were carried out to determine the most efficient carrier gas flow rate during the desorption of volatiles from the adsorbent trap. A range of flow rates from 30 cm^3/min. to 80 cm^3/min. were tested. A flow rate of 50 cm^3/min. gave the highest resolution of components, as determined by GC/MS.

After these initial trials, a range of conditions was established to provide adequate recovery of the volatiles from carton-board materials. Table 4.1 shows the range of conditions used during the purge cycle.

Table 4.1 Purge cycle conditions used for the recovery of volatiles from carton-packaging materials using the dynamic headspace apparatus.

Sample chamber temp.	100	°C
Absorbent trap temp.	20-25	°C
Purge gas flow rate	30	cm^3/min.
Purge gas entry pressure	15	psi
Purge time	10	min.

The conditions used during the desorption cycle for the analysis of carton packaging materials are given in Table 4.2.

Table 4.2 Desorption cycle conditions used for the recovery of volatiles from carton-packaging materials using the dynamic headspace apparatus.

Absorbent trap temp.	200	°C
Carrier gas flow rate	50	cm^3/min.
Desorption time	20	min.
Carrier gas entry pressure	15	psi
Transfer line pressure	15	psi

The transfer line from the apparatus was inserted into the split/splitless injector of the GC operated in splitless mode. No injector purge cycle was adopted to remove residual vapours within the injector port as this removes analyte components transferring from the dynamic headspace apparatus.

The GC method employed for static headspace analyses was used for unprinted samples of carton-board. Printed samples were analysed using a 44 minute GC programme, during which the column was elevated from 40°C to 238°C at a rate of 4.5°C/min.

4.2.1.2 Results and discussion

A number of carton-board materials was sampled for volatiles using the dynamic headspace technique. These included samples of virgin carton-board and samples of carton-board printed with UV-cured lithographic inks and overcoated with a UV-cured varnish. For each analysis, 20 grams of packaging material was placed within the sample chamber.

Figure 4.11 shows the chromatogram for the volatiles collected by the dynamic headspace apparatus from a 20 gram sample of virgin Iggesund Carton Excel HS carton-board using the conditions described above. Figure 4.12 shows the chromatogram for the volatiles collected from a 20 gram sample of Iggesund Carton Silkia carton-board printed with UV-cured lithographic inks and over coated with UV-cured varnish.

Figure 4.11 Volatiles collected by dynamic headspace from a 20 gram sample of Carton Excel HS.

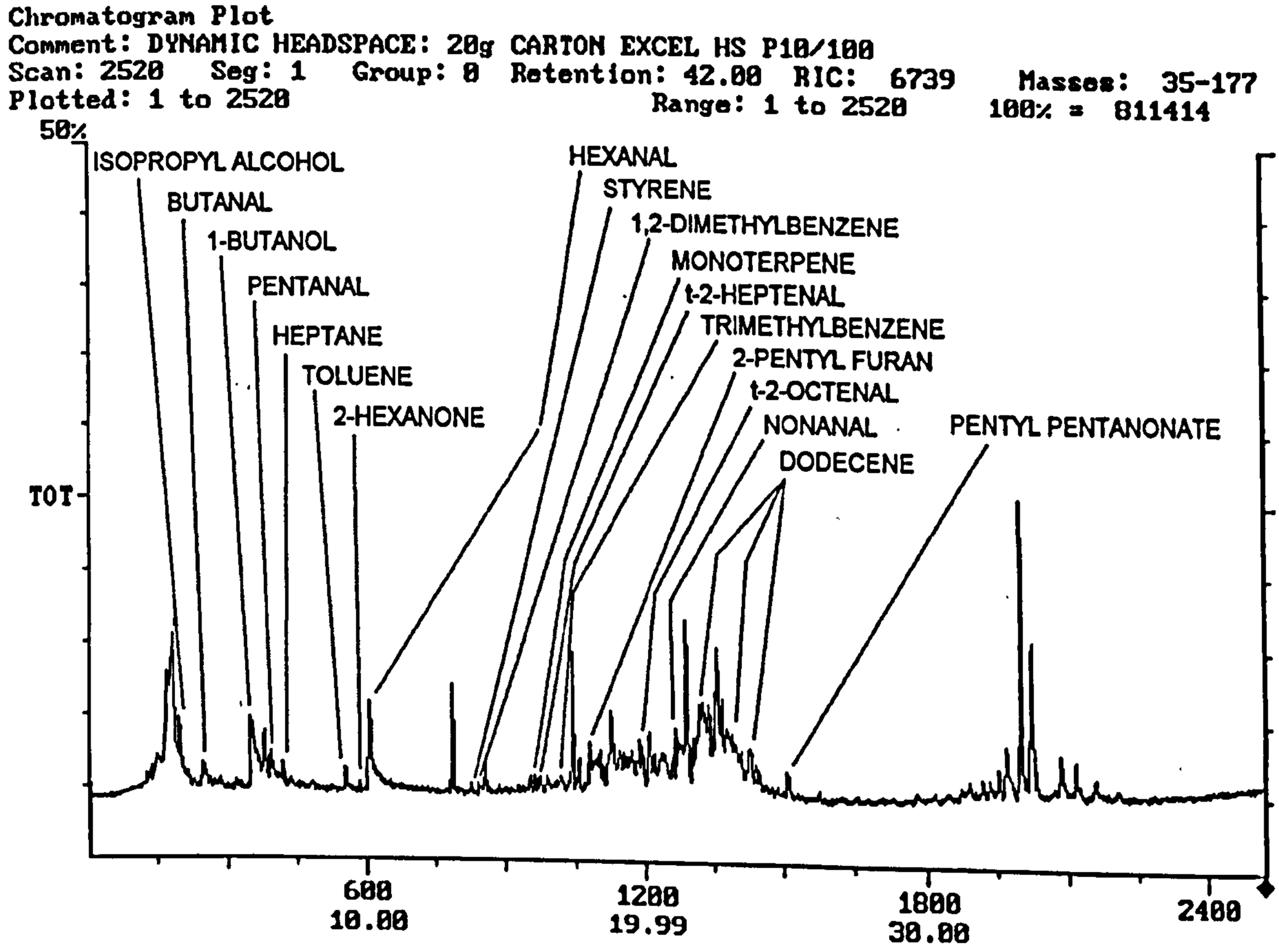
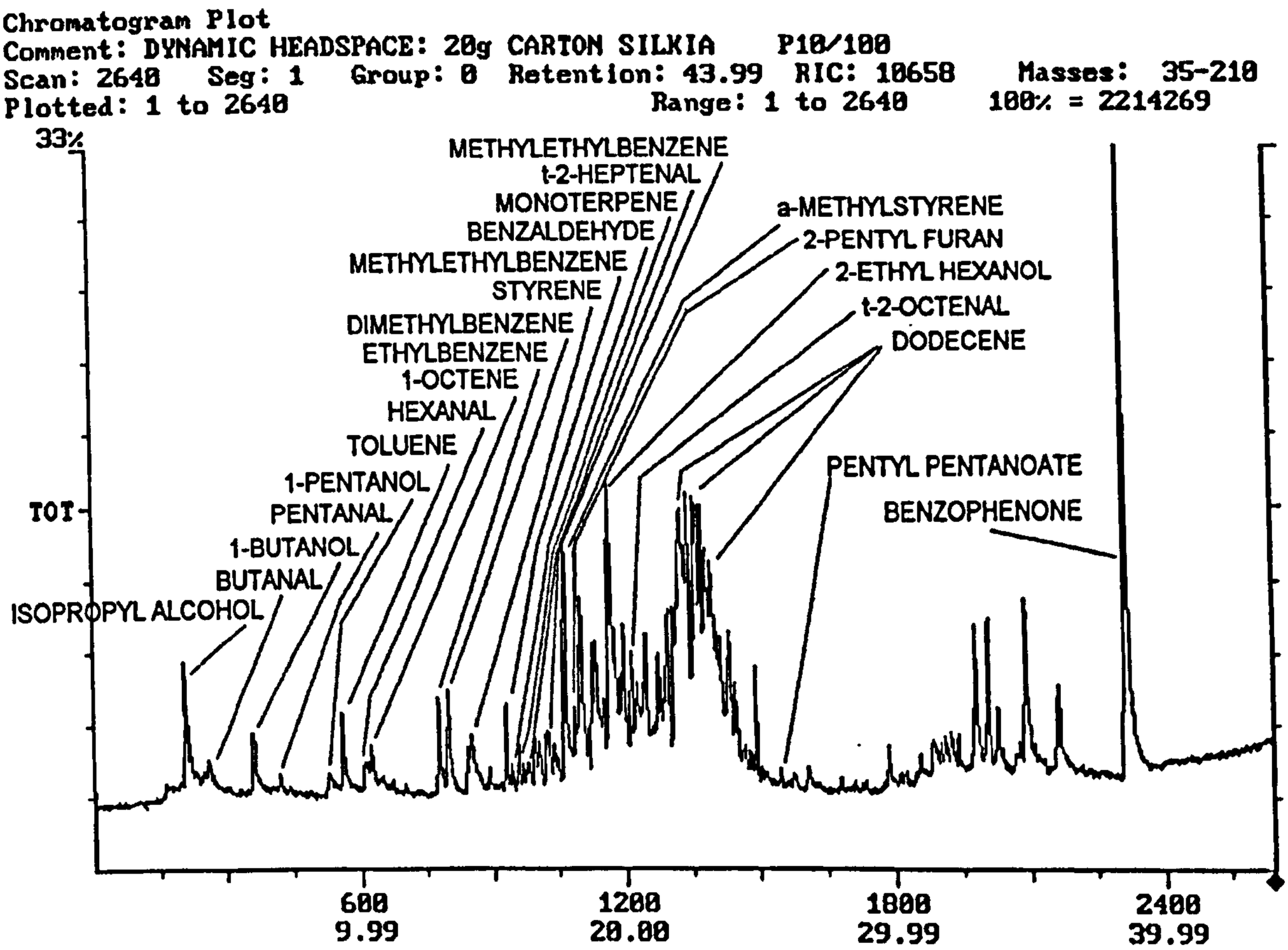


Figure 4.12 Volatiles collected by dynamic headspace from a 20 gram sample of printed Carton Silkia.



The chromatograms are not very different from those for samples of carton-board sampled using static headspace analysis (see Section 3.2). The dynamic headspace apparatus, however, was able to isolate two unsaturated aldehydes from both the unprinted and printed carton-boards. This suggests that the inert atmosphere, within the apparatus during extraction, provides stability against oxidation reactions.

A small amount of benzaldehyde was isolated from the printed carton system. Benzaldehyde, if present in sufficient amounts, can cause significant taint problems within a packaged food. Benzaldehyde was not detected using static headspace analysis.

The total recovery of volatiles from the carton-board materials was lower than the recoveries witnessed for the three individual test compounds used to develop the dynamic headspace procedure. This suggests that the capacity of the adsorbent trap may have been exceeded. The result would be a portion of the volatile component passing through the trap without being adsorbed. Alternatively, preferential adsorption may be enhanced, during which certain

compounds with a higher affinity for the charcoal adsorbent displace compounds with lower affinity.

The compounds recovered using the dynamic headspace apparatus reflect the compounds present in the samples of carton-board at the time of analysis. This is in contrast to the static headspace procedure in which a portion of volatile compounds recovered during analysis result from the decomposition reactions occurring during thermal equilibration of the sample. Using the dynamic headspace procedure, therefore, it should be more easy to correlate the volatile compounds recovered from a carton-board sample with its organoleptic quality.

Samples from the batch of carton-board, represented in Figure 4.12, were evaluated by sensory analysis, using Method 6 taint test, as described in Section 2.3.3. Panellists found that the carton-board sample imparted a 'sweet' taint on water samples, corresponding to a large amount of benzophenone found using the dynamic headspace procedure. A 'plasticy' taint was also found. This can be attributed to the presence of a number of acyclics and hydrocarbons.

4.3 VACUUM DISTILLATION EXTRACTION

Several groups have used vacuum distillation extraction methods in the analysis of volatiles associated with foods (Lea and Swoboda, 1962; Wong *et al.*, 1996a and 1996b). Distillation can be used for the separation of liquid mixtures of different components into a number of fractions of differing composition. Often, the objective is to separate the mixture into its pure components. Alternatively, distillation can be used for the isolation of volatile compounds from non-volatile components within a system of interest.

According to the molecular theory of gases and liquids, a pure liquid is in dynamic equilibrium with the vapour above it. This is because molecules containing a certain amount of energy can overcome the attractive forces of a liquid and pass into the vapour. The existence of a dynamic equilibrium means that a liquid at a given temperature will be in equilibrium with a given concentration of vapour molecules, i.e. a given vapour pressure. The fraction of molecules in the liquid containing sufficient energy to escape is proportional

to the energy level distribution. As the temperature increases, this distribution shifts and the fraction possessing adequate energy increases. Since the Boltzmann energy distribution of the molecule is exponential, as the temperature increases, the fraction of molecules with greater than a specific energy increases exponentially.

Vacuum distillation is necessary when the boiling point of the material to be vaporised is too high at normal pressures for the material to remain stable. By working at lower pressures the boiling point is reduced and thermal decomposition can be avoided.

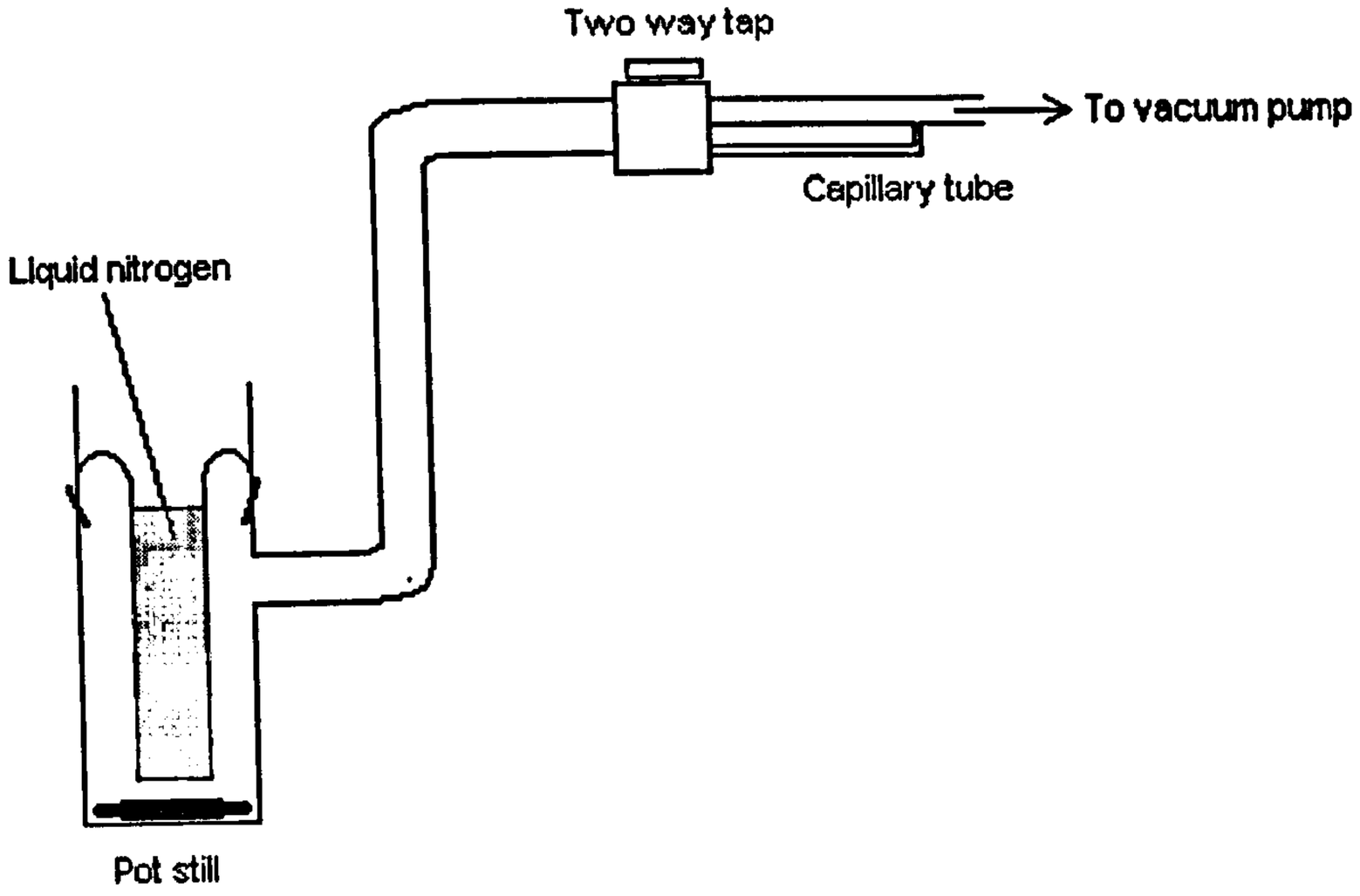
The condensed distillate collected within the cold traps during vacuum distillation is normally extracted with a suitable solvent. This solvent extract is then concentrated down to a smaller volume and analysed by direct injection into a GC. Wong *et al.* (1996a) reports, that after the vacuum extraction of flavour volatiles from fruits, 200 cm^3 of condensed distillate was extracted from two liquid nitrogen traps, with 5 x 40 cm^3 of dichloromethane. These extracts were combined, concentrated using a Kuderna-Danish concentrator, and finally reduced to a volume of about 0.05 cm^3 under a gentle stream of N_2.

Lea and Swoboda (1962) used a pot still technique for the recoveries of aliphatic aldehydes of chain length C_6 - C_{14} from edible food oils. Early tests yielded small amounts of lower molecular weight volatile compounds. Later tests produced greater yields of compounds of lower molecular weights, down to propanal. The vacuum system employed a pot still. This was connected to the vacuum pump via a wide bore glass tube and a capillary tube. The passage of air through each of this tubes was controlled by a two way tap. This vacuum system is illustrated as Figure 4.13 (Lea and Swoboda, 1962).

The distillation procedure was carried out by freezing the oil sample in the bottom of the pot still. The distillation was carried out in two phases. An initial period of distillation in a closed system under low vacuum was followed by a second stage of continuous pumping through the capillary tube. The purpose of this initial procedure was to prevent the carrying of very volatile substances past the condenser in the first rapid evolution of dissolved air and water from the fat. Pumping through the capillary provided a slow vacuum build-up in the system during the second stage, reducing the amounts of volatiles passing the condenser. During the first stage, the oil was allowed to thaw and, during the

second stage, the oil sample was heated by immersion of the still in a water bath.

Figure 4.13 The pot still vacuum distillation apparatus.



After the distillation stage, the condensed distillate is recovered using 10 cm^3 of solvent. The authors claim that propanal, hexanal and dodecanal were recovered at 95-100% from samples of purified oil that had been doped with these three aldehydes.

This vacuum extraction procedure produces almost total recoveries of aldehydes that are present in oil samples. The procedure is quite tedious and requires solvent concentration procedures during which some volatiles may be lost or denatured. It was felt that a vacuum extraction procedure was needed to remove the need for solvent extraction and concentration of the distillate.

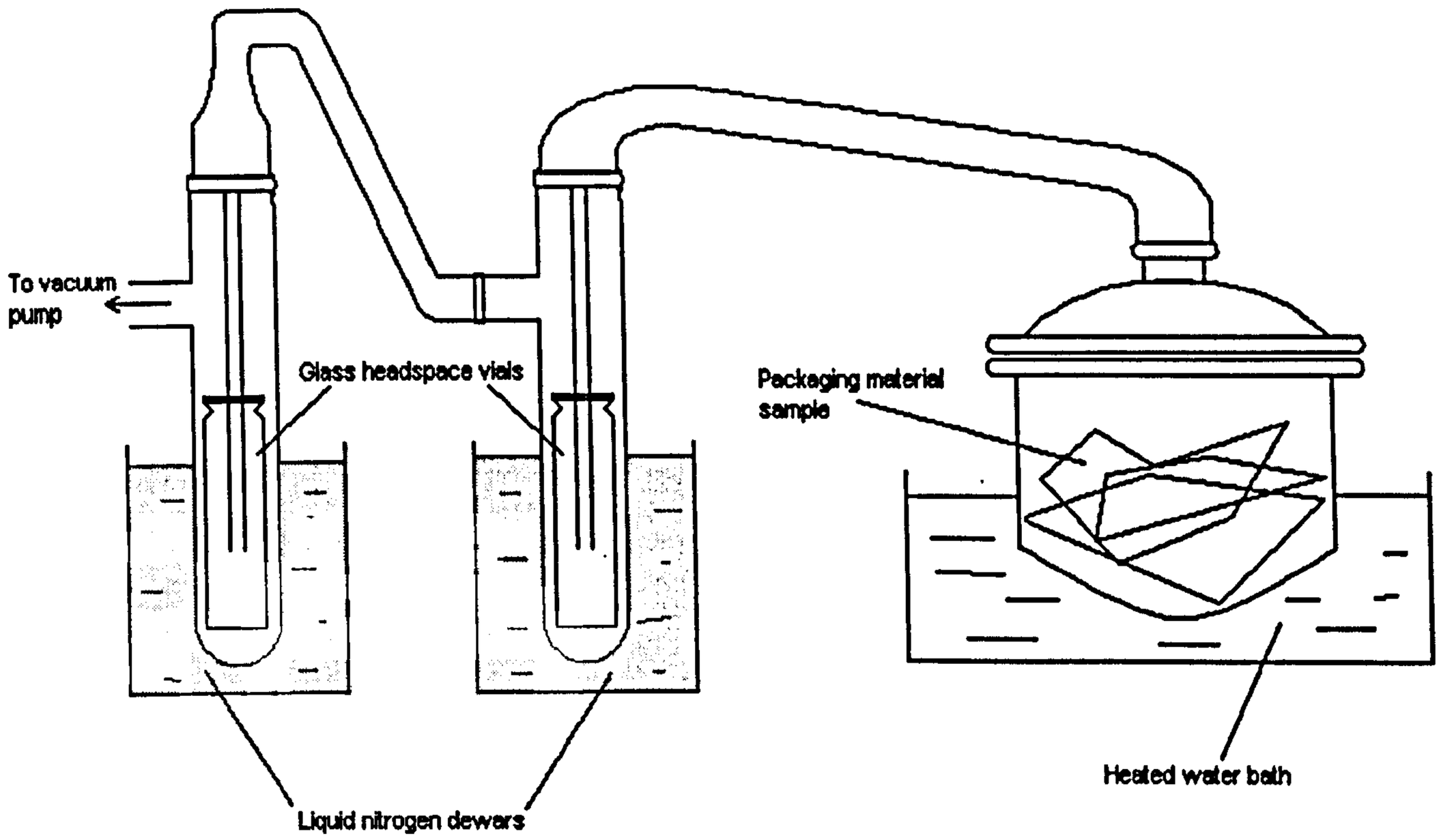
4.3.1 DEVELOPMENT OF THE VACUUM DISTILLATION EXTRACTION PROCEDURE

A vacuum distillation extraction apparatus was developed that did not rely on solvent extraction of the distillate. Figure 4.14 illustrates the system.

The apparatus was of glass construction. The sample chamber was a 1 litre reaction vessel with a single outlet in its lid. A wide bore tube connected this

chamber to the cold traps. These cold traps consisted of a couple of large quickfit tubes with side arms semi-immersed in liquid nitrogen.

Figure 4.14 The laboratory developed vacuum distillation extraction apparatus.



Each cold trap had a 22 cm^3 glass headspace vial placed within it. When the system was evacuated, an inlet tube inserted into the top of each of these vials carried volatiles released from the sample into the vials where condensation occurred.

Two cold traps were used to increase the recovery efficiency. The level of immersion of the cold trap within the liquid nitrogen was important. Too low a level of immersion resulted in reduced condensation of volatile components within the vials. Too high a level of liquid nitrogen resulted in condensation of volatiles within the inlet tube, making recovery difficult.

Evacuation was carried out using a Balzers turbomolecular vacuum pump. This was fitted with a cold cathode pressure gauge which was sensitive to pressures of 10^{-7} mm Hg. Before an extraction, the packaging sample was placed in the sample chamber, at room temperature. The system was then

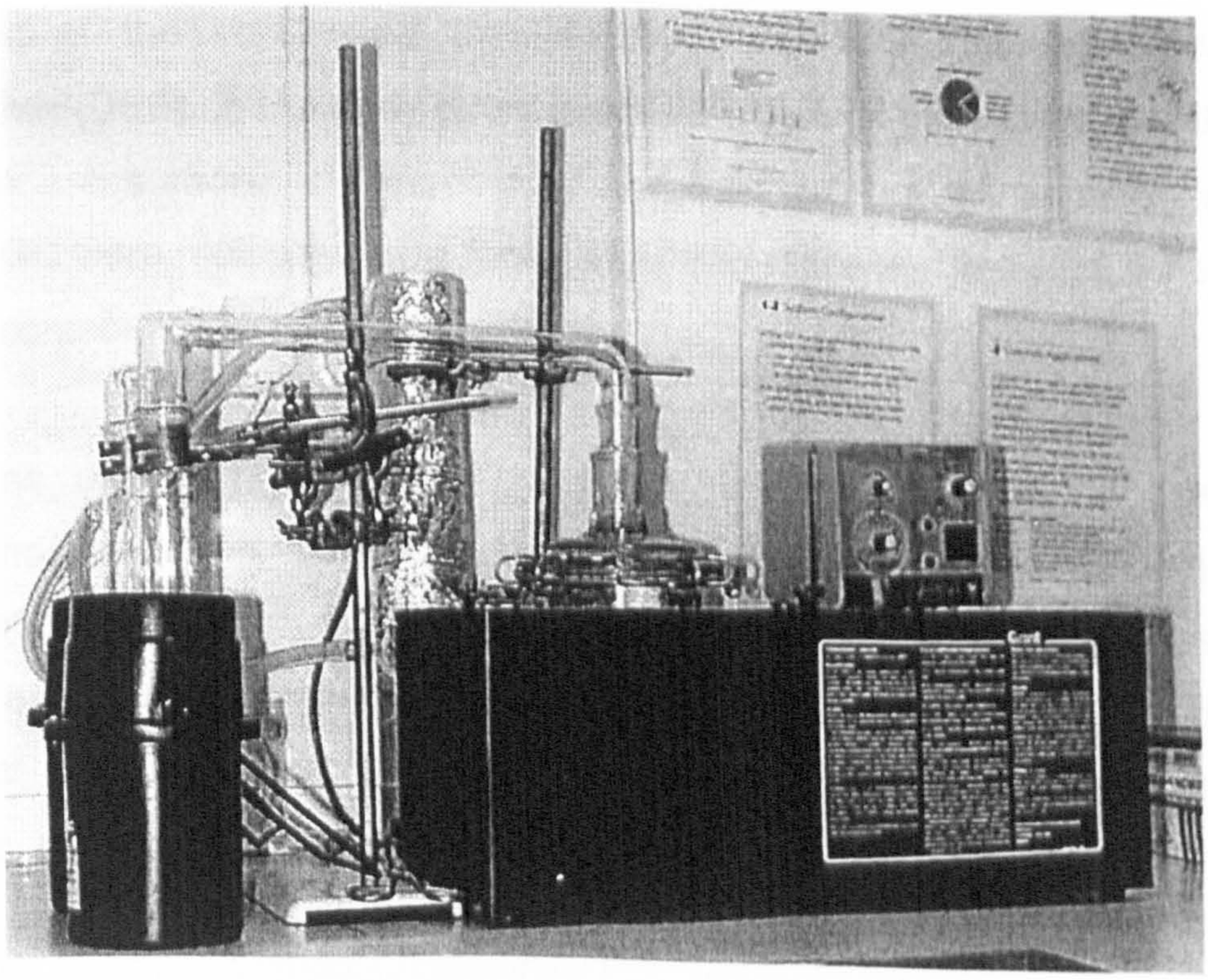
slowly evacuated and the liquid nitrogen promptly introduced to the cold traps. The water bath was then heated to the desired temperature for the extraction.

After the extraction period, the system was isolated from the pump and slowly repressurised. The liquid nitrogen was then removed from the cold traps and the vials containing the condensed distillates promptly sealed with silicone/PTFE septa caps.

Two methods were used for the analysis of the vial contents. The first method involved static headspace sampling of the vial contents after a suitable equilibration period. The second method involved injecting a small amount of solvent into the sealed vials and allowing the solvent to mix with the trapped distillate. A small proportion of this extract was then taken and introduced to the GC by direct injection.

Figure 4.15 shows a photograph of the vacuum distillation extraction apparatus. The sample chamber is immersed in the water bath. The two cold traps are semi-immersed in dewars of liquid nitrogen. An additional cold trap is visible behind the apparatus. This was used to protect the pump from any volatile components that escaped through the collection traps.

Figure 4.15 Photograph of the vacuum distillation extraction apparatus.



4.3.1.1 Experimental

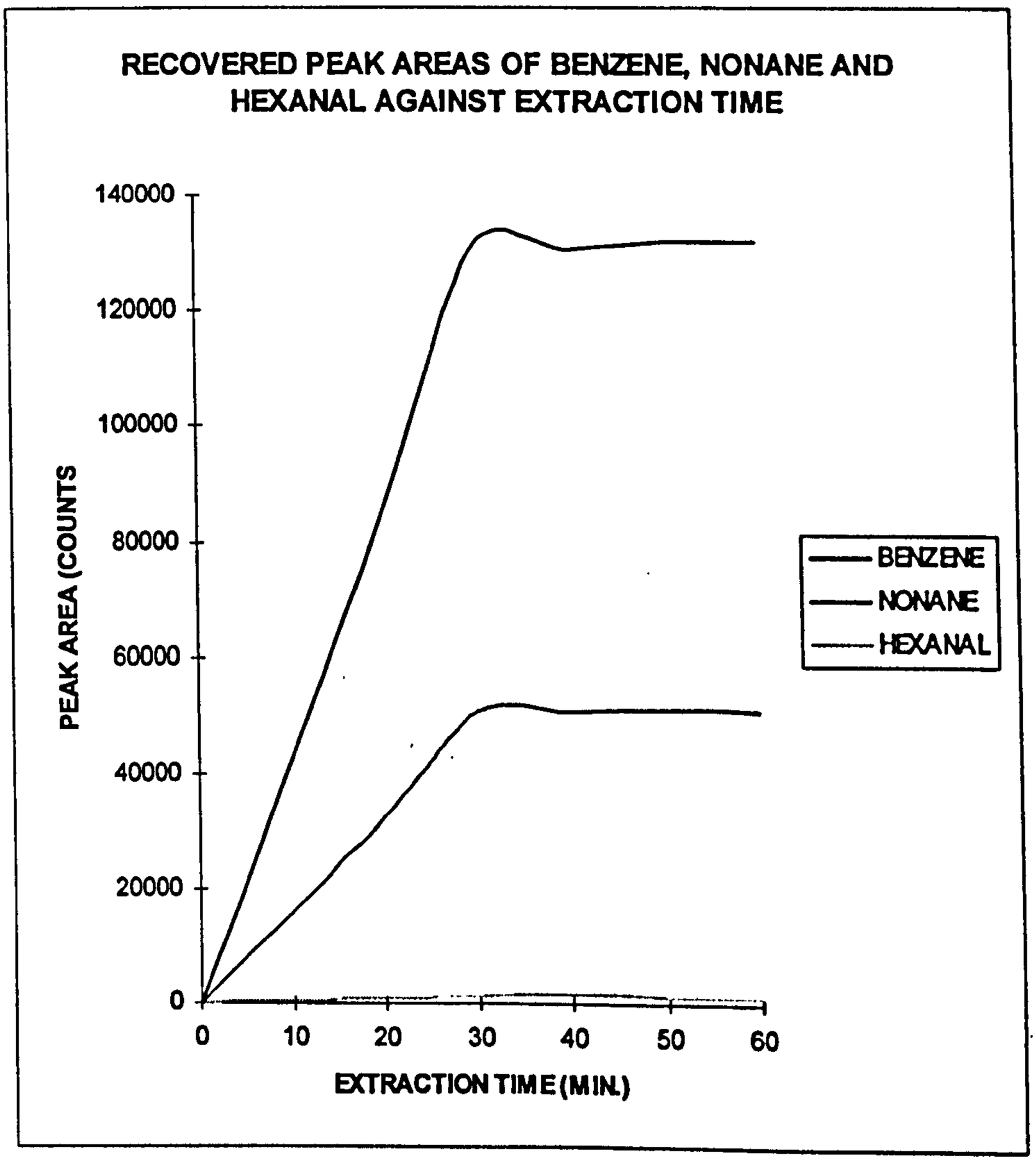
The compounds benzene, nonane and hexanal were used to develop the vacuum extraction procedure. 1 nL of each was impregnated into 10 gram samples of purified CTMP.

Tests were carried out to determine the optimum extraction time and the recovery efficiency of the technique. For all tests, the sample was introduced into the sample chamber at room temperature. The system was then sealed and the vacuum pump activated. After approximately 10 seconds, liquid nitrogen was placed into the cold traps. This delay was used to reduce the amount of atmospheric moisture, oxygen, carbon dioxide etc.. condensing on the traps. The water bath containing the sample chamber, was then heated to 95°C, over 20 minutes.

Figure 4.16 presents the peak areas, against extraction time, for benzene, nonane and hexanal, recovered from the first vial after the sample chamber, using direct headspace analysis of volatiles. Figure 4.17 shows the peak areas, against extraction time, for three compounds recovered, by extraction of the condensed distillate from the first vial, with 200μL of dichloromethane (DCM), followed by direct injection of 2μL of the extract into the GC. Figure 4.18 shows the peak areas, against extraction time, for three compounds recovered, by extraction of the condensed distillate from the first vial, with 200μL of diethylether (DEE), followed by direct injection of 2μL of the extract into the GC. The peak areas shown in Figures 4.17 and 4.18 for the 2μL samples injected into the GC were multiplied by a factor of 100 to represent the total amounts of the test compounds in the 200μL extract.

After an extraction time of 30 minutes, the recovery of the three test compounds levels out. The % recoveries of the these compounds using static headspace analysis of the distillate were 1%, 0.5% and 1% for benzene, nonane and hexanal, respectively. Using DCM to extract the distillate, followed by direct solvent injection, yielded recoveries of 53%, 58% and 61% for benzene, nonane and hexanal, respectively.

Figure 4.16 Recovered peak areas against extraction time for benzene, nonane and hexanal using static headspace sampling of the vacuum extract.



When solvent extraction was carried out using DEE, recoveries of 48%, 51% and 65% were experienced for benzene, nonane and hexanal, respectively. The static headspace procedure for the analysis of the distillate, though reasonably straightforward, yielded extremely low recoveries of the test volatiles. The recoveries experienced using the two solvents were more acceptable. Generally, the DCM produced a higher yield of the test compounds. However, DEE yielded a higher yield of hexanal.

From these initial trials, it was found that an extraction time of 30-60 minutes provided adequate recovery of the volatiles from carton-board materials.

Figure 4.17 Recovered peak areas (multiplied by a factor of 100) against extraction time for benzene, nonane and hexanal using DCM to extract the distillate.

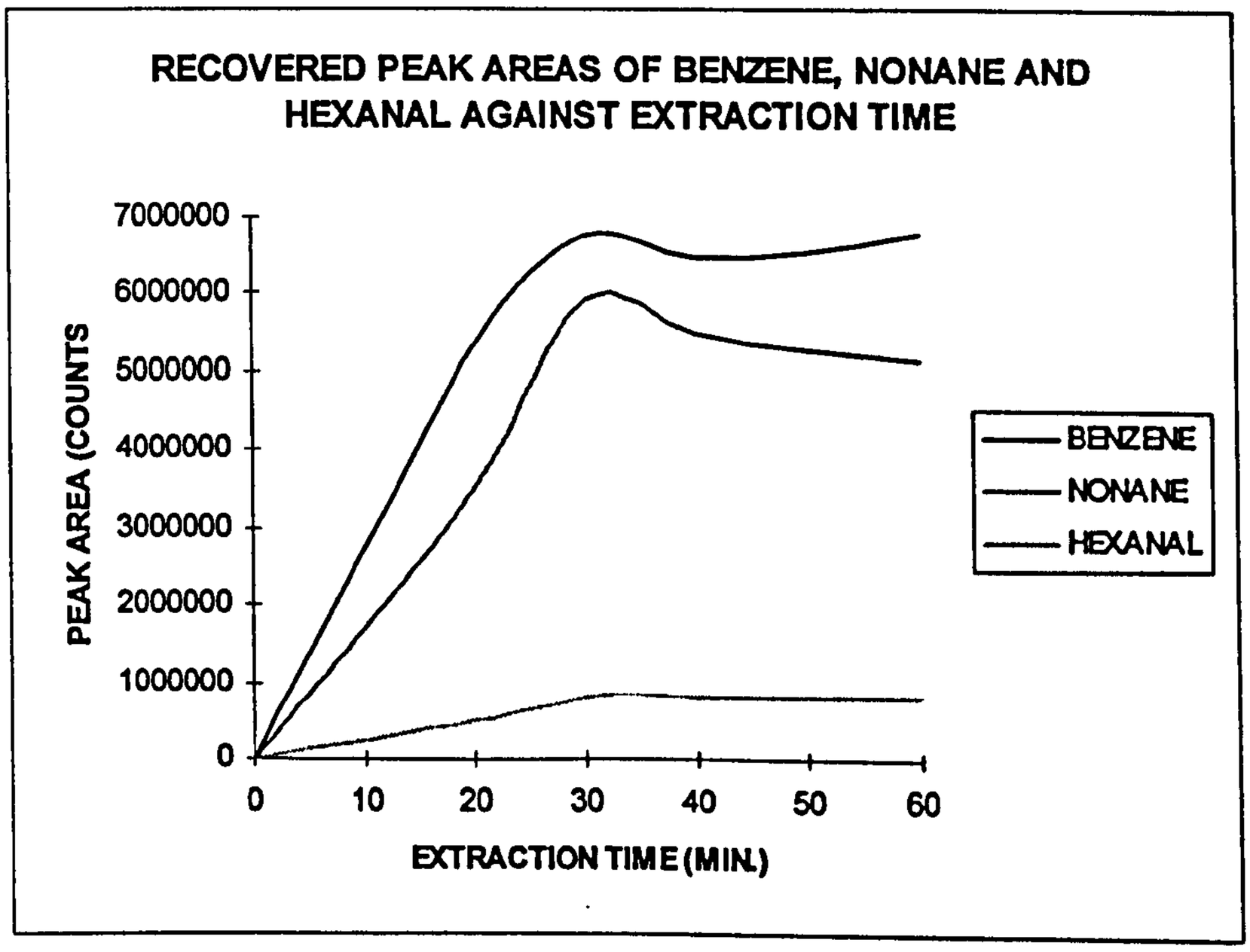
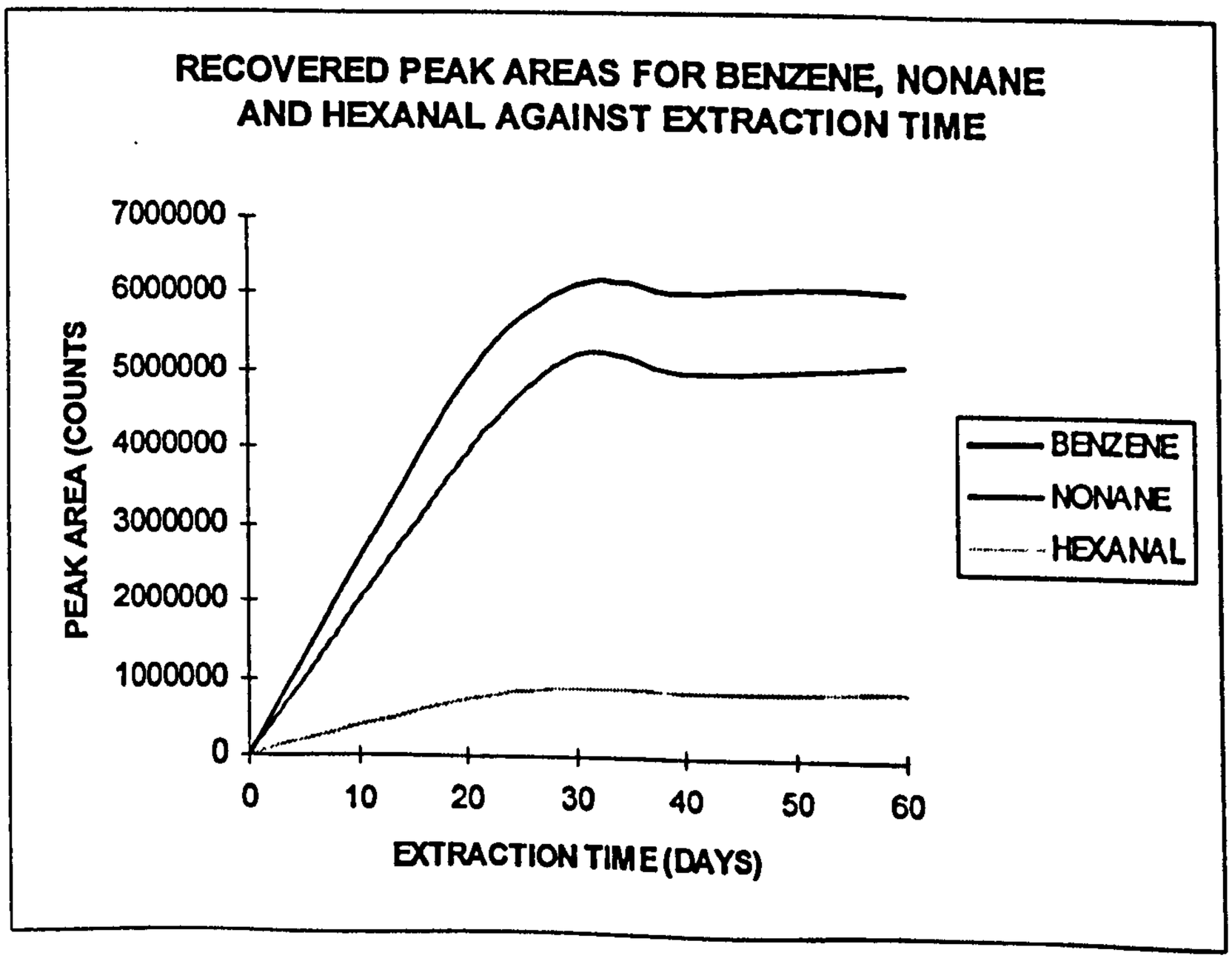


Figure 4.18 Recovered peak areas (multiplied by a factor of 100) against extraction time for benzene, nonane and hexanal using DEE to extract the distillate.



Separation and resolution of components on the GC was carried out using the conditions shown in Table 4.3.

Table 4.3 GC conditions used in the separation and resolution of distillate from vacuum extraction of carton-board materials.

Column Program			Injector		Transfer Line	
Start	40	°C	150	°C	275	°C
End	274	°C				
Time	52	min				
Rate	4.5	°C/min				

The mass spectrometer was set up to scan in the range of 35 - 320 m/z with segment length of 52 minutes corresponding to the GC programme time.

4.3.1.2 Results and discussion

Several carton-board materials were sampled for volatiles, using the vacuum distillation extraction. These included samples of virgin carton-board and samples of carton-board printed with UV-cured lithographic inks and overcoated with a UV-cured varnish. For each analysis 20 grams of packaging material was placed within the sample chamber.

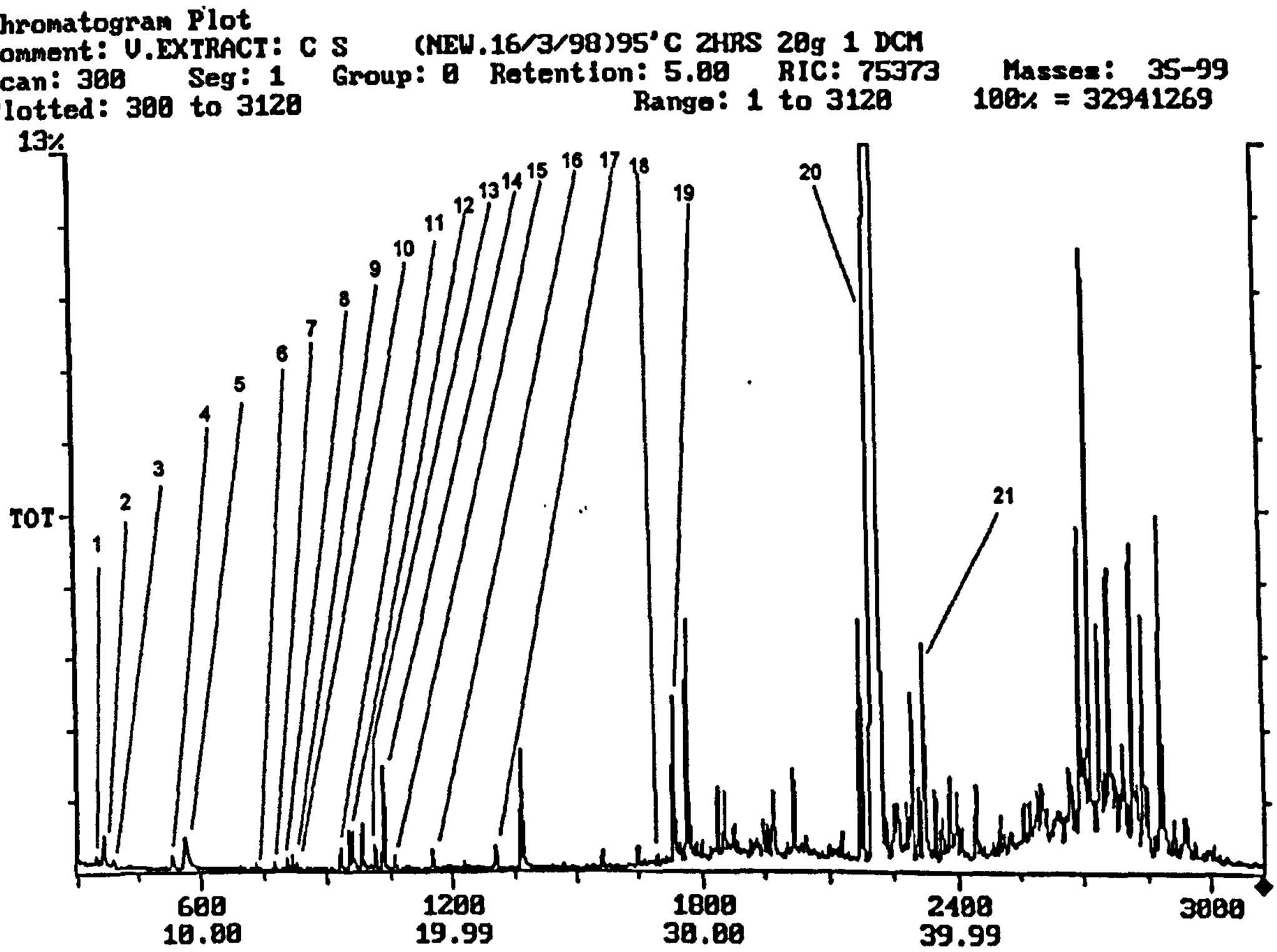
20 gram samples of printed Iggesund Carton Silkia were vacuum extracted. Some of the distillates were extracted with 200μL of DCM and some with 200μL of DEE. 2μL portions of the distillates dissolved in the solvents were taken and analysed by direct injection into the GC.

Figure 4.19 and Figure 4.20 show the chromatograms of the volatiles present in 2μL of DCM used to extract the distillate from the first collection vial and the second collection vial, respectively.

The amount of volatiles escaping from the first cold trap into the second cold trap was proportionality small. The compounds that did reach the second trap

tended to be the more volatile components, with the exception of benzophenone.

Figure 4.19 The chromatogram of the volatiles collected from the first collection vial by the extraction of the vacuum distillate of printed Carton Silkia with DCM.



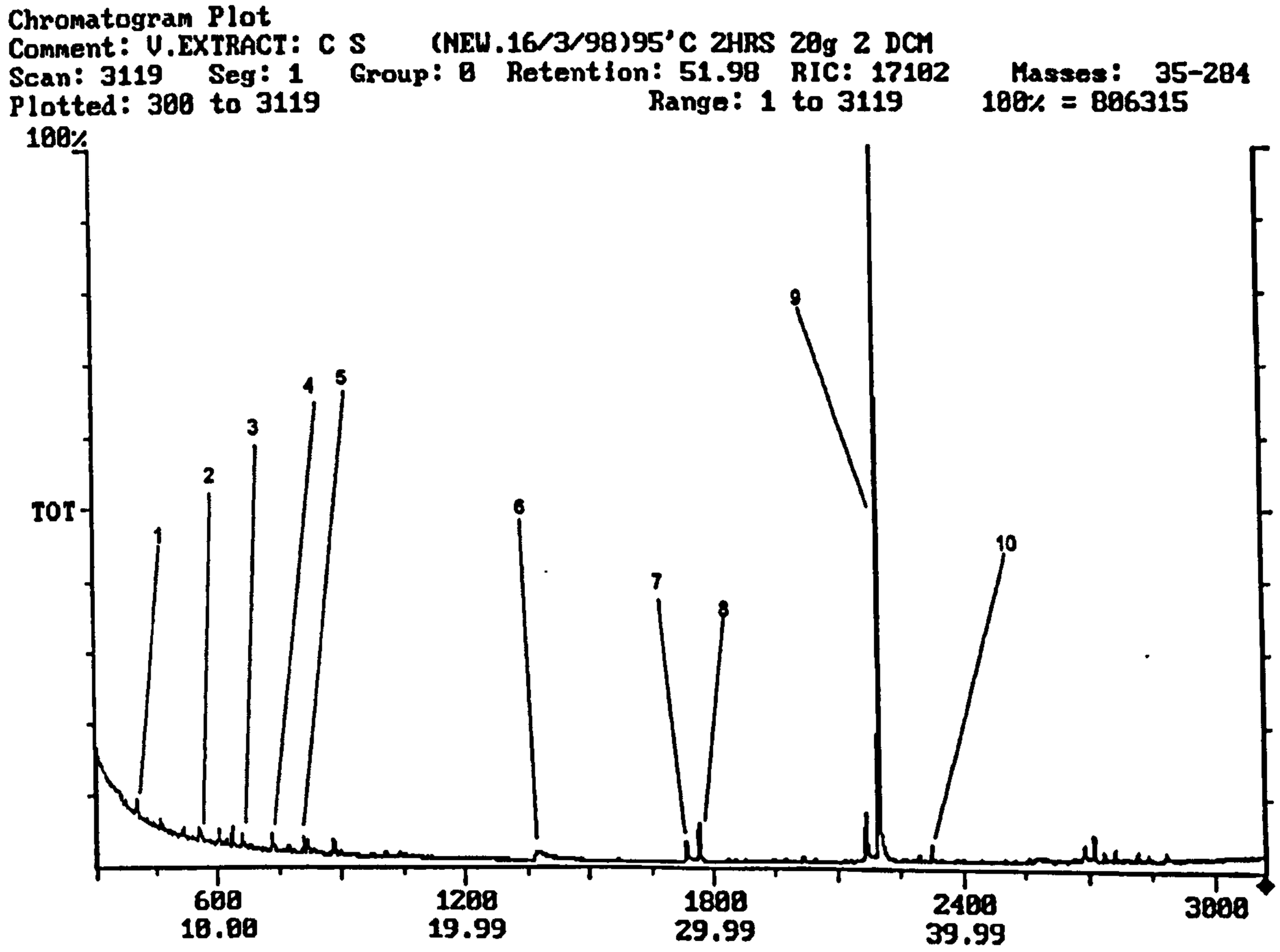
Peak assignment

Peak No.	Compound	Peak No.	Compound
1	1-Butanol	12	Methylmethylethoxypropanol
2	Pentanal	13	Trimethylbenzene
3	Heptane	14	2-Ethyl hexanol
4	1-Pentanol	15	2-Pentyl furan
5	Hexanal	16	Decane
6	Cyclohexanone	17	Nonanal
7	1,2-Dimethylbenzene	18	Cyclohexen-1-yl-benzene
8	Methylethylbenzene	19	Ester
9	Heptanal	20	Benzophenone
10	Styrene	21	Ethyl 4-dimethylaminobenzoate
11	Heptanal		

The majority of compounds usually recovered using static headspace analysis of carton-board samples were identified with the addition of some less volatile

components. The chromatogram acquired from the volatiles in the first trap shows a large number of compounds with longer retention times.

Figure 4.20 The chromatogram of the volatiles collected from the second collection vial by the extraction of the vacuum distillate of printed Carton Silkia with DCM.



Peak assignment

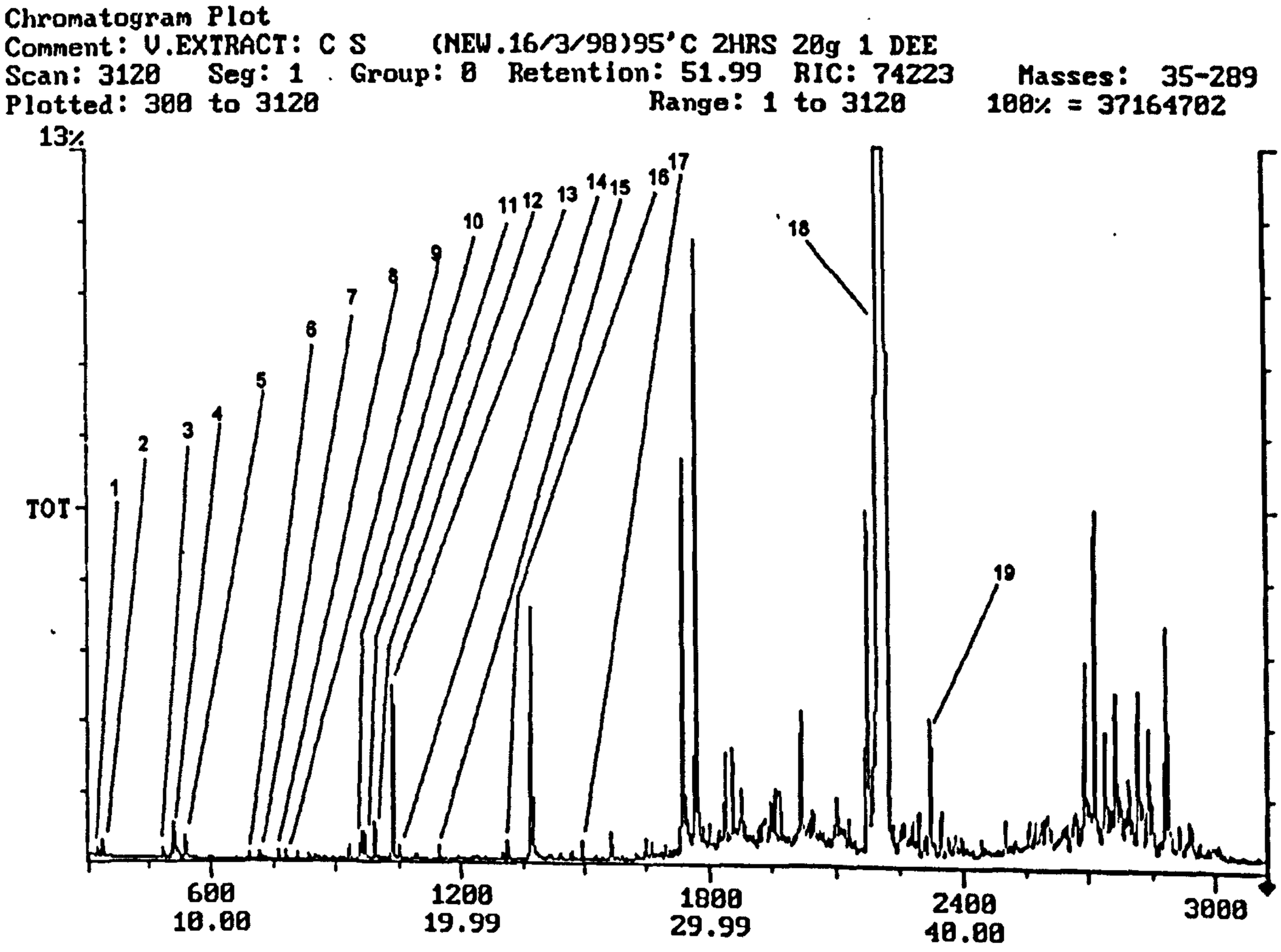
Peak No.	Compound	Peak No.	Compound
1	Pentanal	6	Butoxyethoxyethanol
2	Hexanal	7	Ester
3	Ethylbenzene	8	Ester
4	1,2-Dimethylbenzene	9	Benzophenone
5	Methylethylbenzene	10	Ethyl 4-dimethylaminobenzoate

Figure 4.21 shows the chromatogram of the volatiles present in 2μL of DEE used to extract the distillate from the first collection vial, produced during the vacuum extraction of 20 grams of printed Carton Silkia.

The chromatogram acquired from the volatiles extracted with DEE does not differ significantly from that for the DCM extract. This suggests that these two

solvents have equal effectiveness in extracting the condensed distillate of carton-board materials, despite DCM having Lewis acidic character and DEE have Lewis basic character.

Figure 4.21 The chromatogram of the volatiles collected from the first collection vial by the extraction of the vacuum distillate of printed Carton Silkia with DEE.

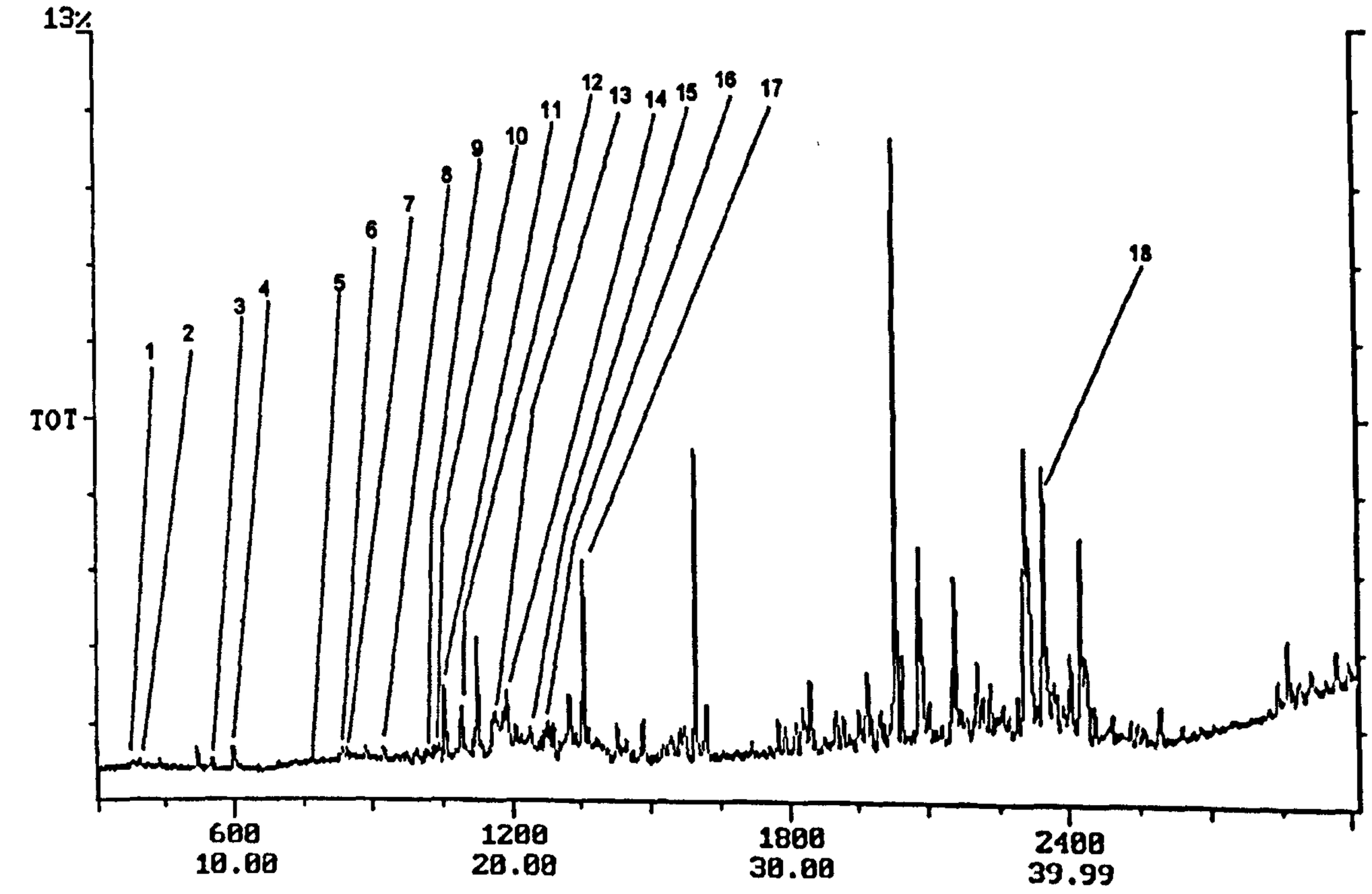


Peak assignment

Peak No.	Compound	Peak No.	Compound
1	1-Butanol	11	Benzaldehyde
2	Pentanal	12	α-Methylstyrene
3	1-Pentanol	13	2-Ethyl hexanol
4	Hexanal	14	2-Pentyl furan
5	Methylethoxyethanol	15	Decane
6	Cyclohexanone	16	Nonanal
7	Styrene	17	Isoborneol
8	1,2-Dimethylbenzene	18	Benzophenone
9	Methylethylbenzene	19	Ethyl 4-dimethylaminobenzene
10	Butoxypropanol		

Figure 4.22 shows the chromatogram of the volatiles sampled by static headspace from the first collection vial distillate produced during the vacuum extraction of 20 grams of printed Carton Silkia.

Figure 4.22 The chromatogram of the volatiles sampled by static headspace from the first collection vial produced during the vacuum extraction of printed Carton Silkia.



Peak assignment

Peak No.	Compound	Peak No.	Compound
1	Pentanal	10	Ethylmethylbenzene
2	Heptane	11	α-Methylstyrene
3	Toluene	12	Trimethylbenzene
4	Hexanal	13	2-Ethylhexanol
5	Ethylbenzene	14	Monoterpene
6	Styrene	15	t-Butylbenzene
7	1,2-Dimethylbenzene	16	Monoterpene
8	Methylethylbenzene	17	6-Methylheptyl-2-propenoate
9	Propylbenzene	18	Benzophenone

Less volatile components were recovered using static headspace analysis of the distillate than were recovered by solvent extraction. Static headspace analysis, however, yielded a higher number of alkylated benzenes.

The presence of alkylated benzenoid compounds, responsible for some 'plasticy' / 'solventy' / 'musty' taints, in the vacuum extracts of carton-board materials, suggest that these compounds are not formed from the decomposition of higher molecular weight components, during thermal equilibration using static headspace analysis of carton-board materials, as was suspected.

The vacuum extraction procedure provides a very stable environment with a very low partial pressure of oxygen, making oxidative breakdown reactions very unfavourable. Due to this very stable environment, the recoveries of lipid oxidation products were found to be lower using vacuum extraction compared to static headspace and dynamic headspace analysis. The dynamic headspace procedure yields the greatest amounts of lipid oxidation products from carton-board materials, despite providing less favourable conditions for oxidation than static headspace analysis. This was thought to be due to the increased recovery efficiency of dynamic headspace compared to the static headspace procedure.

The small amounts of odorous compounds associated with the oxidative decomposition of lipid components within the carton-board samples, recovered using vacuum extraction, explain why panellists did not complain of any significant taint associated with these compounds. Commonly, the presence of odorous oxidation products in a carton-board sample can be characterised, generally, by a 'green' / 'rancid' taint, particularly when water is used as the test substrate.

The large number of higher molecular weight compounds recovered using vacuum extraction are of less significance to taint and odour studies, with one or two exceptions, since these compounds have higher detection thresholds and are less mobile. Benzophenone, in particular, is one exception, with a characteristically strong odour and high migration powers.

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5 GENERAL DISCUSSION

The carton-board packaging system consists of a number of different components. Several of these components have the capacity to form the basis of taint and odour problems. The majority of these components introduce compounds into the packaging system which, if present in sufficient amounts, will cause a taint problem if they are allowed to come in to contact with the packaged food.

Several types of odorous compound and precursors of odorous compounds, were sampled by the techniques of static headspace, dynamic headspace and vacuum distillation extraction. These compounds that originate from the carton-board and the inks and varnishes have been discussed, as have the methods of taint evaluation. Many of the odorous compounds discussed in Section 3 bring about specific taint characteristics to a packaged food.

The most significant class of odorous compound that is formed from the autoxidation and photoxidation of oleic acid and linoleic acid are the aldehydes, the most dominant of which is hexanal. Hexanal has a characteristic 'green', 'grassy', 'fruity' odour. Another significant aliphatic aldehyde that is formed during linoleic acid oxidation is pentanal which has a 'rancid', 'green', 'pungent' odour. Table 5.1 lists a number of aliphatic aldehydes, their sensory characteristics, their odour and taste detection thresholds in paraffin oil and their odour detection thresholds in water.

Table 5.1 Flavour properties and threshold values (in ppm) of some aliphatic aldehydes formed by the oxidation of unsaturated fatty acids (Meijboom, 1964[1] and Grosch, 1987[2]).

Aldehyde	Sensory description	Paraffin oil		Water
		Odour [1]	Taste [1]	Odour [2]
Butanal	sharp, pungent	0.15	0.024	
Pentanal	rancid, green	0.24	0.15	0.024; 0.012
Hexanal	green, grassy, fruity	0.32	0.15	0.008; 0.016
Heptanal	green, soapy	3.2	0.042	0.008; 0.003
Octanal	citrusy, sweet, fruity	0.32	0.042	0.0008; 0.0007
Nonanal	citrusy, sweet, soapy	13.5	0.32	0.008; 0.001
Decanal	fatty, sweet	6.7	1.0	0.008; 0.0001

From butanal to decanal, the odour threshold concentrations range from 7-0.1 ppm in paraffin oil. In water, these threshold values range from 0.7 ppb - 0.024 ppm, indicating the importance of the substrate in which the aldehyde resides. The taste threshold values in paraffin oil are lower than are the corresponding odour thresholds, ranging from 0.024-1.0 ppm.

These aldehydes need only be present in very small amounts to be detected in packaged foods. If enough of a particular aldehyde is present in a carton package as a result of oxidation of unsaturated lipids, sufficient migration may occur into the food, from the carton, to cause a taint problem.

Two unsaturated aldehydes were formed in significant amounts from the oxidation of linoleic acid within the carton-board. Table 5.2 lists the sensory characteristics of these compounds in addition to their odour and taste detection thresholds.

Table 5.2 Flavour properties and threshold values (in ppm) of two unsaturated aldehydes formed by the oxidation of unsaturated fatty acids (Meijboom, 1964[1] and Grosch, 1987[2]).

Aldehyde	Sensory description	Paraffin oil		Water
		Odour [1]	Taste [1]	Odour [2]
trans 2-heptenal	Nutty, soapy, oily	14	0.63	0.051
trans 2-octenal	Woodbug, fatty, oily	7.0	1.0	0.004

The threshold values of these two aldehydes are slightly higher than those of their corresponding saturated homologues. Again, there is a significant dependence on the nature of the substrate in which the aldehyde resides.

The amounts of the above mentioned aldehydes recovered from samples of CTMP and Koppargloss carton-board that had been impregnated with unsaturated lipids, as discussed in Chapter 3, are present at levels that are significantly higher than their taste and odour threshold values in paraffin oil and in water. Typically samples of carton-board and CTMP impregnated with linoleic acid, and its derivatives, produce odours characterised by 'cardboardy', 'green' and 'soapy' at lower oxidation levels, and odours characterised by 'rancid', 'pungent', 'green', 'cardboardy', 'nutty', 'soapy' and 'musty' at higher levels of oxidation. Oleic acid impregnated samples produce a lower degree of

odour due to the smaller amounts of odorous compounds formed during oxidation. These odours can be characterised as 'cardboardy', 'sweet', 'citrusy', 'fruity', 'fatty' and 'soapy'.

If the test systems described were part of a real packaging system, severe taint problems could arise in foods, packaged in these materials. The implications of the presence of linoleic acid, and its derivatives, as opposed to oleic acid, and its derivatives, in carton-board samples are more severe since the formation of odorous volatiles from linoleic acid is much more rapid than in the case of oleic acid. The build up of odorous volatiles in the carton-board system resulting from decomposition of oleic acid is more significant over longer time spans, i.e. for packaged food products with longer shelf-lives.

It is unlikely that such high amounts of aldehydes would form in a real packaging system. However, it has been shown from tests using commercial, untreated CTMP carton-board substrates, and the work of Donetzhuber (1981), Söderhjelm and Eskelinen (1985) and Söderhjelm and Sipiläinen-Malm (1996), that odorous volatiles including aldehydes form over time. Ketones, alcohols, furans, lower fatty acids and hydrocarbons also form from oxidation of unsaturated fatty acids. Kochhar (1993) lists the sensory characteristics of these compounds and their threshold values. The threshold values of these compounds tend to be greater than those of the aldehydes. Thus, they need to be present in higher concentrations to cause a taint problem.

Tests involving the impregnation of unsaturated lipids into CTMP and carton-board matrices showed that oxidation reactions resulting in the formation of odorous compounds were dependent on a number of factors. These included the effect of time and temperature, the availability of oxygen, the relative humidity of the system, the presence of transition metal ions, the presence of photosensitisers and subsequent UV irradiation and the nature of the lipid (i.e. was it in free fatty acid form , alkyl ester derivative form or triglyceride form, and was it mono-unsaturated or di-unsaturated). These factors are, therefore, significant to the organoleptic quality of carton packaging, particularly if the food is contained in the carton package for longer periods of time.

The techniques of dynamic headspace analysis and vacuum extraction yielded more representative amounts of odorous compounds in carton-board materials, than were yielded using the static headspace procedure. This was due to the extraction procedures being carried out in the absence of oxygen.

The low amounts of odorous compounds found in samples of Carton Silkia carton board using the dynamic headspace procedure and the vacuum extraction technique translated to the low detection of taints characterised by 'green', 'rancid' and 'cardboardy', associated with the odorous lipid oxidation products. Samples of Carton Silkia analysed using static headspace analysis yielded greater amounts of oxidation compounds such as hexanal and 2-pentylfuran, the presence of which were not reflected in subsequent sensory evaluation. Studies involving the static headspace analysis of CTMP and carton-board substrates impregnated with unsaturated lipids showed that considerable amounts of volatile oxidation products form during thermal equilibration of samples.

Taint arising from packaging materials is dependent on the odorous compounds present within a packaging material, on the nature of the packaging substrate and the geometry of the packaging system, as well as the particular sensitivities of the human subjects.

Carton-board, as a packaging substrate, is porous. It consists of several layers of interlaced cellulosic fibres and cellulosic fibre fragments. The distribution of fibres and fillers varies according to the way the carton-board is made. Fine fibres and fillers are usually present in larger amounts, in the vicinity of the surface that was facing the wire bed during manufacture. The outermost layer is usually low in fibrous material (fines).

The pores in the fibre network are important to the movement of gases and liquids. Their volume, size distribution and shape differ depending on the carton-board grade. The largest pores are usually found closest to the zone associated with the wire surface (the base layer or back). Volatile compounds may, therefore, diffuse through the base layer more easily than through the top layer.

Cellulose is hydrophilic in nature and has high wettability. Water vapour can migrate into carton-board substrates rapidly. This penetration is reduced by sizing agents and by refining the wood pulp. Sideways spreading of water within a pulp layer may occur and is regarded as an extraction process. The water penetrates into the bulk of the board substrate, where it extracts components and brings them into the food by diffusion. Sensory testing of packaging samples at higher humidities demonstrated this phenomenon, as

indicated by the increases in the level of taint imparted to the test material compared to identical tests carried out at lower humidity.

The penetration of oils and fats into the carton-board is slower, but follow the same path as water (Söderhjelm and Sipiläinen-Malm, 1996).

The strengths of the bonds between migrating compounds and fibres can be indicated by relevant adsorption isotherms. Adsorption is governed not only by the strength of the bonds between the compounds and the surfaces of fibres and pigments but, also, by the extent to which the compounds are solvated by water. Water may also penetrate cellulosic fibres and cause them to swell, which will greatly affect adsorption.

The effect of packaging geometry has a significant effect on the migration of odorous compounds from package to contents. From the sensory testing studies, described in Section 2.3.4, in which milk chocolate was exposed to samples of printed carton packaging material, the degree of taint imparted to the chocolate increased in the order of: the system in which chocolate was not in direct contact with the carton sample, the system in which chocolate was in direct contact with the printed side of the carton, the system in which chocolate was in direct contact with the unprinted side of the carton sample. These findings suggest that the implications of the presence of odorous compounds in a carton material are more severe if the carton is intended for primary packaging of the food, i.e. where food and package are in direct contact, particularly if the food is in contact with the unprinted surfaces.

The sensory testing of unprinted carton-board and printed carton-board showed, in some cases, that the cured ink film, on the surface of the board, provided an effective barrier against the migration of odorous volatiles from the board to the test material. Sensory testing also demonstrated the fact that the ink film was capable of introducing components into the carton-board system that imparted 'inky' / 'varnishy' taints into the food material. The UV curable ink formulations contained low amounts of odorous compounds such as photoinitiators, amine photoactivators and residual solvents from prepolymer and diluent manufacture.

Taints arising from the migration of odorous compounds associated with the oxidation of unsaturated lipids and the presence of synthetic binder systems in carton-board materials, occurring when the food material and the unprinted surface of the carton are in direct contact were detected as 'plasticy', 'green'

and 'musty' during sensory evaluation of these systems. 'Plasticy' taints are attributed to acyclic compounds, such as toluene, 1,2-dimethylbenzene, ethylbenzene, styrene, α-methylstyrene and other alkylated benzenes.

Sensory testing also showed that 'flavour scalping' occurred from milk chocolate to CTMP. This 'flavour scalping' lead to a 'stale' perception. This phenomenon occurred through the air space between the two materials and through direct contact of the two materials. This reverse migration was enhanced at increased relative humidity. During sensory testing when 'flavour scalping' was identified, it was found that taint of the milk chocolate occurred simultaneously, indicated by the detection of a 'cardboardy' taste. The removal of flavour volatiles from the chocolate and, thus, the sensory competitors, reduces the detection thresholds at which the tainting compound(s) are detected, resulting in a more severe taint problem.

The risk to human health that may arise from ingestion of tainting substances is very important point from the toxicological, social, marketing and regulatory points of view. Originally, toxicology was the science of poisons, i.e. very harmful substances. It is now recognised that 'poison' is not a definitive scientific term. Adverse effects depend on the quantity of a substance ingested. For any compound ingested, these effects will lie on a spectrum from negligible at low levels, through a range of increasingly adverse symptoms and consequences at increasing quantity levels, to serious illness and, at sufficiently high dose, death. The dose of a compound at which a specific adverse effect occurs varies greatly. A poison is widely accepted as a substance that causes serious harm at low dose levels. Evaluation of the migration of substances into food is concerned with the assessment of small amounts of substances that may cause adverse effects over a long period of exposure.

The majority of toxicity measurements are carried out on living organisms. These range from bacteria to mammals. Results are obtained in statistical terms and expressed per unit of body weight (mg (kg body weight)$^{-1}$) for a single dose, and mg day^{-1} (kg body weight)$^{-1}$ for a chronic dosage.

For single dose, or acute toxicity, the most important parameters are LD_{50} and LC_{50} which are the lethal (single) dose or concentration that kills, (statistically), 50% of the population. Table 5.3 lists some LD_{50} values. These are based on oral application to a rat population for some of the compounds

that have been associated with carton-board taints (Sigma-Aldrich, 1996-1997). The units are based on mg of toxic compound per kg body weight of rat.

Table 5.3 LD_{50} values for some compounds associated with carton-board taints.

Compound	ORL-RAT LD_{50} value (mg/kg)
Hexanal	4890
Pentanal	3200
Heptanal	3200
trans 2-Heptenal	1300
2-Heptanone	1670
2-Pentylfuran	1200
1-Octen-3-ol	340
Toluene	636
Styrene	2650
α-Methylstyrene	4900
Ethylbenzene	3500

Considering that the average human weighs 60-70 kg, all the compounds listed in the table would need to be present in very high amounts to cause death. Though these values give an indication of toxicity they tend not to be so significant to the migration into food since the amounts of toxic compounds in food are likely to be several orders of magnitude lower. In tests relevant to migration, a chronic dosage is determined, below which toxic effects are negligible, unobserved, or not harmful. The chronic dosage of a particular tainting substance is given in terms of a safe migration level in to food (SML). For toluene the SML value is 0.02mg/kg of human body weight and for ethylbenzene 0.1mg/kg of human body weight. Significantly, these values are not much higher than the detection thresholds for these two compounds indicating the toxicological significance of low level taints.

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6 CONCLUSIONS

- Sensory testing can provide a very effective means of characterising taints imparted to food substrates by packaging materials. This effectiveness depends, however, on choosing an appropriate test for the system under test that reflects the experience of the individuals in the sensory panel.
- Static headspace sampling coupled to GC/MS techniques provides a basis of characterisation of the volatile components within the packaging system.
- It has been shown that commercial samples of wood pulp contain unsaturated species that are capable of undergoing oxidative reactions to form volatile compounds. Many of these compounds are odorous with very low taste and odour detection thresholds.
- Model systems created from CTMP and carton-board substrates that were impregnated with unsaturated lipids yielded a similar array of volatiles to those experienced in the real pulp and carton packaging systems.
- Oxidation reactions resulting in the formation of odorous compounds are of a free radical nature and proceed via hydroperoxide intermediates. The major hydroperoxides formed from the autoxidation of linoleic acid are 9-hydroperoxdes and 13-hydroperoxides. These breakdown to form hexanal and 2,4-decadienal, respectively. 2,4-decadienal rapidly undergoes further oxidation in the presence of oxygen to produce a range of odorous products.
- In these model systems, the free radical breakdown of the unsaturated lipids was affected by a number of factors. These included the storage temperature, the storage time, the availability of oxygen, the relative humidity, the nature of the lipid (i.e. free fatty acid, alkyl ester derivative or triglyceride) and the presence of photosensitisers.
- It was found that the presence of some UV curable inks, and their subsequent irradiation with UV light, brought about photoxidation within the system resulting in significant increases in the amounts of odorous compounds recovered. This photoxidation process involved the generation of singlet oxygen by an appropriate photosensitiser. It is thought highly likely that the photosensitising agent present was the photoinitiator, benzophenone.

- In addition to the compounds formed from oxidative decomposition of unsaturated lipids, odorous compounds can originate from other sources. Examples of such alternative sources are the synthetic binders used for the surface preparation of carton-board substrates and the inks and varnishes that contain odorous components such as photoinitiators, photoactivators and residual solvents associated with the ink/varnish vehicles.
- The equilibration period, employed during static headspace analysis of volatiles within carton packaging, can cause the accelerated formation and decomposition of odorous compounds, resulting from the oxidation of unsaturated lipids. Recoveries of volatiles from packaging materials, using dynamic headspace and vacuum distillation extraction techniques can be more representative of the volatile content of the package under ambient conditions. This is because these techniques provide a more inert environment to preserve compounds that are susceptible to oxidative attack. Dynamic headspace analysis and vacuum extraction procedures can, also, provide much higher recoveries of volatiles than is achieved by static headspace analysis.

FUTURE WORK

- The development of the sensory testing procedures described in Chapter 2 into a means of quality assurance testing for particular carton systems using recognised odour descriptors that can be used to gain a descriptive assessment of the system.
- The assessment of various conditions and treatments that can reduce the rate and extent of the formation of odorous compounds via the oxidation of unsaturated fatty acid precursors.
- The continued development of the dynamic headspace procedure for the characterisation of odorous volatiles present in very low amounts in carton-board samples.
- To investigate additional volatile compound sampling techniques such as steam-distillation extraction, solvent extraction procedures, supercritical fluid extraction and any other appropriate techniques.