LOSSES OF THIAMINE ASCORBIC ACID AND LYSINE IN THERMALLY STERILISED FOODS

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

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JULY 1982

TO

MY PARENTS, SISTERS AND BROTHERS

TO

ISMAEL

WITH

LOVE AND RESPECT

ABSTRACT

The major objective of this investigation is the comparative nutritional evaluation of thermosterile foods in pouches and in cans during sterilization treatment and during storage.

To achieve this objective, four products (whole brussels sprouts, whole new potatoes, pork casserole and rice pudding) were developed and produced in pouches and in cans. All variables were maintained constant in the production of these food products and equivalent heat sterilization treatments were applied. Available lysine, ascorbic acid and thiamine (vitamins C and B_1) were selected as the nutrients for this investigation. Those nutrients were determined immediately before and after the sterilization treatment and at several intervals during storage.

Pouched processing improved the retention of ascorbic acid and thiamine in relation to canning. Storage conditions produced mainly the same effects in ascorbic acid and thiamine retention independently of type of package for pork casserole, but larger losses were obtained for the canned products for brussels sprouts and new potatoes.

Available lysine losses due to the sterilization treatment are small ($\simeq$ 15%). Difficulties with the sterilization process control and the poor sensitivity of the analytical technique employed seriously question the significance of the comparison between the canned and pouched products.

For all samples the use of longer processing times to obtain higher sterilization treatments produced larger destruction of nutrients. Also the use of lower storage temperatures increased the nutrient retention.

Large losses of ascorbic acid were obtained in pouched brussels sprouts during storage at three storage temperatures (5°C, 20°C and 37°C), and the ascorbic acid losses were greatly

increased at 37°C storage conditions. A series of experiments were conducted to investigate these results. Peroxidase activity was found to be responsible for these losses. It was found to be present in the pouched samples but not in the canned products. It was determined that processing times of over 20 minutes at 121.1°C and 116°C were required to inactivate the enzyme. The results suggest that the improved heat penetration of pouches which permit the achievement of a microbiologically safe sterilization treatment in short times can also be insufficient for the inactivation of heat resistant peroxidases.

ACKNOWLEDGEMENTS

I wish to thank my supervisor, Mrs J. Ryley for her encouragement and discussion throughout the entire course of this work. I am also grateful to Professor D. S. Robinson for the opportunity to join the Procter Department.

I am deeply indebted to Mr. J. Lamb for his continuous involvement and helpful discussions, and I would also like to thank Dr. G. Stainsby, Dr. J. McKay and Mr. G. Glew for their encouragement and concern.

I am grateful to Mr. N. A. M. James, Mr. L. Guevara and Mr. P. Lappo for their assistance and hours of helpful discussion.

I am grateful to Mr. M. W. F. McCaw and Mr. K. J. Wilson of the Procter Department workshops for their excellent engineering services.

My appreciation is extended to all the members of the Procter Department for their consideration and encouragement and to my typists Mrs. J. N. Green and Miss S. Krawjewski for typing the manuscript.

Finally my thanks go to my friends for their consideration and help specifically to Miss G. Rao and Mr. J. Gasson who assisted me in the preparation of this thesis.

TABLE OF CONTENTS

Appendix

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

a	half the length of the brick or radius of the cylinder
A	Arrhenius frequency factor
AA	Ascorbic acid
AO-12	Acid Orange -12
b	half the width of the brick or half the length of the cylinder
B	process time, measured from the corrected zero time
c	half the thickness of the brick
c;	initial concentration of microorganisms
c_o	concentration of microorganisms at time t
CC	coefficient of correlation
c.t.	correction term
C.V.	coefficient of variation
C.V.T	come-up time
C.Z.T	corrected zero time
D	decimal reduction time
DAA	dehydroascorbic acid
DB	dye bound
DBC	dye binding capacity
DCP	2,6-dichlorophenol-indophenol
DKG	diketogulonic acid
DNP	dinitrophenylated
E_a	Energy of activation
f	heat penetration factor
F	equivalent sterilization value for a given microorganism at a given temperature
F_o	equivalent sterilization value at 121.1° C for <u>clostridium botulinum</u> (Z = 10 min)
FDNB	2,4,6-fluoro-dinitro benzene

g	number of degrees below retort temperature at the center of the container at the end of the heating cycle
HAR	histidine and arginine
HARL	histidine, arginine and lysine
I	temperature deficit-retort temperature minus initial temperature
IT	Initial temperature
J	lag factor
JI_h	Pseudo initial temperature
K	rate constant
K_F	pouch flatness coefficient
l	maximum thickness of the sealed pouch
L	maximum length across the pouch surface
L_r	Lethal rate
LSD	least significant difference
MCC	methoxy carbonyl chloride
MIV	o-methylisourea
OPDA	o-phenylene-diamine
R	gas constant
RT	retort temperature
t	time
T	Temperature
TDT	thermal death time
TNBS	2,4,6-trinitrobenzene-1-sulphonic acid
TNP	trinitrophenylated
U	thermal death time at retort temperature
$\bar{x}_i$	mean
z	temperature change required to reduce D or TDT by a factor of ten
α	thermal diffusivity
σ	standard deviation

subindices

c	cooling cycle
h	heating cycle

CHAPTER 1

INTRODUCTION

The effects of heat sterilization on the quality and the nutrient retention of foods has always been a major concern for food scientists and technologists; particularly since an important change in eating habits has been taking place, i.e. a decline in the consumption of fresh products with a subsequent increase in the consumption of processed foods (Hurt,1979, and Mencacci, 1975). This concern has constantly prompted studies into ways to enhance nutrient and quality retention during thermal sterilization processes. One of the promising developments for thermally processed foods is the development of the "retortable flexible pouch" or "flexible can", since it has been generally assumed from theoretical considerations that, two of the major advantages of pouch thermosterilization are the improved quality and nutritional value of the food. However, relatively few evaluations of the food quality in thermosterilized pouches have been reported and the only comparison of nutrient retention in thermal sterilized foods in pouches and cans reported (Thorne, 1976) suggests that although there is improved retention of ascorbic acid in potatoes in pouches in relation to canned potatoes,this is mainly an effect of reduced leaching losses and not a sterilization gain. Furthermore, he reported better overall retention during canning than during pouch processing.

These findings were not in agreement with the computer simulation studies of Teixeira et al (1975), who concluded that variations in the container geometry which permit a more rapid heating of the product can be very effective in achieving a significant improvement in the nutritional value of thermally

processed foods. He proposed that this geometry would approximate a flat disc shape. These conclusions support the generally held view that pouch processing would improve nutrient retention due to the reduction in processing time.

Therefore nutrient evaluation of pouch processing was considered an important topic for further studies. Since cans are the most successful heat sterilizable hermetic container, canned food was selected as a comparative product for these studies.

The use of shelf stable foods is reported to be growing in Japan (Tsutsumi, 1975) Europe (Mencacci, 1975) and U.S.A. (Heintz 1980) due to their consumer convenience and storability. However there has been no mention in the literature of studies in the nutrient retention of pouch processed foods during storage. Consequently it was also considered important to study the nutrient retention of pouched foods during long term storage, and that useful results in providing new directions for further research could be best obtained from a study carried out in real food systems which had commercial potential as menu items.

Therefore the objectives of this investigation are:

1. To study the nutrient retention of thermal sterilized foods in pouches .
2. To compare the nutrient retention of foods in pouches and in cans with equivalent sterilization treatments.
3. To investigate and compare nutrient retention of pouched and canned foods during long term storage.

CHAPTER 2

LITERATURE REVIEW

This chapter has been divided into four main sections. The first section deals with the thermal sterilization of food with particular reference to pouch technology and evaluation of sterilization processes for both canning and pouch processing. The second section deals with the stability of vitamins C, B_1 and proteins, while in the next section the effect of thermal sterilization processes on these nutrients are discussed. The fourth and last section deals with the assay methods for vitamin C, B_1 and available lysine.

SECTION 1

FOOD PRESERVATION BY THERMAL STERILIZATION

Natural foods are biological systems which undergo gradual deterioration from time of harvest or slaughter, and the seasonal production of food has always been a problem. To overcome this limitation and ensure an adequate food supply all year round, foods have to be preserved. The application of heat to foods is one of the most important methods of preservation, particularly in hermetically sealed containers. Traditionally, the containers used for this method of preservation include cans, glass jars and deep drawn aluminium, of these the top can is the most widely used.

The effects of heat sterilization on the nutritional and quality factors of foods has been a major concern for the food scientists. This concern has incited study of ways to increase quality and nutrient retention. Two results of these research efforts have been aseptic canning and HTST processsing. A third, a more recent result, has been the development of the "flexible retortable pouch". Although according to Lampi (1977) the prime objectives in the retortable pouch development for the army Natick research group were not the improvement of quality and acceptability but to meet the army feeding requirements, (which included fitting into field clothing packets, being soft and lighter in weight for easy carrying). However, as reported by Teixeira, et al (1975), in computer simulation studies to improve thiamine retention in thermal processing of foods, the change of geometry of the containers to a flat disc shape can be very effective to achieve a significant improvement in the nutritional value of thermally processed foods. This consideration supports the

development of flexible retortable pouches, as a method to improve quality and nutrient retention.

2.1 Flexible Packaging of Thermosterilized Foods

Two main types of "flexible retortable pouches" or "flexible cans" were developed in the last decade; those containing aluminium foil, and those which do not use aluminium foil (Rees, 1974). The former has been the most successful of the two and it is widely used in Japan and Europe, (Copley, 1978).

2.1.1 Materials and Package Construction

The flexible retortable pouch (or pouch for simplicity) has a bag shape, i.e. a rectangular 4 seals flat package, and is constructed from a laminate material consisting of three layers, (Cage and Clark, 1980; D.R.G. group, 1975; Ghosh, et al, 1977; Komatsu et al, 1975 and Szezeblowski, 1971): as shown in Figure 2.1.

- An internal layer or polyester or polyamide to import gas-tightness, strength for handling and printability.
- A central laminate of aluminium, impermeable to light and moisture.
- An inside ply of polypropylene, polyethylene or copolymer that gives strength for handling, heat seal and is inert with food.

Some of the typical retort pouch materials were listed by Lampi, (1973), in his extensive review, with specific data about the materials used in laminates in common use.

2.1.2 Characteristics

Resistance to damage from rough handling was extensively investigated by Burke and Schulz, (1972) in a comparative study

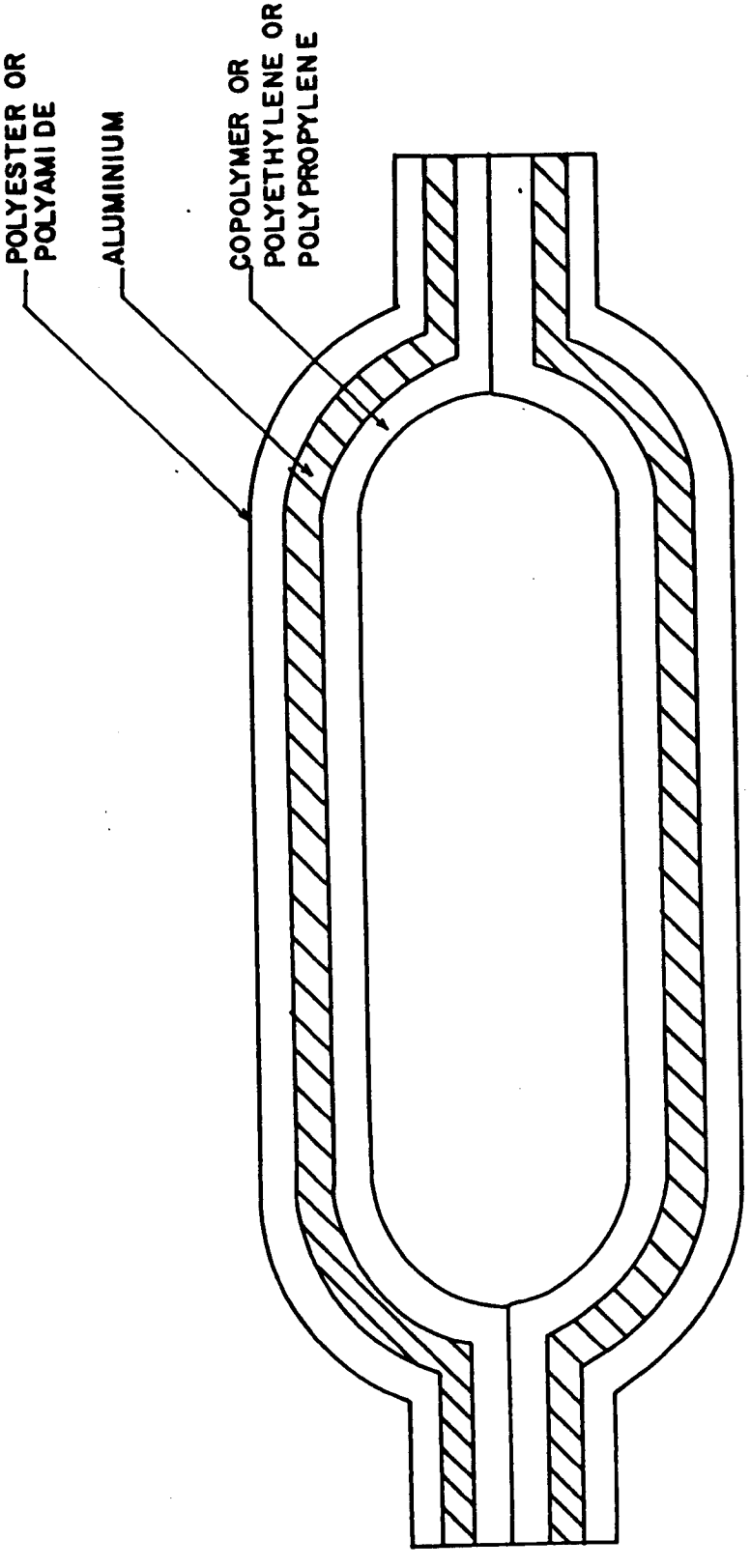


FIG.2.1 POUCH CONSTRUCTION AND MATERIALS

of the performance of pouches and cans. They concluded that pouches are capable of withstanding drop and vibration hazards at least as well as cans.

Szezeblowski and Rubinate, (1965), studied the resistance of pouch materials to food simulated solvents during food processing and found three films for which the total extractives were below the USDA and FDA limits.

Bacterial permeability was studied by various authors (Evans et al, 1978; Keith and Bee, 1980; Maunde r et al, 1968; Ronisvalli et al, 1966; Szezeblowski and Rubinate, 1965; and Szezeblowski, 1971). Szezeblowski (1971), after tests in which he buried the pouches in soil and left them hanging under tropical conditions, (85 - 95°C with 95% relative humidity) reported that the pouch was impermeable to bacteria. Biotests were applied by Bee (1979), Maunder et al (1968) and Ronisvalli et al (1966) by immersing damaged and undamaged pouches in suspensions of micro-organisms. They found that contamination was due to damage in the package as breaks in the film, seal rupture, holes in the film but not to the properties of the material. Maunder (1968) reported that after vibration, carton drop, 30 individual drops and static load (200 lb x 3 min) from 441 packages tested, 43 (10%) failures were detected.

Gas and water vapour transmission were investigated by Lampi (1973), Hu et al, (1968) and Turtle (1976). They concluded that pinholes and foil breaks can greatly influence the transmission of gas and water vapour. Lampi (1973) stated that oxygen permeability and water transmission rates of 0 cc/m ^{2}/24 hr/atm in undamaged pouches.

Pouches are known to withstand normal processing temperatures

(Mapp and Nieboer, 1972, D.R.G. group, 1975, and Turtle and Alderson,1968). Komatsu, et al, (1975), reported that pouches can stand temperatures in the heating medium well exceeding 130°C. Tsutsumi, 1975, reported the use of processing temperatures of 135°C.

The normal heat seal ranges reported by Lampi, (1973 and 1979), are between 160°C to 260°C.

2.1.3 Food Quality

Retortable pouches have been widely used for production of commercial products in England (Anon., 1976a), France (Anon, 1979a), Canada (Anon., 1975a, 1975c), West Germany, United States (Andres and Duxbury, 1972; Anon., 1973 and 1979b) and Japan (Tsutsumi, 1975). Some of the advantages claimed for the use of pouch processing in food production are briefly outlined below:

(i) Short process time. The extended pouch surface in relation to its volume, permits a fast penetration of heat, (Guedez and Bates, 1975, Hermans, 1977, Komatsu et al, 1975). Teixeira, 1975 using computer model simulation of thiamine retention demonstrated that containers with large surfaces produce better retention due to fast heat penetration rates.

(ii) Better organoleptic properties and overall quality, (Mermelstain, 1978). Tung, et. al. (1977) produced sweet corn in butter sauce in pouches processed at 121°C for 25 min and organoleptically compared them with an equivalent frozen product, by taste panels. The results show that the retort pouch product was highly acceptable and better sensory rankings were obtained for it. However, Platt (1979) compared eight products for consumer acceptance in both pouches and cans. He found no significant differences in the overall quality ratings.

(iii) Suitable for HTST processes. Some authors (Tsutsumi, 1975, Guedez and Bates, 1975, and Komatsu, 1975) state that this container can stand temperatures of the heating medium well exceeding 130°C which will bring a further reduction in process time, thus improving nutritional and quality retention of thermally processed foods.

(iv) Shelf stability. The pouch does not require refrigeration or freezing and is shelf stable as long as canned foods, (Norman, 1979, and Kramer (1979), Nebesky, (1977) and Shultz, (1972), reported that stability of pouch processed food can be as long as 8 years at 70°F, (21.1°C) or two years at 100°F (37.7°C).

(v) Fruits can be packaged with or without syrup or brine. Mapp and Nieboer (1972), Mermelstain (1978), Ashover and Borker, (1977), claimed that it is not necessary to add brine/syrup solution to the solids to aid heat penetration. The pouch can be vacuum sealed and the container itself is "wrapped around" the product. They also suggest that as there is no brine/syrup to leach out the flavour or ingredients, pouch processing may improve nutrient and quality retention. Copley, 1978, suggests that packaging without syrup or brine permits up to 40% savings in weight and volume.

(vi) Convenience for the consumer. As mentioned by an anonymous author (1979c) "the retortable pouch combines the advantages of the metal can and the plastic boil-in-the-bag". Since, like the boil-in-the-bag products, retort pouches can be safely and easily reheated in boiling water, normally five minutes are enough, (Anon., 1978, and Andres, 1979). Also packages are easily and safely opened by cutting with scissors, or even some companies like Reynolds Metals Co., have produced "tear off" pouches.

These two consumer convenience characteristics together with

long shelf life at normal canned food storage conditions were considered of importance in a study for the development of alternative methods of supplementary nutrition to elderly people in their own homes, carried out by the Catering Research Unit of Leeds University, (Armstrong et al, 1980). They developed an electrically operated dry heater to facilitate even further this operation for elderly subjects.

(vii) Cost reduction. Natick research laboratories carried out several product tests and reported cost reductions due to energy saving during processing of pouches, and space savings during the storage of pouch materials, (Anon., 1979d, Schultz, 1977, and Ryan, 1979), compared to both cans and frozen one-dish meals in aluminium foil pans (Brandt, 1978).

Schultz (1977) reported that the energy required to produce a 226 g (8 oz) pouch was 1934 BTU/container, while it was 3,560 BTU/container for a 226 g (8 oz) can.

2.1.4 Thermal Sterilization

It has been generally accepted that processing of flexible retortable pouches presents no great problem (similar to canning), provided certain basic recommendations are followed. According to several published guideline papers in pouch processing, (Broady, 1970, Hermans, 1977, Komatsu, 1975, Lampi, 1973, 1979, Mancacci, 1975, Michaels et al, 1979, and Platt, 1979), some of the most important are: air removal, counter pressure and pouch shape, which will be discussed briefly below.

Air removal

The enclosed air in the pouch must be kept to a minimum as it greatly influences the heat penetration rate, particularly in horizontally positioned pouches. Here the air may form a layer

that separates the upper pouch wall from the food thus creating an insulating effect. This means that most of the heat received by the product will penetrate through the lower pouch wall consequently increasing the necessary process time to achieve "commercial sterility", (or sterility for simplicity).

The residual air in the pouch can also increase the internal pressure of the pouch increasing the possibility of seal damage or bursting. These two problems can be reduced by increasing the pressure during processing, forcing the pouch walls to contact the food.

Counter pressure

The application of air overpressure, i.e. an excess pressure above steam pressure at heat process temperature, is essential in order to control thermal internal expansion of the pouch, thus avoiding seal damage or in extreme cases bursting of the package.

Yamano and Komatsu (1969) detailed various relationships between factors affecting packaging expansion such as residual gas volumes, process temperatures, internal pressure,etc., to produce a mathematical equation to calculate the required counter pressure to prevent failure for any process temperature. Later, Whitaker, (1971), reported also a procedure to calculate the overriding air pressure necessary to preserve the integrity of thermally processed pouches.

There are also several methods for the determination of residual gas volume of a retort pouch. These methods are of two types, destructive such as direct measurement method (Shappe and Werkowski, 1972) and calculation of direct measurement (Evans, et. al, 1978) and non-destructive such as neutral buoyancy technique (Yamaguchi,et.al, 1972) and pressure bouyancy (Lampi, 1973).

Pouch shape

One of the main advantages of pouch processing is the reduction in process time due to the extended pouch surface in relation to its volume. Therefore, to obtain a better heat transfer, the pouch should be preferably processed in a horizontal position as in this position the product can spread out over a maximum surface while keeping its thickness to a minimum. The use of retort racks has also been suggested (Komatsu, et. al, 1975, and Evans, et. al, 1978) to ensure that the pouch thickness does not significantly increase during processing. Komatsu, et. al., (1975) defined a coefficient of flatness (K_F) as a function of the maximum thickness (1) in the sealed package, in centimetres and maximum surface across the surface of the container (L), in centimetres:

$$K_F = \frac{1}{L} \qquad \text{eq. 2.1}$$

He proposed that K_F should be maintained at a level not exceeding 0.15 to 0.25 for processing temperatures between 130° to 250°C for HTST pouch processing as a measure to ensure package integrity.

The pouch thickness and shape (due to its position in the retort) is also important for the determination of the heat process, since the location of the thermocouples for process monitoring will be determined by the pouch shape. The variations in pouch thickness can cause severe under processing of some areas and over processing in others. Therefore strict control of pouch thickness is recommended through the heat processsing cycle to ensure adequate and standard heat treatment. This can be partially achieved by the use of restricting racks or shelves, (Evans et al, 1972, and Roope and Nelson, 1982).

2.1.5 Processing equipment

Counter pressure above the process equilibrium pressure can be achieved commercially in two ways, by water processing with superimposed air pressure (similar to that used for glass containers), or by homogeneous mixed steam-air processing. Several technical developments are occurring to meet the needs of pouch processing such as air-steam retorts (Milleville, 1980a, 1980b) hydrostatic sterilizers (Anon., 1975d and 1978d) fill-form and seal units (Anon., 1974 and Pinto, 1972); Hydropac processors (Heid, 1970); plate heat exchanger retorts (Haveghorst, 1975) and even systems for aseptic pouch packaging (Davis and Maunder, 1967).

Also several studies on the use of the existing canning retorts with little or no modification for pouch processing have been reported to solve the economic problems involved in establishing a retort pouch line either for processing or research., (Roop and Nelson, 1981, and Castillo, et. al., 1980).

2.1.6 Post-process contamination

In spite of its advantages the retortable pouch also has some disadvantages, the most important problem being post processing contamination of pouched foods. Post process contamination of pouches can occur through:

(a) Seals which are imperfect as a result of wrinkles, or enclosure of food materials in the seal area (Davis and Maunder, 1967, Evans and Thorpe, 1978, Lampi, et. al, 1976, Michaels and Scham, 1978, Rubinate, 1964 and Shenkenberg, 1975).

(b) Punctures and foil breaks in the packaging material due to rough handling (Hu, et. al, 1971, Maunder, et. al, 1968,

Shenkenberg, 1975 and Szezeblowski, 1971).

(c) Delamination during processing due to defective pouch material or over processing (Komatsu, 1975, and Shenkenberg, 1975).

In an effort to solve these problems, several studies have been carried out providing different answers.

Ashover and Borker, (1974), Evans, et. al.,(1978), Michaels and Schram,(1979), and Turtle and Alderson,(1971), have proposed a series of recommendations for pouch processing and post-processing handling to improve pouch performance, they may be summarised as follows:

(i) Seal inspection after filling by mechanical or visual means

(ii) Control of pouch position and thickness during processing.

(iii) Cooling water must be chlorinated.

(iv) Post-processing pouch handling equipment must be sanitised carefully.

(v) Pouches must be dried and over-wrapped individually as soon as possible.

(vi) Processed pouches must be handled mechanically at all stages up to and including cartoning and over-wrapping.

This last recommendation has been dropped in later studies. Michaels and Schram,(1979), conclude that by means of seal inspection and hygienic post-process handling, lower failure rates should be attainable for large scale production.

As an answer to the technial problems of inspecting seals before processing, new types of sealing machines were designed (Mencacci, 1975 and Anon, 1975c) and inspection units to spot defective seals by infrared scanning were developed (Lampi, et. al, 1969, 1979 and Ordway and Schultz, 1972).

2.1.7 Performance

Nevertheless, Maunder et al, (1968), Rubinate, (1964), and Szezeblowski ,(1971), have reported that failure due to rough handling are below 5% and Duxbury ,(1973), and Turtle and Alderson ,(1968), agree that the failure rate of pouches is of the same order as for cans and closing the gap to match the Japanese final defect rate of 0.02%, (Tsutsumi, 1975).

2.2 Heat Process Determination

The heat treatment of foods in hermetically sealed containers aims at the destruction of microbiological life and inhibition of enzymic activity, with the least possible effect on the quality and nutritive value of the product.

A combination of several factors dictate the type and severity of thermal processes applied to the food to produce commercial sterility. Among these factors are the pH of the food, type and number of micro-organisms present, mode of heat penetration into the product, type and size of container.

Foods are normally divided according to their acidity in four groups (Desrosier, 1970, and Stumbo, 1965): high acid foods with pH < 3.7: acid foods with pH values between 3.7 and 4.5: medium acid foods with pH values between 4.5 and 5.0 and low acid foods with pH > 5. Recently some authors (Karel et al 1975; Leniger and Beverloo, 1975; and Jackson and Shinn, 1979) consider only three types of foods, high acid foods (pH < 3.7), acid foods ($4.5 \geq pH \geq 3.7$) and low acid foods (pH > 4.5). These limits have been carefully chosen based on micro-biological considerations. Spore forming bacteria do not grow at pH less than 3.7 and 4.5 is slightly lower than the minimum pH at which *Clostridium botulinum* can grow and produce toxin.

Due to extreme effects of *Clostridium botulinum* toxin, this micro-organism is assumed present in all low acid foods intended for canning, and the thermal sterilization process aims at its destruction.

2.2.1 Microbiological considerations

It has been generally accepted that the thermal destruction of micro-organisms follows first order kinetics, which can be expressed as:

$$\log c_e = \log c_i - \frac{kt}{2.303} \qquad \text{eq. 2.2}$$

where

c_e = concentration of micro-organisms at time t

c_i = initial concentration of micro-organisms

k = first order reaction rate constant

t = time

A graphical representation of this equation (Figure 2.2) will be a line intersecting the y-axis (log of concentration of survivors) at c_i with a slope equal to -k/2.303. This curve is known as the survivor curve and the time required for the curve to traverse a log cycle is called the "decimal reduction time" or D value, which is the time required at constant temperature to reduce the population of a micro-organism by 90%. The relationship between k and D can be represented

$$D = \frac{2.303}{k} \qquad \text{eq. 2.3}$$

by substituting in equation 2.2

$$t = D(\log c_i - \log c_e) \qquad \text{eq. 2.4}$$

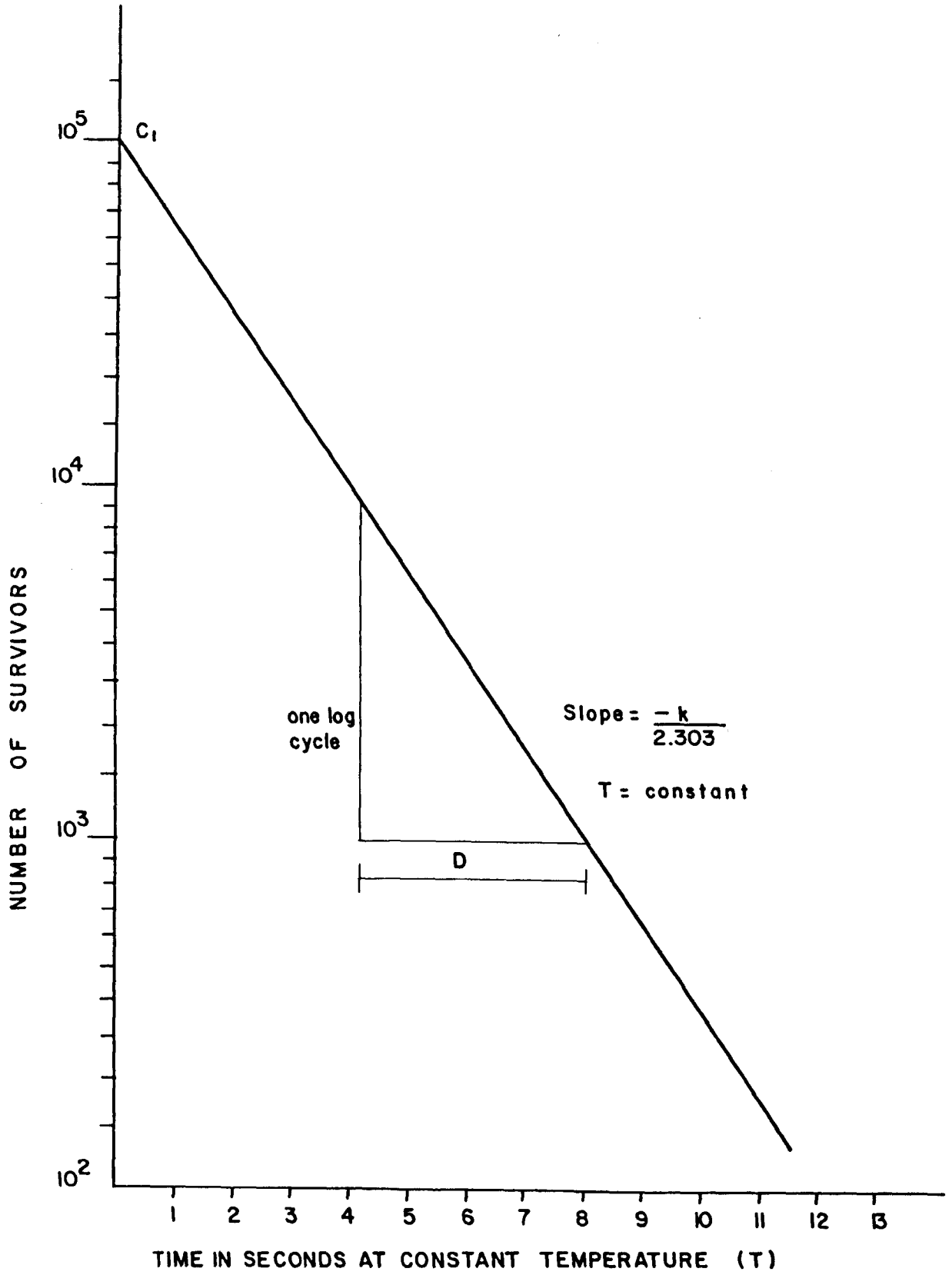


FIG. 2.2 SURVIVOR CURVE

The dependence of k on absolute temperature can be described by the Arrhenius equation as follows:

$$k = A \exp(-Ea/RT) \qquad \text{eq. 2.5}$$

where

A = the Arrhenius frequency factor, which is a constant (min^{-1})

Ea = Activation energy (J/mole)

R = gas constant (J/mole)

T = absolute temperature (K)

applying logarithms to both sides:

$$\ln A = \ln k + (Ea/RT) \qquad \text{eq. 2.6}$$

This mathematical relation can also be represented by a line with a slope equal to Ea/R when plotting ln A as 1/T.

Although the Arrhenius equation is one of the best ways in chemistry of representing the temperature dependence of k, in the canning industry for the evaluation of microbial destruction the thermal resistance (D) or the thermal death time curves (TDT) are used instead. Their graphical representation is shown in Figure 2.3, where it can be seen that:

$$\log (TDT_1/TDT_2) = \frac{1}{Z} (T_1 - T_2) \qquad \text{eq. 2.7}$$

or:

$$\log (D_1/D_2) = \frac{1}{Z} (T_1 - T_2) \qquad \text{eq. 2.8}$$

where:

Z = temperature change required to reduce D or TDT by a factor of 10. It characterises the micro-organisms since it represents a measure of its heat sensitivity.

D = the time required to reduce by a factor of 10 the

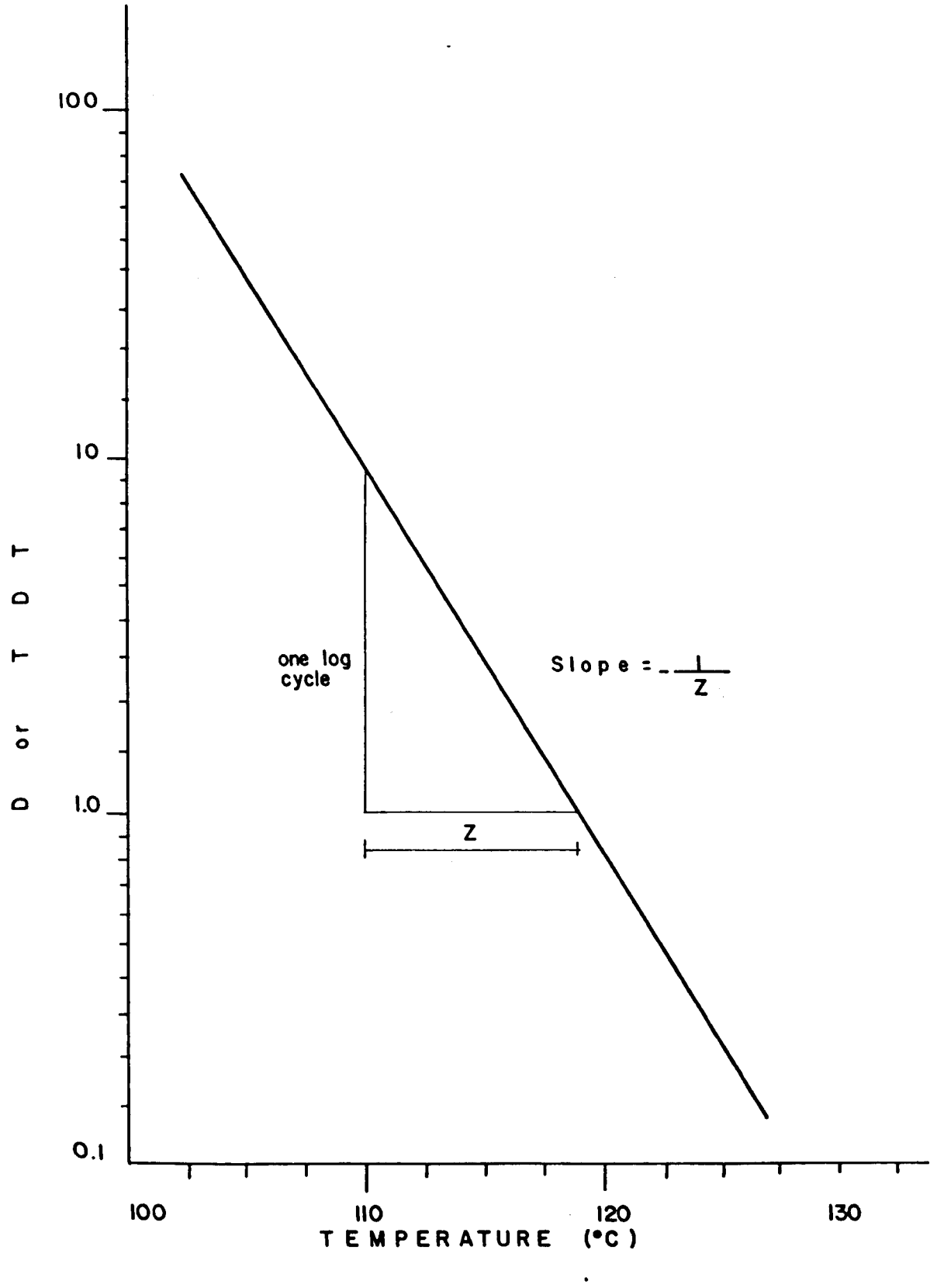


FIG.2.3 THERMAL RESISTANCE OR THERMAL DESTRUCTION TIME CURVE.

number of survivors of any given micro-organisms

TDT = the time required to destroy any population of any given micro-organisms. It is normally a multiple of D, and for microbial inactivation is represented by the symbol F.

These three terms, decimal reduction time (D), thermal death time (TDT or F) and Z-value are the basis for thermal processing evaluation in the food industry. Several reviews on the methods available for the evaluationn of the sterilization processes have been published in the literature (Brennan et al, 1976; Charm, 1978; Desrosier, 1970; Jackson and Lamb, 1981; Jackson and Shinn, 1979; Joslyn and Heid, 1963; Hayakawa, 1977; Helman and Singh, 1981; National Canners Association,1976 and Stumbo, 1965).

2.2.2 Process determination

To characterize the destruction of microbiological life, the concept of F_o-value has been widely used in the food industry. F_o-value is a measure of the lethality of a sterilization process and it is measured in minutes (see TDT definition above). For its complete definition it is referred to a standard temperature and a specific micro-organism (which is characterized by its Z value). The reference temperature most commonly used is 121.1°C and the micro-organism most important in canning is *Clostridium botulinum* which has an average Z value of 10°C. This will be represented as $F^{10°C}_{121.1°C}$ or for convenience as F_o. Therefore an F_o-value of 5 minutes will represent the lethal effect caused in *Clostridium botulinum* spores if they were instantaneously heated to 121.1°C, held for 5 min at 121.1°C and instantaneously cooled down.

It has been established by statistical considerations (Stumbo 1965) that the minimum sterilization process should be such as to reduce any population of heat resistant *Clostridium botulinum* spores by 10^{12}.

Therefore, recalling equation 2.4:

$$t = D(\log c_i - \log c_f)$$

$$F_o = t = D(\log 10^{12} - \log 1)$$

$D = 0.21$ from tables (Karel, et. al., 1976)

$$F_o = 0.21\ (12-0)$$

$$F_o = 2.52 \text{ min}$$

So the minimum accepted sterilization treatment in terms of F_o-value is 2.52 min for the canning industry.

Several methods have been developed to evaluate the steril - izing effect of a thermal process. The two most widely used are the "General method" proposed by Bigelow, et. al.,(1920), and the "Mathematical method" or "Formula method" originally developed by Ball, (1924). The applicability of these methods depends on the type of heat penetration curve exhibited by the product.

Three types of heat transfer mechanisms in cans were described by Jackson and Olson, (1940), when conducting experiments with bentonite solutions: convection (1% bentonite) conduction (5% bentonite) and combination of the two (3.25% bentonite). They also reported the types of heat penetration curves obtained with these bentonite solutions which were described as single (convection or conduction) and broken type (combination of the two) heat penetration curves. These two types of heat penetration curves have been used to characterize foods for the selection of the sterilization process evaluation.

(a) General method

The general method is based on the knowledge of the time-temperature relationship at the cold spot of the container. According to the National Canners Association ,(1978), this method is useful for determining the exact lethality of a particular process and it is particularly valuable when the heat penetration curve on semilogarithmic paper can not be represented by one or two lines. To obtain the F-value of the process, with this method a manual integration of the lethality of the process is carried ou t based on the several basic considerations. One of the most important assumptions is the construction of a hypothetical thermal death time curve passing through 1 min at 121.1°C for a micro-organism with a Z = 10°C. This assumption permits the calculation of the sterilizing capacity of any process in terms of minutes at 121.1°C;by recalling equation 2.7.

$$\log(TDT_1/TDT_2) = -\frac{(T_1 - T_2)}{Z}$$

using Ball's hypothetical TDT curve the point defined will be

$TDT_2 = 1$ min

and $T_2 = 121.1$ min

by substitution

$$\log(TDT_1/1) = -\frac{(T_1 - 121.1°C)}{Z} = \frac{(121.1°C - T_1)}{Z}$$

$$\frac{TDT_1}{1} = 10^{\frac{121.1 - T_1}{Z}}$$

lethal rate is defined as

$$L = \frac{1}{TDT} \qquad \text{eq. 2.9}$$

therefore

$$L = \frac{1}{10^{\frac{121.1 - T_1}{Z}}} \qquad \text{eq. 2.10}$$

With these formulae the lethal effect on a micro-organism at any temperature can be transformed to equivalent lethal effect at 121.1°C. Therefore the sterilizing effect of the process can be obtained by the integration of the lethal effects

$$F_o = \int_{\text{beginning process}}^{\text{end process}} L\,dt \qquad \text{eq. 2.11}$$

This method cannot be used to predict process times of F_o values for the same food in a different process or a different container. However, this method can be complemented by the mathematical method and it can be used regardless of the shape of the heat penetration curve.

(b) Mathematical method

The mathematical method is based on the assumption that the can centre temperature will approach retort temperature during the sterilization process with a logarithmic rate. This logarithmic approach is expressed by the following formula (as proposed by Ball, 1924)

$$B = f_h \log(J_h\ I_h/g) \qquad \text{eq. 2.12}$$

where

B = process time, measured from the corrected zero time

f = heat penetration factor of the heating cycle and the time for the temperature deficit I_h to pass through one log cycle

U = thermal death time (TDT) at the surrounding temperature or retort temperature RT

I_h = temperature deficit - retort temperature minus initial temperature (RT - IT) during heating cycle

g = number of degrees below retort temperature at the centre of the container at the end of the heating cycle

j_h = log factor of the heating cycle $j_h = jI_h/I_h$

jI_h = Pseudo initial temperature - interaction of the corrected zero time and the asymptote to the heat penetration curve

CUT = Come-up-time - time for the retort to reach process temperature

CZT = corrected zero time - 0.58 of CUT

A similar expression can be defined for the cooling cycle but instead of using h as subscript, c should be used which stands for cooling.

A series of tables and curves relating these variables are available. Plots showing the relationship between f_h/U ratio with log of g at different m + g and Z values are normally given in text books or tables relating f_h/U ratio to g at fixed J_c and Z values. The latter are the most widely used since it is known that small variations in m + g value (< 10°C) have a negligible effect on the F-value (< 1%).

This method can be employed for any condition of can size, initial temperature and retort temperature when heat transfer within a can is known for a particular set of conditions and the semilogarithmic heat penetration curve is known to be represented by a straight line. This method gives reliable results when changing can size only when the heat transfer is mainly by conduction.

Based on experimental heating curves of conduction packs

Olson and Jackson (1942) showed that the heating rate, designated as f_h, is a function of the thermal diffusivity and the dimensions of the container. They proposed several equations for the determination of thermal diffusivity, (α), according to the geometry of the container. The equation of a rectangular object (brick) is as follows:

$$\alpha = \frac{0.933}{(1/a^2 + 1/b^2 + 1/c^2)\ f_h} \qquad \text{eq. 2.13}$$

where:

α = thermal diffusivity

f_h = slope of the semilogarithmic heating curve

2a = length of the brick

2b = width of the brick

2c = thickness of the brick

and for a finite cylinder:

$$\alpha = \frac{0.398}{(1/a^2 + 0.427/b^2)\ f_h} \qquad \text{eq. 2.14}$$

where:

2a = diameter of the cylinder

2b = length of the cylinder

Since the thermal diffusivity for conduction-heating food products varies only slightly in the range of temperatures used and it is independent of container size or dimensions, it can be considered the same for any product packaged in two different sizes of container. Therefore, conversion factors for f_h-values from one size of container to another can be calculated from the following expressions:

$$\frac{fh_1}{fh_2} = \frac{(1/a_2^2) + (0.427/b_2^2)}{(1/a_1^2) + (0.427/b_1^2)} \qquad \text{eq. 2.15}$$

and

$$\frac{fh_1}{fh_2} = \frac{(1/a_2^2) + (1/b_2^2) + (1/c_2^2)}{(1/a_1^2) + (1/b_1^2) * (1/c_1^2)} \qquad \text{eq. 2.16}$$

Equations 2.14 and 2.15 have been widely used for cans and conversion factors are available in tables for the commercially available can sizes (National Canners Association, Vol I, 1976)

Retortable pouches approximate to a rectangular shape when liquid, or liquids with small pieces of solid, or paste like food products have been packaged into them. Consequently, equations 2.13 and 2.16 can be used for the calculations of thermal diffusivity and conversion factors for different pouch dimensions.

Later Schultz and Olson (1938) suggested a method to convert heat penetration data from one can size to the other when heat is mainly transferred by convection. They developed a term called "can factor" which is used to convert the f_h-value of a product from one can size to the other. These can factors are also tabulated in several books ,(National Canners Association, 1968), for common can and jar sizes used in industry.

(c) F_o-value integration method

Both the general and the mathematical method are based on the probability of survival at one point, the cold point of the container. Stumbo (1965) developed a mathematical procedure to evaluate the process time based on the probability of survival in the whole container, and thus reduce overprocessing. However this procedure is only applicable to conduction heated foods. A full treatment of the method is presented in his book.

Various other methods have been reported in the literature for the evaluation of average lethality and mass average retention. Most of these methods are attempting to refine the mathematical basis of temperature prediction and require correspondingly complex solution techniques. Accurate prediction of temperature enables temperature dependent changes to be predicted and optimum processing conditions selected. The mathematical models developed have only been adequate for changes following first order kinetics in conduction heated packs and most of them have mainly been confined to thiamine in model systems and pureed food materials (Castillo, 1979; Hayakawa, 1977; Mulley, 1975; and Teixeira et al, 1969). However it has been mentioned that D and Z and E_a values are subject to biological variations for any food products. This variation can affect the sterilization evaluation. In a study to evaluate the effect of this variation in the prediction of temperatures in canned foods, Lenz and Lund (1977a, 1977b) compared several methods of sterilization process evaluation of conduction heated foods. The methods compared were the general method (Bigelow and Esty, 1920); the mathematical method (Ball and Olson, 1957) as modified by Stumbo, (1973); the method of Teixeira et al (1969); the method of Herndon et al (1968); the method of Flabert and Dettour, (1972) lethality-Fourier number method (Lenz and Lund, (1977) and the method of Hayakawa, (1970). They found that the lethality fourier number method was equivalent to the improved mathematical method and they were the best methods for predicting temperatures in canned foods.

One of the major shortcomings of most of the available methods for calculating average lethality or retention of nutrients is that they are based on using the TDT equation for the relationship between reaction rate and temperature instead of the more

accurate Arrhenius equation.

Reichart, (1979), reported that the death rate of micro-organisms may be described as a first order kinetics only at certain micro-organism concentrations. He and Hayakawa, et. al, 1981, proposed new methods for the determination of heat destruction parameters of micro-organisms such as the D-values. All these methods need also major computational effort to solve the complex equation proposed. Therefore their use at present is limited and outside the scope of this work.

SECTION 2

RELATIVE STABILITY OF NUTRIENTS

It has been generally agreed in reviews and books on the subject that the most sensitive nutrients to food processing and storage are vitamins and proteins.

2.3 Vitamins

Vitamins naturally occurring in foods may be present in several forms with different stabilities. Although careless handling of foods such as bruising and inappropriate storage conditions and preparation processes such as peeling and trimming can greatly reduce the vitamin content of fresh foods, one of the main causes of loss during preparation and processing of foods is leaching of water soluble vitamins.

The extent of leaching of water soluble vitamins is dependent on various factors such as the state of the food, pH, time and temperature of the water treatment, solid to water ratio presence of other damaging or protective substances in the water, etc. The processes where leaching can occur include blanching, washing, cooking, cooling, canning in brine or syrups, etc.

Some processing additives are known to damage vitamins as in the case of sulphur dioxide (SO_2) which is used to prevent both enzymic and non-enzymic browning, but damages thiamine, (Oguntona and Bender, 1976).

Many vitamins are easily oxidised and are sensitive to heat but factors such as pH, water activity, oxygen and metal concentrations, temperature and others, influence their stability. To summarise these effects, Harris and Karmas (1977) tabulated

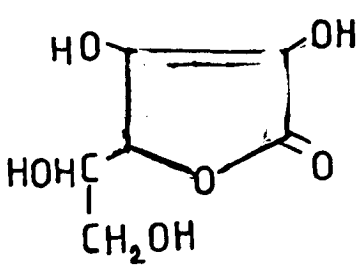
the relative stabilities of vitamins under various conditions, where it is shown that vitamin C can be entirely lost and vitamin B_1 can be reduced to 20% of its original value after cooking. Most authors agree that these two vitamins are the most sensitive to thermal processes, due to their heat sensitivity and water solubility.

2.3.1 Vitamin C

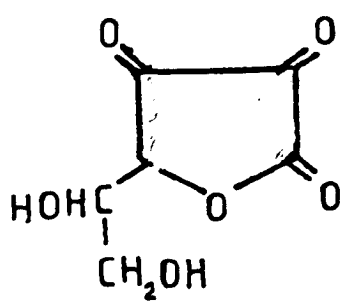
The chemistry, occurrence in foods, and stability of vitamin C have been extensively reviewed by the American Medical Association, 1974 b, Archer and Tannenbaum, 1979, Bauerfeind and Pinkert, 1970, Bender, 1966 and 1978, Benterund, 1971, Birch and Parker, 1974, Chichester 1973, Dyke, 1965, Harris and Karmas, 1975, Harris and Von Loesecke, 1960, Hollingsworth, Kramer, 1977, Lee, 1977, Lund, 1977 and 1979, Paul, 1977, Priestley, 1979 and Sebrell Jr. and Harris, 1972).

Structure

Vitamin C is present in foods as L-ascorbic acid or reduced form and as L-dehydroascorbic acid or oxidised form. Both forms are physiologically active. Ascorbic acid and dehydroascorbic acid are optically active, but only the L-isomer is physiologically active. The chemical name of the compound is L-threo-2,3,4,5,6-pentahydroxy-2-hexenoic acid-4-lactone, or L-threo-hexono-1,4-lactono-2-ene, which is shown below, together with the oxidised form.



L-ascorbic acid



L-dehydroascorbic acid

Ascorbic acid is a powerful reducing agent and strongly acidic but dehydroascorbic acid is not acidic. Both are highly soluble in water. Ascorbic acid is very sensitive to various modes of degradation. Factors that can influence the degradation mechanisms and amount present in foods are briefly mentioned below.

Occurrence in Foods

According to Oliver (1972), most of the vitamin C contribution in foods comes from fruits and vegetables. The distribution of ascorbic acid within one individual fruit or vegetable is extremely variable (skins, pulp, leaves) and also the vitamin C content of any type of vegetable or fruit is influenced by several factors such as variety, cultural practice, maturity, climate, etc., as reviewed by Harris and Karmas, (1975), Harris and Von Loesecke, 1960 and Nagy, 1980. There are no reports of naturally occurring D-isomers (which are without physiological activity).

2.3.2 Stability of Vitamin C

The degradation of ascorbic acid in foods is known to be dependent on several environmental factors, like temperature, pH value, presence of air, light and heavy metal ions, enzymes, time, ascorbic acid concentration, presence of other reductants and/or oxidants and oxidation-reduction potential of the system. According to most authors, the main mechanisms of vitamin C loss are enzymic degradation and aerobic and anaerobic reactions of a non-enzymic nature.

Enzyme Destruction

There are several enzymes present in foods which can oxidise vitamin C. These enzymes include cytochrome oxidase, ascorbic acid oxidase and peroxidase (Reed, 1975).

Peroxidases are important in processed foods because of their reported heat stability, since they can survive blanching and HTST processes, (Adams, 1978; Gibriel et al, 1979). They are known to be responsible for many off flavours in some foods, (Burnette, 1977 and Baardseth and Slinde, 1980). Reed (1975) stated that it has been generally accepted that if peroxidase is destroyed, then it is quite unlikely that other enzyme systems will have survived. Furthermore, with reference to conventional canning processes (116°C to 121°C), Karel (1975) stated that the inactivation of enzymes during blanching is not a primary objective since the produce generally receives a thermal process severe enough to inactivate enzymes. So for this project they are of minor importance. Nevertheless, it should be mentioned that the oxidation of ascorbic acid by peroxidases needs the presence of peroxides and substances which are able to form quinones in presence of peroxidase activity. The oxidation of ascorbic acid normally proceeds until all the peroxides are reduced (Saunders, et al, 1964, and Tauber, 1950).

Aerobic Degradation

In the presence of oxygen, ascorbic acid, (AA), is known to degrade to dehydro ascorbic acid, (DAA), in the first step. Levadoski, et. al. (1964) using ^{14}C-labelled ascorbate confirmed the findings of Herrmann and Andrae, (1951), that monodehydro-ascorbic acid is formed as an intermediate in the autoxidation of AA to DAA. This autoxidation is greatly influenced by the

presence of oxygen but according to Khan and Martell, (1967a), is not proportional to the amount of oxygen concentration at low partial oxygen pressures (0.40). They explained this effect by suggesting the possibility of a different oxidative pathway. One possibility is direct oxidation by hydroperoxyl radicals or hydrogen peroxide (Archer and Tannenbaum, 1979).

It has been demonstrated by Spanyar and Kevei, (1963), that the aerobic degradation of AA is catalysed by the presence of heavy metals such as copper (Cu) and iron (Fe). Khan and Martell (1967a, 1967b) discussed the mechanism for the metal catalysed oxidation of AA, taking into consideration the findings of Spanyar and Kevei, (1963), who reported that in the Cu^{++} catalysed decomposition of AA to DAA, H_2O_2 is formed in measurable quantities, (see Figure 2.4).

It is known that dehydroascorbic acid is very reactive and unstable and undergoes hydrolytic cleavage of the lactone ring to give 2,3-diketogulonic acid (Mooser and Neukon, 1974 and Hay et al, 1972). The effects of pH, temperature and oxygen on the degradation of DAA were emphasised by the work of Velisek et al (1976), who isolated several volatile products from the decomposition of DAA in different phosphate buffer solutions of pH 2,4, 6 and 8, either heated under reflux for 3 hours or left at 25°C for 200 hours. Fifteen products were identified. The concentration of five main degradation products, i.e. 3-hydroxy-2-pyrone, 2-furancarboxylic acid, 2-furaldehyde, acetic acid and 2-acetylfuran depended on the pH value and temperature but not on the presence of oxygen.

Anaerobic Degradation

The anaerobic ascorbic acid degradation is generally less

understood than the aerobic degradation.

In early work, Kurata, et. al., (1967), presented experimental evidence that under storage conditions degradation of ascorbic acid through DAA formation was not the only pathway of degradation occurring. They suggested the possibility of two different mechanisms of degradation taking place at the same time, i.e. aerobic and anaerobic degradation. Latter, Kurata and Sakurai (1967a, 1967b) and Kurata and Fujimaki (1976a, 1976b) suggested both an oxidative and a non-oxidative mechanism of AA degradation. In the first steps for the anaerobic degradation of AA, according to their suggestions, AA reacts via its keto tautomer which is in equilibrium with its ion. The ion undergoes delactonisation to diketogulonic acid, (DKG).

Several authors have tried to elucidate the mechanisms of degradation of AA in model systems by the identification of several decomposition products. Some of these decomposition products have been included in the pathways of aerobic and anaerobic decomposition of AA proposed by Bauernfeind and Pinkert (1970) and Archer and Tannenbaum (1979), which are summarised in Figure 2.4. These products include 2,3-diketogulonic acid, (1)*, by Nofre et al., (1960); xylosone, (2)*, ethylglyoxal, (3)*, and 2-keto-3-deoxy-L- pentonc-γ-lactone(4)*, by Feather, et. al., (1972), Kurata and Sakurai, (1967a, 1967b) and Kurata and Fujimaki, (1976b); Furoic acid, (5)*, by Mooser and Neukom, (1974); Furfural, (6)*, by Kurata and Sakurai, (1967b) and Velisek et al., (1976); and 2,5-dehydro-2-furoic acid, (7), by Huelin et al., (1971), (* are shown in Figure 2.4).

Some of these decomposition products (5, 6 and 7) have already been identified in foods such as orange and grapefruit powders, by Shaw, et. al., (1970) and Tatum, et. al., (1967 and 1969).

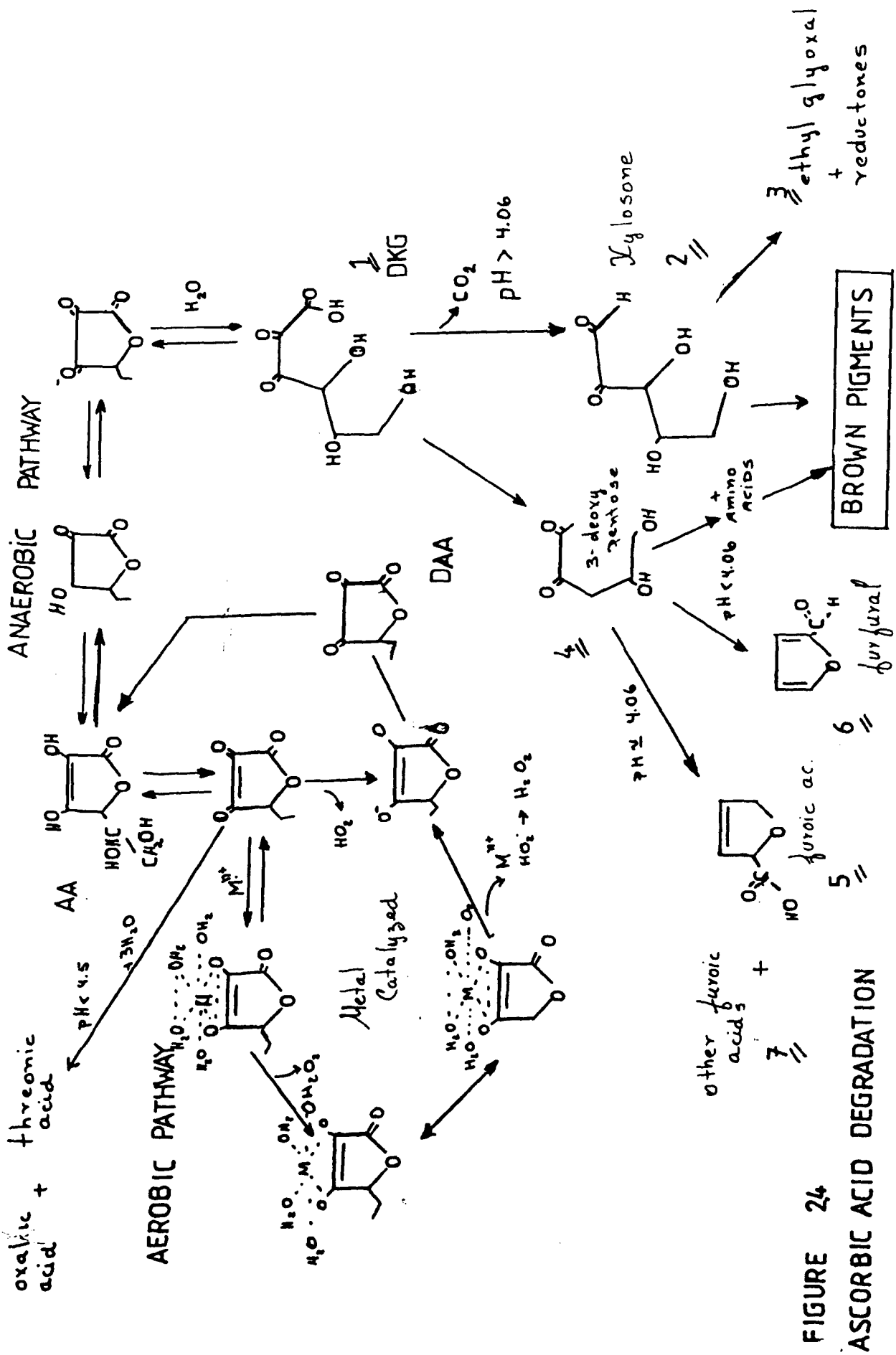


FIGURE 24
ASCORBIC ACID DEGRADATION

However, any degradation beyond DKG formation is of no nutritional importance for vitamin C physiological activity which is already lost at this point. But the degradation products are of importance in food systems since they can interact with proteins (see section 2.4) in non-enzymic browning reactions as suggested by various authors (Mauron, 1973 and 1980, Hurrell 1980 and Dworschak, 1980). These reactions are not well understood at the present time but definite experimental evidence of their occurrence has been presented by Kurata and Sakurai (1967a and 1967b), Namiki et al., (1974), Yano et al., (1976 and 1978) and Ranganna and Setty, (1974).

2.3.3 Factors affecting Stability of Vitamin C

The mode of ascorbic acid degradation in food systems is dependent on several environmental factors, as mentioned above, which will be discussed briefly below:

Oxygen

The presence or absence of oxygen determines the main type of ascorbic acid destruction mechanism - aerobic or anaerobic.

The rate of destruction of ascorbic acid is dependent on the oxygen concentration, as shown by Joslyn and Miller (1949), who studied the autoxidation of ascorbic acid in sugar solutions. They found that under conditions of limited oxygen the rate of oxidation was reduced but when oxygen was totally eliminated AA degradation rate was reduced considerably.

Those findings agree with the recommendation of Waletzko and Labuza, (1976), who recommended that foods should be packaged in a low oxygen environment, or if possible without oxygen, to increase the shelf life and the ascorbic acid retention

of intermediate moisture foods.

The concentration of oxygen on canned foods was clearly summed up by Herrman et al., (1978), who reported that in fruit nectars two types of parallel reactions take place, an oxidative degradation and an anaerobic or non-oxidative degradation, during processing and the beginning of the storage period.

Heavy Metals

Spanyar and Kevei, (1963), reported that copper is very destructive in the presence of oxygen but shows almost no effect in its absence. They also reported that iron had a milder prooxidant effect. The same results were reporteod later by Kyzlink and Čurdova in 1975 when studying the effects of antioxidants in foods.

Khan and Martell (1967a, 1967b) reported that the rate of cupric ion catalysed oxidation was found to be first-order with respect to the concentration of molecular oxygen and that the rate shows an inverse dependence with pH.

Lee et al., (1977), studying the effects of metal catalysis in canned tomato juice, concluded that the rate of AA destruction increased with copper concentration, and that the energy of activation of the AA degradation in the presence of Cu in canned tomato juice had a maximum value at pH near the pK of ascorbic acid.

Sattar et al., in 1977 studied the effect of various metals (Cu^{2+}, Fe^{3+}, Zn^{2+}, Ni^{2+}, Pb^{2+} and Mn^{2+}) at various concentrations. In the presence of abundant oxygen, only Cu^{2+} and Fe^{3+} increased AA decomposition with increasing concentrations. They also reported that under limited oxygen availability the addition of Cu^{2+} on the oxidation of AA had only a small effect.

Oxygen availability had no effect on Fe^{3+} catalysis of AA oxidation. All the other metals presented negligible effects on the rate of destruction.

Summarising it can be said that metal catalysis is very effective in the aerobic destruction of vitamin C, but it has little or no effect in the absence of oxygen. Copper ions are the most damaging, followed by iron, Zinc, Nickel, Lead and Manganese ions show only slight catalytic effects but more information is needed on these metals. Canned foods are likely to change from low oxygen concentration to negligible or total anaerobic conditions during storage. High levels of oxygen will only be present during blanching.

pH value

Khan and Martell, (1967), stated that in the pH range of 2 to 5 the rate of ascorbic acid destruction increases with increasing pH in the presence of oxygen. Furthermore, Archer and Tannenbaum, (1979), stated that the pH rate profile for the aerobic uncatalysed degradation of AA is an S shaped curve which increases continuously throughout the pH value corresponding to the pK_1 of ascorbic acid. The curve tends to flatten at a pH value close to 6.

The nature of the oxidation products also shows dependence on pH under aerobic conditions. Production of furfural is known to be favoured at acid pH values (Velisek, et.al., 1976); oxalic acid and threonate are known to be formed during aerobic oxidation under strongly alkaline conditions (Hay, et.al., 1972).

It has been claimed (Lin and Agalloco, 1979) that for both aerobic and anaerobic degradation of AA, the activation energy generally decreases with increasing pH.

Lee et al., in 1977, studied the effect of pH on the kinetics of aerobic ascorbic acid degradation in canned tomato juice. They concluded that both the rate constant and the energy of activation changed with pH, reaching a maximum at pH of 4.06, (which is near the pKa_1 of ascorbic acid), and that the destruction of AA under anaerobic conditions would not follow a true first order reaction.

Hueslin et al., (1971), showed that for the anaerobic destruction of AA the pH of the solution also influences the decomposition products formed. They divided the pH range products in three sections, below 4.06, pH near 4.2 and higher pH values than 4.2. They concluded that reactions occurring in the pH range of foods, i.e., at a pH near the pK_1 of AA and in the presence of fructose, the major decomposition product would be 2,5-dehydro-2-furoic acid, which has been identified as an ascorbic acid thermal decomposition product in citrus dehydrated powders, (Tatum, et. al., 1969), temperature range between 40°C and 215°C.

From this information it seems clear that at the pH values of canned and raw foods, i.e., low and medium acid foods, $3.5 \leq pH \leq 7.0$, the destruction rates will be expected to be moderately high, for both aerobic and anaerobic destruction pathways. Also it seems reasonable to assume that acidification of the food product will increase vitamin C retention.

Temperature

Many authors have studied the effect of temperature on the rate of ascorbic acid destruction and have reported increased rate of loss of AA with increased temperature, (Karel, 1979;

Kramer, 1977; Labuza, 1972; Lee et al., 1977; Lin and Agalloco, 1979; Mannheim and Nehama, 1979; Nagy, 1980; Sattar et al., 1977, and Waletzko and Labuza, 1976).

Nagy and Smoot, (1977), studied the effects of temperature on the retention of vitamin C in canned orange juice. They claimed that at temperatures above 27° C a marked increase in the rate of vitamin C destruction takes place.

Saguy, et. al., (1978) reported that the effect of temperature on anaerobic ascorbic acid destruction rate follows the Arrhenius equation in the concentration of grapefruit juice. They also found a drastic increase in the rate of reaction at elevated temperatures.

From a comparison of the existing data on the effect of temperature in both aerobic and anaerobic destruction of vitamin C and their own results, Lee, et. al., (1977), concluded that anaerobic destruction of AA is affected by temperature less than AA under aerobic conditions.

Moisture Content

Most of the work done on the relation between moisture content and ascorbic acid degradation has been in dehydrated foods (low in moisture content). They agree that an increase in moisture content increased ascorbic degradation. Karel and Nickerson, (1964), reported that water uptake was the major single factor in AA destruction in dehydrated orange juice during storage and that the destruction rate was the same in the presence of air or under vacuum. Mizraki and Karel, (1978), stated that temperature and moisture content were the main factors affecting AA destruction in tomato powder.

Labuza and Lee, (1975), studied the destruction of AA as a function of water activity. They reported several important points:

- The destruction rate of AA degradation increased with increasing moisture content and water activity.
- The relation between AA retention and a_w followed a first order reaction
- Ascorbic acid was more easily oxidised in a desorption system than in an adsorption system.
- Energy of activation showed no consistent effect. It increased with increasing water content in some foods (seaweed) but the opposite effect occurred in others (mixed cereal).
- Viscosity and dilution of the aqueous phase were proposed as the factors affecting the increased AA destruction in high a_w systems.
- It was shown that above a certain level of moisture content (>45%), the half life of AA approached a constant value.

The range of moisture content studied by Lee and Labuza, (1975), ranged from 5% to 55%. Maximum increases in destruction of AA were reported between 5 and 25%. It seems reasonable to assume that in most canned foods, the moisture content of the sample will not be an extremely important factor as for dehydrated foods according to Karel and Nickerson, (1964).

Ascorbic Acid Concentration

Gould , (1978), reported that the initial concentration of ascorbic acid in canned fortified tomato juice affected the rate of loss of AA during storage at $0^{\circ}C$, $12^{\circ}C$, $24^{\circ}C$ and $36^{\circ}C$. He also concluded that as the fortification level increased from 0 to 48 mg of AA/cm^3, a decrease in percent retention was observed

and the data obtained did not fit the Arrhenius equation satisfactorily. He suggested that other factors acting in tomato juice may have altered the results from exact adherence to the Arrhenius equation.

Later, other authors (Saguy, et. al., 1978) reported that in concentration and thermal processes, the initial concentration of ascorbic acid had no significant effect either on the rate of deterioration or on the mechanism of destruction. They confirmed that the AA destruction followed a first order reaction and the Arrhenius equation with concentration dependent parameters.

The ascorbic acid degradation kinetics in anaerobic conditions seems to be a first order reaction with respect to the concentration as reported by several authors. Hueslin, et. al., (1971) in model systems; Lee, et. al., (1977) in canned tomato juice; Lathrop and Leung, (1980b) in canned peas and Karel, (1977) in dehydrated tomato juice. However, in aerobic systems there seem to be some discrepancies. Herrmann, et. al., (1978) reported a second order reaction in pasteurised banana nectar during storage; while Joslyn and Miller, (1943), in model systems reported a first order reaction. So more research is needed on this subject, since as reported by Kurata, et. al., (1967) and Herrmann, et. al., (1978), anaerobic destruction occurs as a parallel reaction when aerobic destruction of AA is also taking place. The difficulty in separating both types of reactions in aerobic conditions may be the cause of the discrepancies.

Other factors

Spanyar and Kevei (1963) reported that cysteine in high concentration protects ascorbic acid from metal catalysis. Hay et al., (1972) in an extensive review on the chemistry of

AA mentioned that products such as thiosulfates, thioureas, thioacetals and N-hetorocyclics inhibit the oxidation of AA. Oguntona and Bender (1976) demonstrated that sulfur dioxide reduces ascorbic acid losses. The hydroxy acids, citric and malic stabilise vitamin C by chelating prooxidant metals and increasing acidity (Nagy, 1980 and Joslyn and Miller, 1949).

Recently the effect of light on ascorbic acid degradation has been investigated by several authors. Decomposition products from the photochemical oxidation of ascorbic acid had been reported (Hay, et. al., 1972). Singh, (1976), reported increases on the rate constant of ascorbic acid destruction with increases in light intensity in an infant formula during storage. Sattar, et. al., (1977) presented experimental evidence on the effect of light on AA destruction. From their results they concluded that ascorbic acid per se is not affected by light alone, in the absence of catalysis. It was destroyed by the photosensitizing effect of riboflavin and some other photosensitizing agents present in foods. However, the light effect is of no importance for canning processes.

From data presented some general recommendations to increase vitamin C retention during blanching and canning can be formulated.

- Presence of oxygen should be avoided, even in small quantities.
- Presence of copper and iron ions should be avoided, especially when oxygen is present in the system.
- Acid pH should be preferred.
- Temperatures of storage should be kept low.
- Moisture content effects are relevant and of importance only for foods with low a_w.

- Light is of no importance for canned foods.

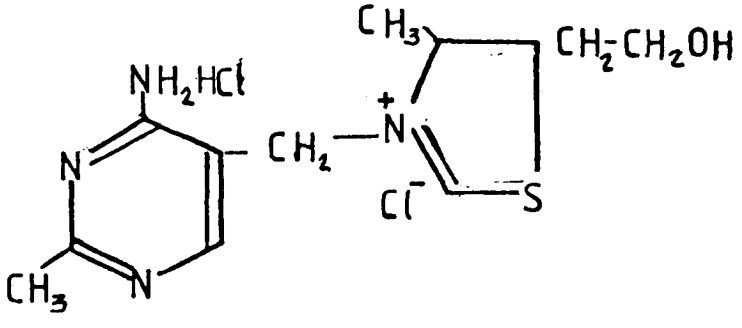
2.3.4 Vitamin B_1

The subject has been extensively reviewed by American Medical Association, (1974b); Archer and Tannenbaum, (1979); Bender, (1966, 1978); Benterud, (1971); Blake, (1977); Chichester, (1973); Dyke, (1965); Farrer, (1955); Hollingworth, (1972); Jansen, (1972); Karel, (1979); Kramer, (1977); Landen, (1972); Ratnatunga et al., (1978); and Tannenbaum, (1976).

Structure

The two most common names of vitamin B_1 are thiamine and aneurine.

The crystalline vitamin B_1, thiamine chloride hydrochloride is a substituted pyrimidine with a substituted thiazole connected by a (CH_2) methylene group. The chemical name of the vitamin is 3-(4-Amino-2-methyl pyrimidyl-5-methyl)-methyl-5-hydroxyethyl thiazolium chloride hydrochloride. Its structure is shown below:



The vitamin crystals are very soluble in water, less soluble in alcohol, and slightly soluble in ether or any other fat solvents.

Occurrence in Foods

It is known that thiamine is present in biological materials in various forms which include free thiamine and phosphorylated forms (mono-, di- and triphosphoric esters).

The most abundant form in plant tissue is free thiamine according to Jansen (1972) and Hennesse and Cerecedo (1939). Ochoa and Peters 91938) found that in animal tissues such as liver and kidney, cocarboxylase was the predominant form while in muscle tissues free thiamine concentrations equalled or exceeded that of cocarboxylase.

2.3.5 Stability of Vitamin B_1

It has been mentioned that thiamine is one of the least stable vitamins during food processing and several factors affect its stability in buffer systems and food products which will be mentioned briefly.

pH value

The sensitivity of thiamine to heat depends strongly on the pH of the solution. As stated by Jansen (1972), thiamine in solution is fairly stable to heat at pH values below 5. It is destroyed by autoclaving at pH values higher than 5 and at pH of 7 or more by boiling or storage at room-temperature.

Using phosphate buffer solutions, in the pH range of 4.5 to 7.0, Felliciotti and Esselen, (1957), demonstrated that the rate of thiamine destruction increased with increasing pH. They also found that the most pronounced change in reaction rate occurred between pH values of 6.5 and 6.0. Recently Mulley et al., 1975b, studied the effects of pH in phosphate buffers (4.5 - 6.5)

on the D value. They demonstrated that when the pH of the solution exceeded 6.0, the stability of the thiamine molecule dropped suddenly as evidenced by a sharp decrease in the D value. Also they showed that at low pH values (below 6.2), when the pH of the solution was gradually increased, there was an equally gradual drop in the stability of thiamine.

Farrer, (1945), plotted log of k (rate constant) against the corresponding pH. The curve obtained showed a change of slope or breaking point. From these results he concluded that the change in destruction rate was a function of the ionic composition of the solution and that the velocity coefficient was inversely proportional to the hydrogen concentration.

More recently, in studies of the mechanisms of thermal breakdown of thiamine, Dwivedi and Arnold, (1972), used ^{35}S labelled thiamine. They reported that heating of thiamine solutions at pH 6 or below resulted in cleavage of thiamine at the methylene bridge between the thiazole and pyrimidine constituents. They concluded that decomposition of thiamine in neutral or slightly alkaline conditions was governed by the pseudo base form of thiamine which undergoes thiazole ring opening to give an ionized thiol. For thiamine in acid conditions a protonated form was present which was more stable than thiamine. The acid-base equilibria of thiamine proposed by them is shown in Figure 2.5

Temperature

Rice and Beuk, (1945), reported that at temperatures above 77 ^{o}C the rate of thiamine loss in pork was constant at a given temperature and increases as the temperature increases. Farrer, (1955), showed that thiamine destruction in buffer

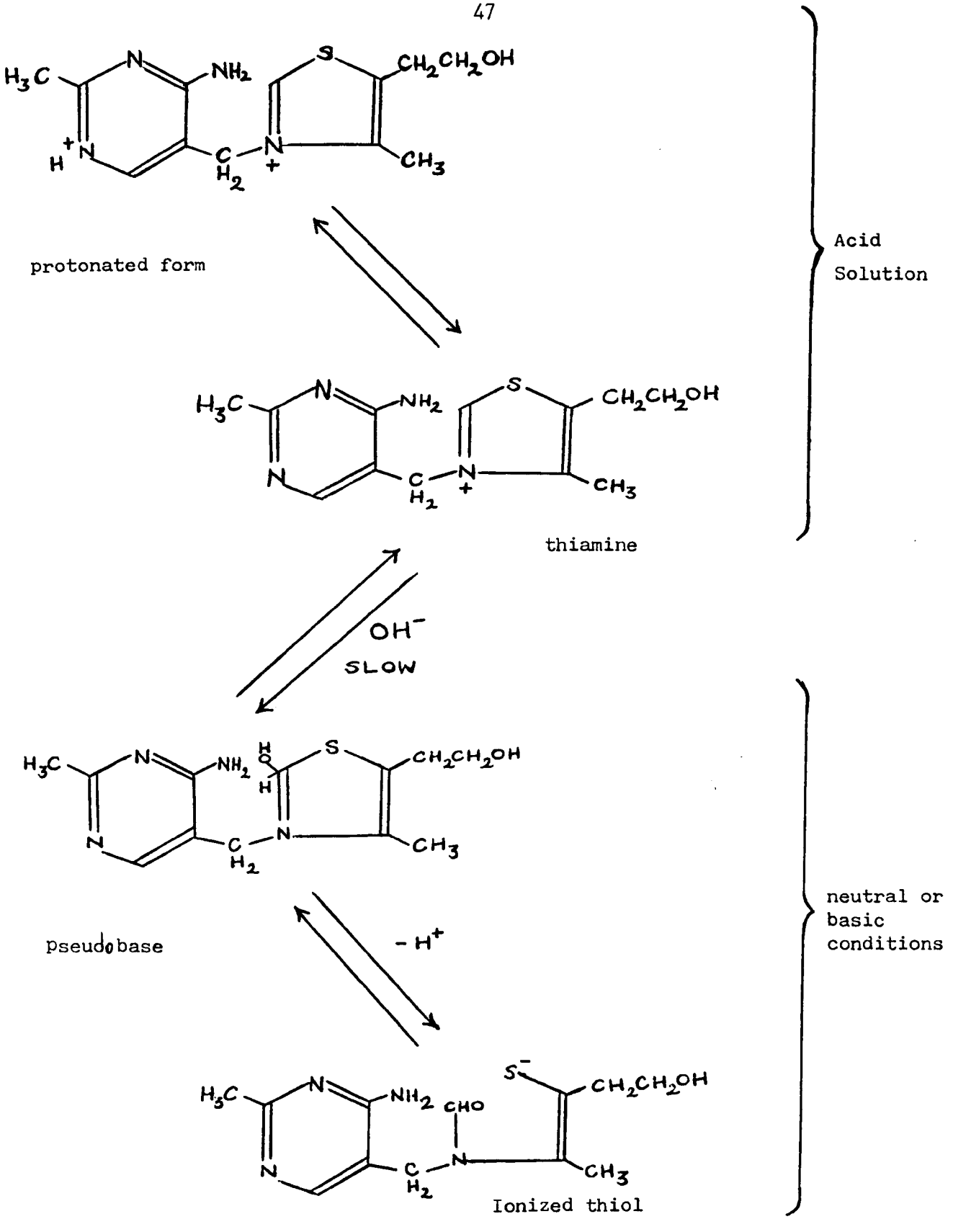
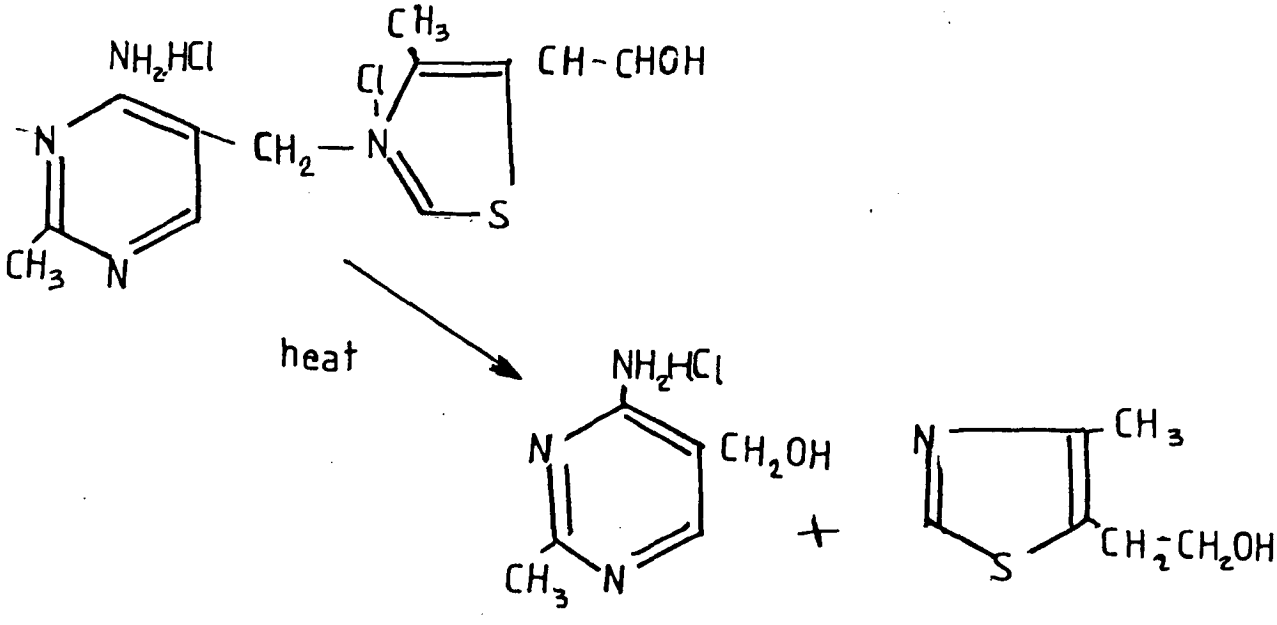


Figure 2.5. Acid base equilibria of thiamine.

solutions at temperatures between 50 - 110°C followed the Arrhenius equation. Felliciotti and Esselen, (1957), when studying the thermal destruction rates between 104°C and 112°C of thiamine in phosphate buffer, pureed meats and pureed vegetables concluded the reaction involved in the cleavage of the thiamine molecule by heat satisfied the first order Arrhenius equation. More recently Mulley et al., (1975a) corroborated that the thermal destruction of thiamine in buffered and food systems can be followed by simple kinetic methods, assuming a first order reaction.

Farrer, 1955, reported that the thermal destruction of thiamine resulted in cleavage of the methylene bridge and expressed the reaction as follows:



2-methyl-5 hydroxymethyl 4 amino pyrimidine hydrochloride

4-methyl-5 hydroxyethyl thiazole

It has been generally accepted that the thermal destruction

of thiamine leads to the formation of a characteristic odour which is involved in the development of the "meaty" flavour of cooked foods. These compounds are believed to come from the thiazole moeity decomposition, (Arnold, 1969; Dwivedi and Arnold, 1972).

Some of the probable reactions involved were summarised by Archer and Tannenbaum, (1976) as shown in Figure 2.6.

Time

Farrer (1955) emphasised that the destruction of vitamin B_1 was greatly increased if the time of heating was increased from 1 hour to 4 hours as demonstrated by the work of Sherman and Burton (1926), and his own work.

Much work has been carried out since to show the inter-relationship of time and temperature on thiamine retention. Several authors (Barreiro 1979; Bendix et al., 1951; Benterud, 1971; Farrer, 1955; Karel, 1979; Mulley, 1974; Mulley et al., 1975; Ralchouska, 1976; Rice and Beuk, 1945; Saguy and Karel, 1979; and Teixera et al., 1975) have reported kinetic data such as "D", "Z", "Ea", and K which show the inter-relationship of time and temperature on thiamine retention.

Form of the vitamin

Farrer suggested that some of the discrepanices existing in the literature on the stability of thiamine in foods may be caused by differences in the stability of the different forms of thiamine present and their relative concentrations. Several authors have attempted to explain the increased stability of thiamine in biological materials in terms of the presence of the

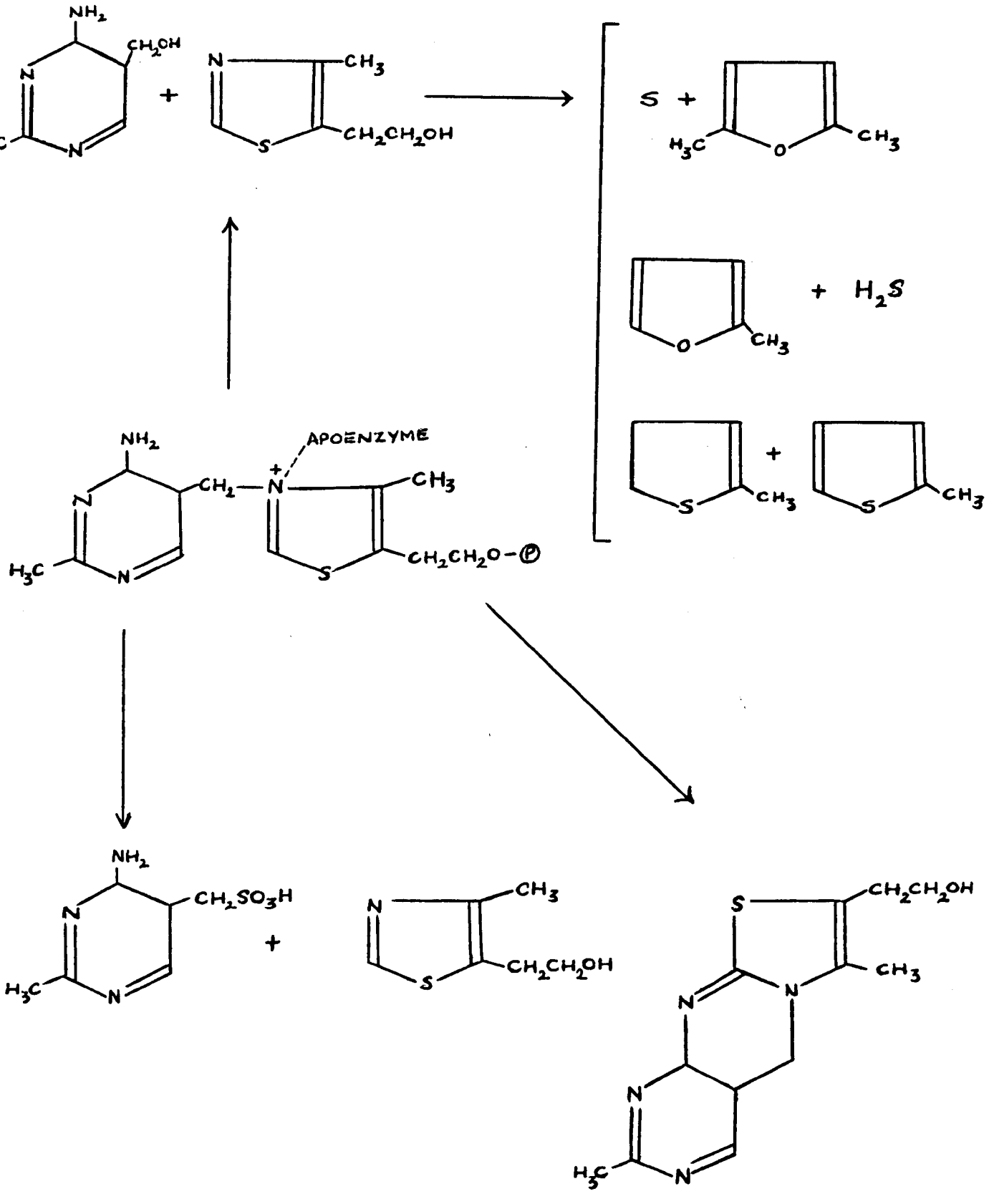


Figure 2.6 Degradation of thiamine
Simplified Scheme.

co-carboxylase form (Hennessy and Cerecedo 1939; Felliciotti and Esselen, 1957, and Karel, 1979).

As a result of their studies Felliciotti and Esselen, (1957), concluded that the thermal destruction of thiamine in (four meats and four vegetables) foods was dependent on the inter-relationship of pH and the relative portions of the free and combined forms of the vitamin. Later Mulley, et. al., (1975a) showed that under identical conditions, co-carboxylase was destroyed more rapidly than thiamine hydrochloride. They suggested that the faster destruction of co-carboxylase was linked with the pyrophosphoric acid group of the molecule which appeared to destabilise the co-carboxylase molecule in some way.

Concentration of the vitamin

Since thiamine is present in foods in various forms, the overall stability of the vitamin will depend on the relative concentrations of the various forms. However, Mulley et al., reported that when free thiamine and co-carboxylase are present together, the increased instability of co-carboxylase becomes apparent only when its concentration in the mixture exceeds 35.0%. They concluded from a literature review on the amounts of co-carboxylase present in foods, that it appeared that the amount of co-carboxylase normally present in foods, will not greatly influence thiamine destruction rates.

Farrer (1948) reported that concentrations of thiamine and co-carboxylase below 10 mg/cm^3 were without effect on their rates of destruction at 100° C. At higher concentrations, the reaction velocity tended to decrease. These findings were later confirmed by Van Zante (1970).

Heavy metals

Farrer (1947) and Booth (1943) reported that heavy metals such as copper, iron, zinc and nickel can influence thiamine destruction by catalysis, particularly metals that can form complex anions with constituents of the medium.

Tanaka (1966a, b) successfully isolated a thiamine complex having an empirical composition of $C_{12}H_{16-17}O_2N_4SCu$ suggested that the slow decomposition of thiamine by copper may occur by the formation and degradation of copper complexes.

Oxygen and oxidising agents

It is generally agreed that vitamin B_1 is fairly stable to oxidation. Farrer and Morrison (1949) reported that oxygen could accelerate the thermal destruction of thiamine at temperatures above 70° C. They also reported that oxygen affected the first order reaction rate of thiamine. However, several other authors agree that the destruction of thiamine is thermal, not oxidative, (Farrer, 1955; Mulley et al., 1975a).

Moisture Content

Work on the moisture content effect on foods has been limited to dried products. Most authors agree that increased losses of thiamine occur at high levels of moisture content. (Farrer, 1975; Rice et al., 1944, and Ratnatunga et al., 1978). No particular effect has been reported on canned foods.

Electrolytes

It is well known that the typical degradative reaction of thiamine involves a nucleophilic displacement at the methylene

bridge. Therefore strong nucleophiles such as sulfite (HSO_3^-) can destroy vitamin B_1, (Oguntona and Bender, 1976). Farrer, (1955), stated that there was a close association between the anionic constitution and pH in catalysing the pseudo unimolecular decomposition of thiamine. Furthermore, he suggested that this same effect operates in more complicated systems like foodstuffs.

Yagi and Itokawa (1979) reported that when rice was boiled in tap water the thiamine was cleaved by the residual chloride into hydroxymethyl pyrimidine and thiazole moiety. This effect is increased when the pH is increased.

Non electrolytes

Rice et al., (1944), reported the stabilising effect of cereals on thiamine during the storage of dehydrated pork. Leichter and Joslyn (1969) reported that starch and casein showed a protective effect on the destruction of thiamine by sulphite. The mechanism of protection is not clear. It has been suggested that the sulphydyl groups of the proteins participate (Wada and Suzuki, 1965).

Since vitamin B_1 has an amino group, it has been suggested (Hurrel, 1981 and Spencer, 1973) that it may undergo Maillard reaction with reducing sugars. But no evidence has been presented on this subject.

To summarise, the data available in the literature suggests that thiamine retention can be improved in fresh and canned foods by following the procedure below.

- Temperature should be kept low for storage.
- Acidic pH should be used for processing foods, preferably below 6.

- Time for thermal treatments should be kept to a minimum.

- Fortification with thiamine seems highly beneficial since free thiamine is more stable than the naturally bound co-carboxylase form.

- Metals ions should be avoided.

- Oxygen presence and moisture content have no important effect on thiamine destruction in canned foods or fresh products.

- Nucleophiles should be avoided such as HSO_3^-, Cl^-, etc.

2.4 Proteins

The nutritive value of a protein for an animal organism depends on numerous factors besides the level of protein consumed: quantity and availability of each essential amino acid present, extent and rate of digestion of the protein and nutritional history of the particular organism.

In the context of food processing all these factors have been reviewed extensively by Adrian, 1974; American Medical Association, 1874a; Anglemier and Montgomery, 1976; Bender, 1966, 1978; Carpenter, 1973; Cheftel, 1979a, 1979b; Chichester, 1973; Hollingsworth and Martin, 1972; Hurrell, 1980; Hodge et al, 1976; Mauron, 1973, 1979; and Morrison and McLaughlan, 1973.

They have clearly established that processing can alter the nutritive value of a protein favourably by mild heat treatments or unfavourably by both mild and severe heat treatments. The nutritive value of proteins may be improved by the inactivation of growth inhibitors such as antitrypsin substances in legume seeds or by increasing digestibility of some proteins when

denaturation facilitates access for the proteolytic enzymes.

Damage to the nutritional value of proteins can be classified into five types: denaturation of proteins, leaching of soluble proteins, loss of amino acids availability, destruction of amino acids, and loss of protein digestibility.

Mild processes alter the quaternary, tertiary and secondary structure of food proteins. This change in protein structure is known as "denaturation" which sometimes results in the loss of desirable functional and organoleptic properties of the food, but it is regarded as of negligible or no nutritional importance, although denaturation may improve protein digestibility.

There can be losses of soluble proteins leached out into the processing water (Lee, 1958). According to Bender, (1966) those losses are not important since they occur in very small amounts and affect mainly vegetables which are not an important source of protein, and as stated by Mauron, (1973), when it does affect meat and fresh products (like in normal cooking) no appreciable loss in nutritionally valuable nitrogeneous compounds occurs, since they are usually recuperated in the broth or gravy which is usually consumed.

Loss of amino acid availability, amino acid destruction and loss of protein digestibility are of nutritional importance as they involve chemical modifications of the primary structure of proteins.

According to Hurrell, (1980), proteins are the most reactive of the macronutrients in food and reactions between food proteins and other components are the major chemical interactions that occur during processing and storage. Those chemical interactions result mainly from the various effects of pH values, temperature, moisture content, presence of oxidants, carbohydrates, lipids

and metals and reactions with chemical additives.

The chemical reactions affecting the nutritional value of proteins can be divided in four groups: reactions involving protein only, reactions between proteins and carbohydrates, reactions between proteins and lipids, and reactions with other molecules such as aldehydes, sulfites, etc.

2.4.1 Reactions Involving Proteins Only

Oxidation

A marked reduction in the availability of tryptophan, Methionine and lysine was reported by Miller et al., (1965) when heating cod containing 14% moisture at 116°C for 27 hours. In explaining Miller's findings, Mauron (1973) discussed the possibility of the oxidation of methionine to methionine sulphoxide as the only known oxidative reaction leading to inactivation of amino acids by severe heating of proteins alone, (Cheftel, 1979).

Desulphydration

It is well known that cystine and cysteine are destroyed when a protein with low carbohydrate content is severely heated in the presence of moisture. Losses of cysteine have been reported in canned meat after sterilisation, (Beuk, et. al., 1948); pork heated at 110°C for 24 hours, (Donoso, et. al., 1962) and cod heated for 27 hours at 116° C (Miller, et. al., 1965a) besides others. Some authors (Bjarmason and Carpenter, 1970; Miller et al., 1965; Stenberg and Kim, 1977), explain these cysteine-cystine losses by desulfurisation reactions, with the liberation of H_2S and other sulphur-volatile compounds and the production of carbonyl compounds. Such products may lead to the formation

of unstable residues of dehydroalanine which may react with other residues to form compounds such as lysinoalanine.

Deamination and Isomerization

Liberation of ammonia due to deamination of amide groups, during amino acid decomposition and during the cross-linking of proteins requires intense heating conditions which are not normally present during thermal processing. The same applies for isomerization of L-amino acids into unavailable D-amino acids. However there had been reports of isomerization of amino acids during roasting conditions (180°C to 300°C).
Nevertheless, these changes are of secondary importance in thermal sterilisation processes.

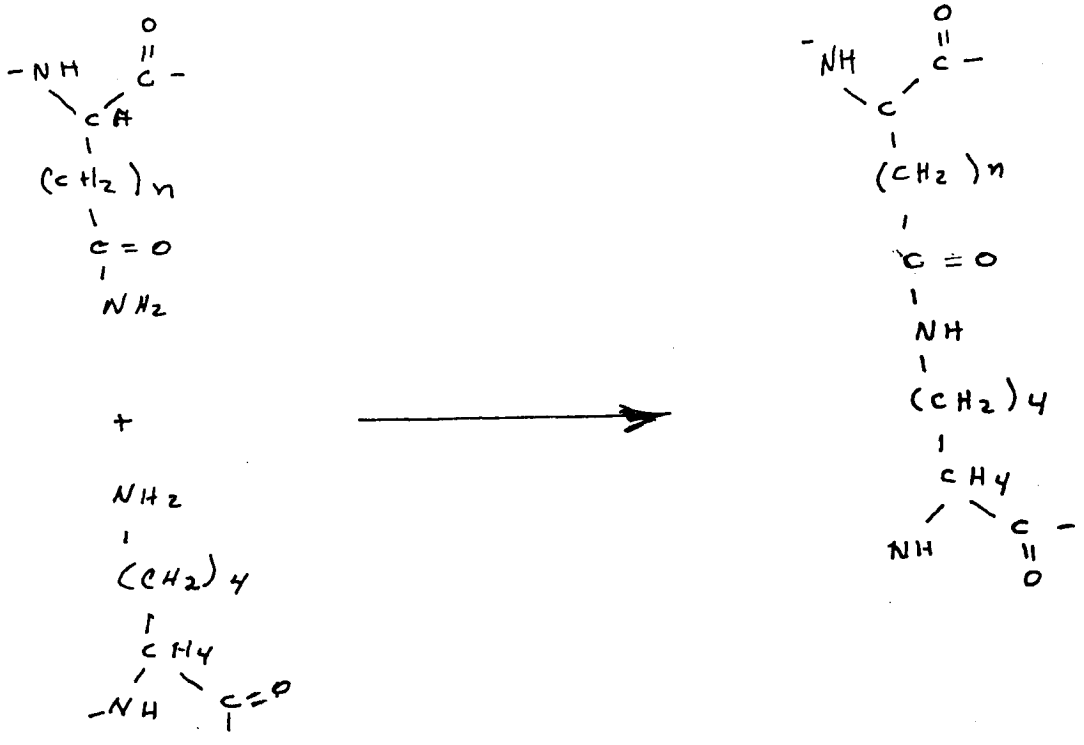
Protein Cross-linking

Proteolytic enzyme resistant cross-links can occur between several amino acids in a protein reducing its biological availability. These types of cross-links have been found in naturally occurring proteins such as keratin, elastin and collagen.

Several workers, (Anatharaman and Carpenter, 1965; Bjarnason and Carpenter, 1970; Beuk,et.al., 1948; Donoso,et.al., 1962; Ellinger and Boyne, 1965; and Miller,et.al., 1965a, 1965b, 1965c), reported severe losses in the nutritional value when proteins with low carbohydrate content were severely heated under dry or moist conditions. In all cases, the decrease in protein quality was larger than the decrease in amino acid content. Some of these authors suggested some type of unavailability which was later explained in terms of protein cross-links

(Bjarnason and Carpenter, 1970; Carpenter, 1973; Donoso et al., 1962; and Hurrell and Carpenter, 1977).

Bjarnason and Carpenter (1970), established the formation of ξ-N-(γ-L-glutamyl)-L-lysine and ξ-N-(β-L-aspartyl)-L-lysine with liberation of ammonia when albumin was heated (110°C - 145°C) for 27 hours. They suggested that protein intermolecular cross-linking was mainly caused by the reaction of -amino groups of lysine with amide groups of aspartic acid and glutamic acid residues and proposed a mechanism of protein-protein interaction which is shown below:



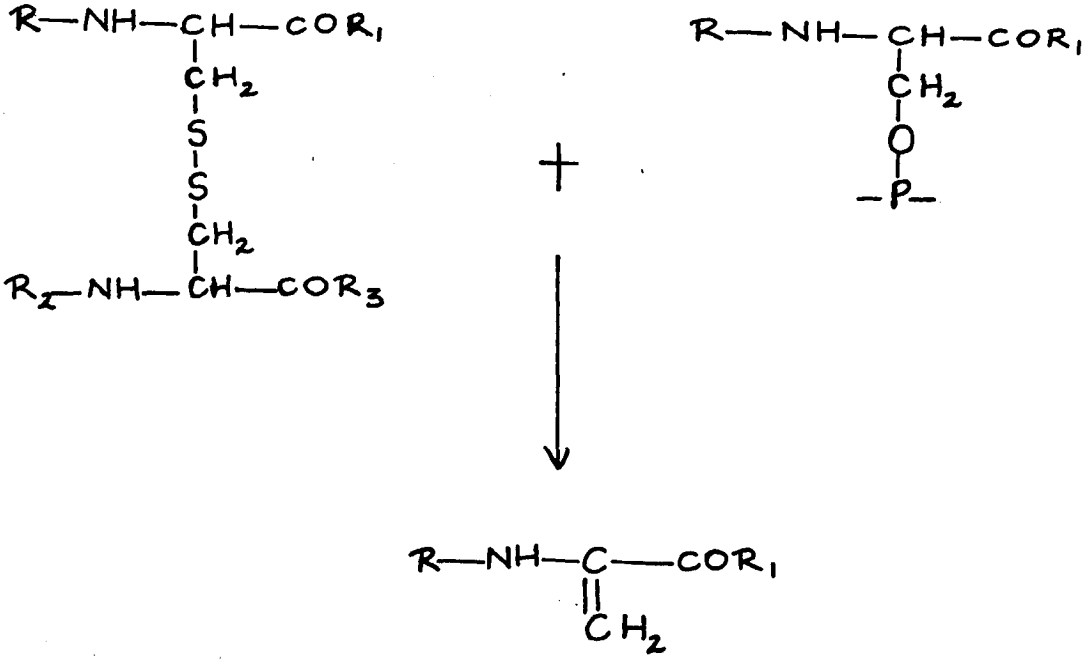
n = 1 as paragine group n = 2 glutamine group

Ref: Bjarnason and Carpenter (1970).

Other types of cross-linking may occur in heated proteins with low carbohydrate content as suggested by Mauron,(1973), who theoretically proposed some other possible cross-links, but such links have not yet been found in heated proteins.

Protein Cross-linking under Alkaline Conditions.

It has been mentioned that heat treatment of proteins may cause the desulphydration of cysteine which according to several authors (Bjarnason and Carpenter, 1970; Cuq,et.al., 1974; Hurrell and Carpenter, 1977; and Sternberg and Kim, 1977) together with glucosidically bound seryl residues in proteins undergo β-elimination reaction forming an unsaturated intermediary compound called dehydroalanine, as shown below;



This reaction is more prominent in alkaline heat treated proteins. Dehydroalanine (DHA) can then condense with other

amino acid residues to form new amino acids such as with:

- Lysine residues to form Lysinoalanine
- Cysteine residues to form Lanthionine
- Ornithine residues to form Ornithinoalanine
- Aspartic acid or glutamic acid residues to form β-aminoalanine.

Some of these aminoacids are known to be responsible for some intra or inter-molecular changes and have been identified in alkaline treated proteins (Bjarnason and Carpenter, 1970; Carpenter, 1973; Cuq, et.al., 1974; Hurrell and Carpenter, 1977, and Sternberg and Kim, 1977). Although alkaline treated foods are almost non-existent in thermally processed foods, lysinoalanine has been reported in processed foods that have never been submitted to alkaline treatments. Sternberg and Kim,(1977), reported that lysinoalanine was present in normally cooked foods (after frying, broiling, boiling and baking) such as chicken thigh, frankfurter sausages, and canned evaporated milk. Aymord, (1976), found lysoalanine in canned corned beef and Raunio, et. al., (1978), reported lysinoaline in very small quantities in canned baby foods. Commercially produced sodium caseinate also contains lysinoalanine according to Aymord (1976), and Sternberg and Kim, (1977).

It can be summarised that protein-protein interaction reduces the nutritive value of protein by:

1) amino acid destruction (mainly cysteine).
2) reduction of amino acid availability (mainly lysine, histidine, arginine and sulphur amino acids).

or 3) by reducing the rate of protein digestion due to steric hinderance or by blocking the sites of enzyme attack.

2.4.2 Protein-Carbohydrate Interaction

The complex group of reactions occurring between proteins (amino groups) and carbohydrates (carbonyl groups), or sometimes with lipids (aldehydes from fatty acid oxidation) leading to browning and flavour production are generally known as Maillard reactions. They are part of the non-enzymic browning reactions which include Maillard reactions and Caramelisation. These reactions are very important in flavour development (Nursten, 1981) and are developed on purpose during baking, frying, toasting and roasting, to improve the organoleptic properties of the food.

In food proteins, the Maillard reactions are very important as they take the place during processing and long term storage even at low temperature.

The chemistry of the Maillard reactions is not yet fully understood. Hodge, (1953) presented an excellent review on the subject in which he divided Maillard reactions in three stages: Initial stages, intermediate stages and advanced stages, which can be summarised as follows:

Initial Stages:

(1) Colourless products.

(2) Formation of Amadori compounds.

(3) Loss of nutritional value.

Intermediate Stages:

(1) Buff yellow products.

(2) Five different pathways for formation of hydroxymethyl furfural, reductones, methyl reductones, α-carbonyls and Strecker aldehydes.

(3) Interaction with other nutrients such as vitamin C and probably vitamin B_1 and undamaged amino groups.

(4) Interaction with lipid degradation products.

Advanced Stages:

(1) Red brown and dark brown colour formation.

(2) Production of caramel like and roasted flavours and melanoidins.

(3) Production of some toxic melanoidins.

The characteristics of each one of these stages will be described briefly.

Initial Stages

It is at this stage when the nutritional value of the protein is damaged. The Amadori compounds have no nutritional value (Mauron and Finot, 1969, and Mauron et al., 1970).

There is general agreement on the chemistry of the early Maillard reactions. The carbonyl-amino reaction between a reducing carbohydrate and the free amino group of an amino acid is a condensation process. The condensation product loses a molecule of water and is converted to a Schiff's base which by isomerisation is transformed into the nitrogen substituted glucosylamine. This glucosylamine undergoes Amadori rearrangement and is thus converted into the 1-amino-1-deoxy-2-ketose. These steps (Pathway A were summarised by Hodge (1953) as shown in Figure 2.7.

Intermediate Stages

Hodge (1953) proposed three main pathways (B, C, D) for the intermediate stage Maillard reactions to occur. The first pathway (B) according to Dworschak, 1980; Hodge, 1953, 1976; Mauron, 1973, 1981 and Nursten, 1981, begins with the 1,2-enediol of the Amadori compound to form the 3-deoxyhexosone which by

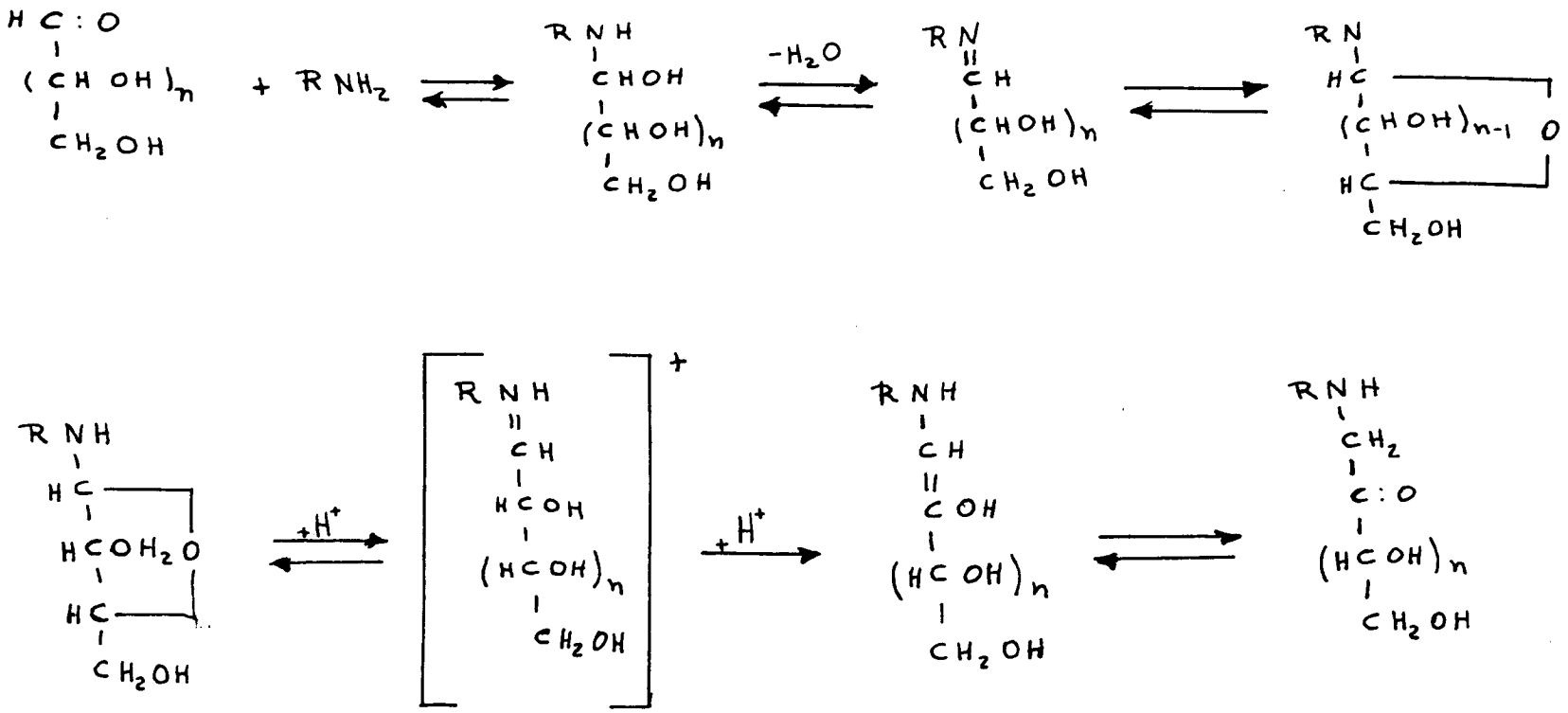


Figure 2.7 Initial Steps of the Maillard Reaction (Hodge, 1976). Pathway A.

dehydration yields flavour compounds such as 2-furaldehydes. The second pathway (C) starts from the 2,3-enediol of the Amadori compound, after the total elimination of the amine yields a methyl decarbonyl intermediate which further reacts to give fission products such as c-methylaldehydes, ketoaldehydes, dicarbonyls and reductones. The reaction products also include flavour components such as acetaldehyde, pyruvaldehyde, diacetyl and acetic acid. These two pathways (B and C) were represented by Hodge (1979) as shown in Figure 2.8.

In both pathways the reactions that follow are very complex but it is known that they result in the production of dark-brown nitrogen containing pigments and heterocyclic nitrogen compounds such as pyrazines, pyroles and pyridines. Also, from the methyl dicarbonyl intermediate (pathway C), several cyclic enolones such as maltol, and isomaltol are produced, (see Figure 2.10).

The third pathway (D) involves the Strecker degradation of α-amino acids (Hodge, 1967), which is an oxidative degradation of α-amino acids to the next lower aldehyde by interaction with α-dicarbonyl (Davidek, et. al., 1974) and some other compounds (3-deoxyglucosone, pyruvaldehyde, glyoxal) which are products of pathways B and C or with ascorbic acid as shown by Namiki, et. al., (1978), Ranganna and Setty, (1974), and Yano, et.al., (1976 and 1978).

The products of this degradation include carbon dioxide and various flavour compounds such as pyrazine, tetramethyl pyrazine and other pyrazine derivatives, which are important for cooked foods (Nurstein, 1981). A simplified diagram of the Strecker degradation is shown in Figure 2.9.

Mauron, (1981), in his review mentioned two more possible pathways (E, F) for the intermediate stage Maillard reactions

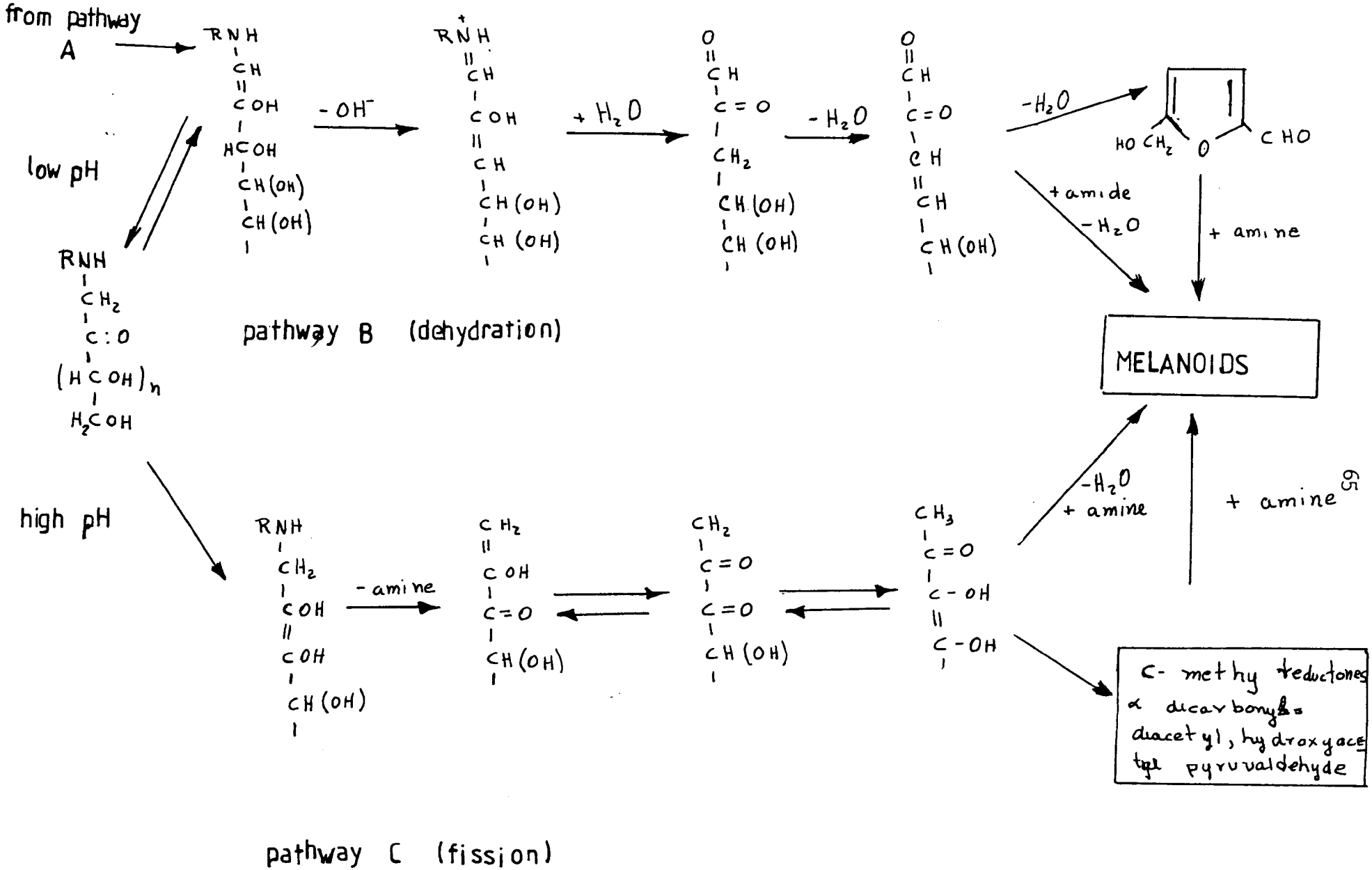


Figure 2.8 Degradation of Amadori Compounds (Hodge, 1976)

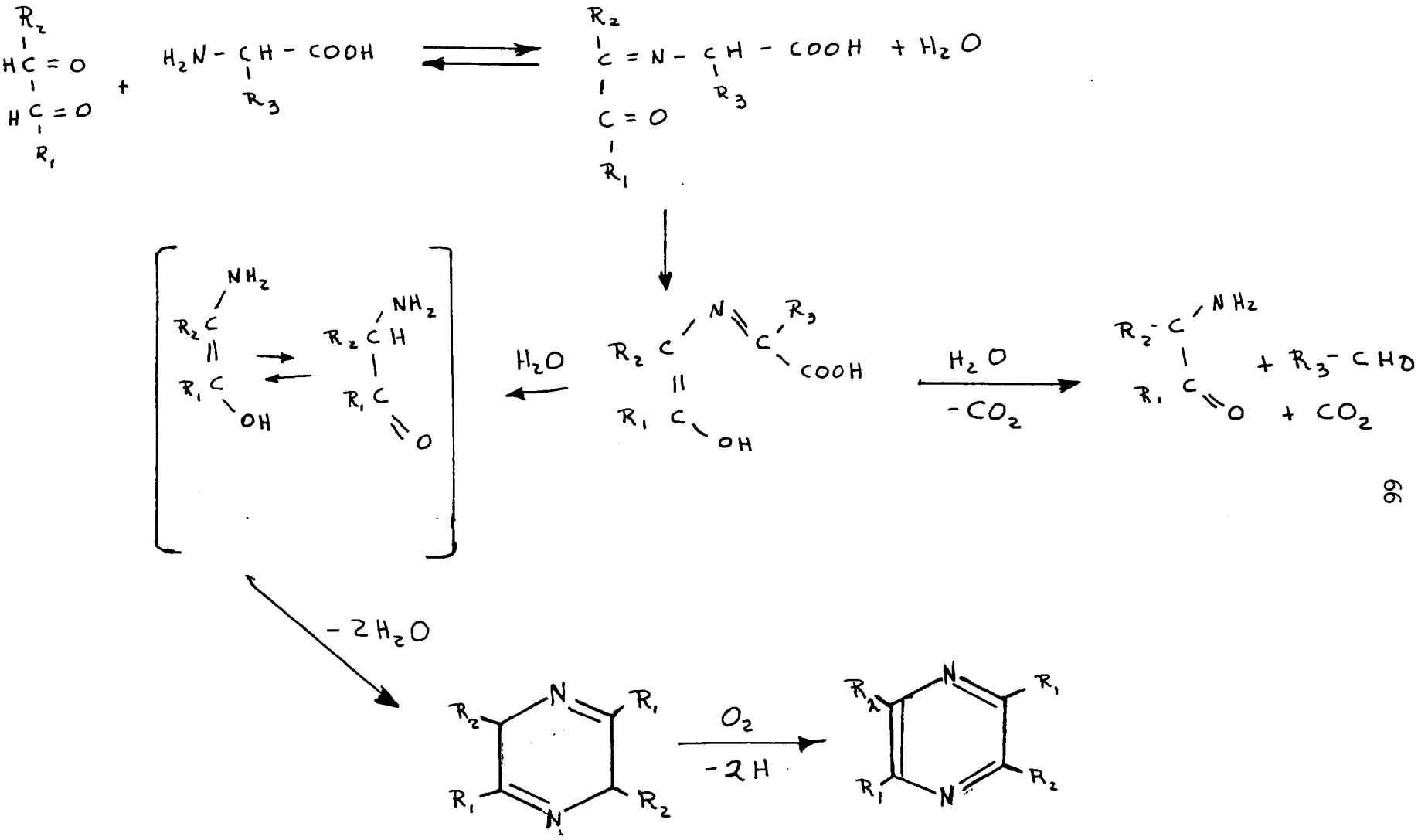


Figure 2.9 Strecker Degradation (Hodge, 1976)

to occur. The fourth pathway (E) was proposed by Hølterman (1966) and begins from the Schiff base which by transamination yields a non reducing amino sugar and an oxoacid which react with a new amino acid by the Strecker degradation. The fifth pathway (F) proposed by Burton and MacWeeny, (1964), starts with the Amadori compound which regenerates the amino acids while the sugar moiety is dehydrated to 5-hydroxy-methyl-furfural which proceeds reacting as in pathway B.

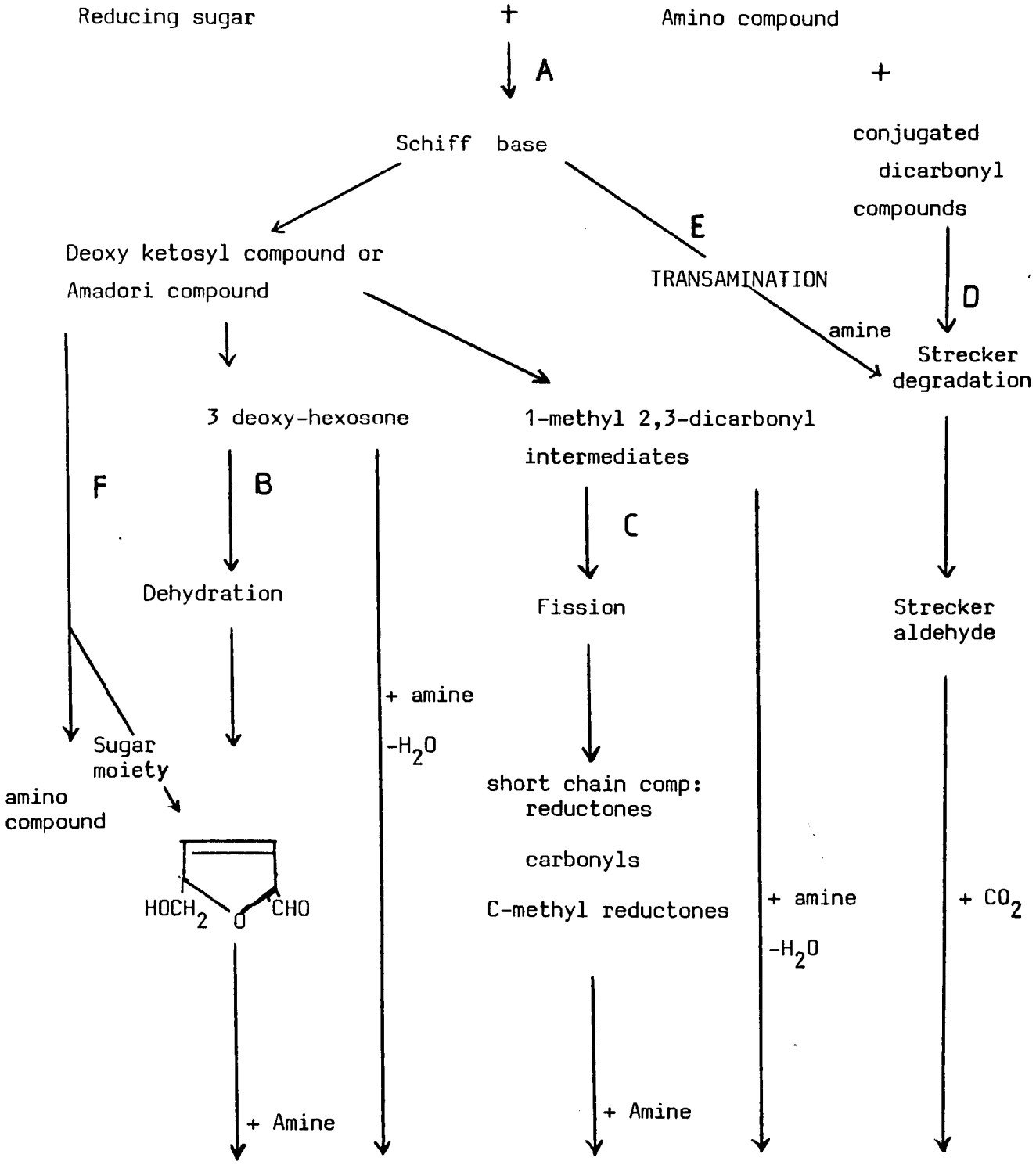
All the five proposed pathways are shown in a simplified scheme of the Maillard reactions shown in Figure 2.10 which is an adaptation of the diagram presented by Mauron (1981).

Final Stages

The chemistry of the final reactions that produce the brown pigments called melanoids is not well understood. However polymerisation of the intermediate stage products is generally believed to be the mechanism for the production of the melanoids. Melanoids are compounds with high molecular weights (above 1,000) usually with reducing power, strongly coloured and with antioxidant properties.

Factors affecting Protein-Carbohydrate Interactions

The occurrence of pure protein foods with no carbohydrate content in canned foods is very rare. In common thermosterilised products proteins and carbohydrates are usually in contact. Therefore the conditions for Maillard reaction to occur are frequent. It has been generally assumed that Maillard reactions have relatively high activation energies so when a food is moderately heated, Maillard reactions occur before any other type of protein damage, (Lund, 1976, 1977 and Granborg, 19).



Formation of Melanoids
Polymerisation of intermediate compounds,
production of N-heterocyclics

A, B, C, D, E, F pathways of chemical reactions mentioned in text.

Figure 2.10 Simplified scheme of the Maillard reactions.

Some of the factors affecting this reaction are mentioned briefly below.

Carbohydrate

It is generally assumed that non-reducing sugars such as sucrose and raffinose must be split to reducing sugars before acting in Maillard reactions. According to Hodge, (1976), pentoses are more reactive than hexoses. Lewis and Lea, (1950), reacted casein at 37° C and 70° relative humidity with various sugars. They found that the reactivities of xylose, > arabinose, > glucose, > lactose, > maltose > and fructose decreased in this order. However Granborg (19), when studying Maillard reaction damage in canned model systems found that the reactivities decreased in the order dextrose, > fructose, > lactose > and saccharose. However he found that this order was not generally applicable such as with whey protein. Therefore, it is reasonable to assume that other protein-sugar systems would present varying responses.

Moisture Content

In a classic study of casein and glucose at 37° C, Lea and Hannan (1949) found that the rate of amino acid reactive groups disappearance was maximal at moisture contents of 15% - 18%. Later, Dworschak (1980), proposed a possible mechanism for this effect. He suggested that the increase of water up to an optimal quantity promotes the active groups into a "convenient nearness", but further, in increasing the water content, reduces the rate due to a hindering effect because of the decrease in concentration.

Most of the canned foods have higher moisture contents

so the effect of humidity would not be greatly reduced when compared with dehydrated or intermediate moisture foods.

pH value

It is generally agreed that low pH values inhibit the Maillard reaction while high pH values enhance it, as shown by Lea and Hannan (1949), who reported that the reaction rate increased as the pH increased from 3 to 7. They found only a small increase in the browning rate in alkaline conditions.

The pH of most canned food is in the range of maximum changes therefore pH value may be an important factor to consider in thermo-sterilised foods in this context.

Temperature and Time

The Maillard reaction is known to occur during both storage and heat treatments, as shown by several authors who reported losses of protein quality in casein-glucose mixtures at 37 °C and 70% RH after 30 days

(Lea and Hannan, (1949) at 85 °C for 40 min (Rao, et. al., (1963) at 130 °C for 2 min Reineccius, et. al., (1978), between others.

Lea and Hannan (1949) showed that in casein-glucose mixtures the rate of disappearance of amino acid reactive groups was dramatically increased when the temperature was increased from 0 °C to 70 °C. Mauron et al., (1960) showed that the losses of several amino acids in a high protein biscuit was proportional to time and temperature of heating. Narayana Rao and McLaughlan (1967) demonstrated when heating casein-glucose mixtures at 121 °C that for an

increase in the time of heating there was a corresponding decrease in the availabilities of lysine and methionine.

Narayana Rao et al., (1963) showed that damage to lysine and methionine for different heating treatments increased with the severity of the treatment.

So it can be concluded that heat is not indispensible to the development of the reaction. It is slow at room temperature but the rate increases as temperature rises. So canned food can be affected either during processing or during storage if the other conditions (pH, moisture content, etc.) are favourable.

Protein-Lipids Interactions

It was demonstrated that interaction of proteins and lipids reduced the protein digestibility and availability by Lea et al., (1966); Karel, (1973 and Tooley and Lawrie (1974), using fish meals with lipids. They showed that no lysine availability losses occurred when herring meal was processed under nitrogen but 12% of available lysine was lost when heating was carried out in the presence of oxygen. Also Szebitko, et al., (1979) demonstrated that tryptophan and lysine were damaged during autoxidation of lipids under accelerated conditions using milk and soyabean oil mixture.

It is well known that lipids with a high content of unsaturated fatty acids undergo oxidation in the presence of oxygen. This autoxidation of unsaturated fatty acids yields peroxyradicals, hydroperoxides, epoxides and carbonyl compounds, (Dugan, 1976). Carbonyl compounds and epoxides may react with amino groups of proteins by Maillard reactions

(intermediate and advance stages) to produce brown pigments (Namiki et al., 1974). Peroxides and hydroperoxides might oxidize methionine and cysteine residues in food proteins as shown by Cuq et al., (1974). Karel, 1973, suggested that the free radicals produced during autoxidation of lipids could react with proteins forming protein-protein and/or protein-lipid cross-links which could reduce the protein digestibility. However, for canned foods, this type of damage is of no importance since the conditions for lipid oxidation are not present and because lipid oxidation makes the food unacceptable from the organoleptic point of view as stated by Szebiotko et al., (1979).

To summarise, a simplified scheme of the reactions of lipids with other food components is shown in Figure 2.11 as proposed by Karel (1973).

Interactions between proteins and other molecules

Aldehydes

Hurrell and Carpenter, (1978) in studying protein-formaldehyde reactions, reported that formaldehyde reacted with several amino acids chains that included lysine, cysteine and histidine, with subsequent losses of amino acids availabilities. They also reported reduction in protein digestibility and suggested that cross-linking was the cause. This reaction is important in smoking processes since formaldehyde is a wood smoke constituent (Dvorak and Vognarova, 1965), but not for canned foods.

Quninones

Quinones are formed from the oxidation of polypeptides

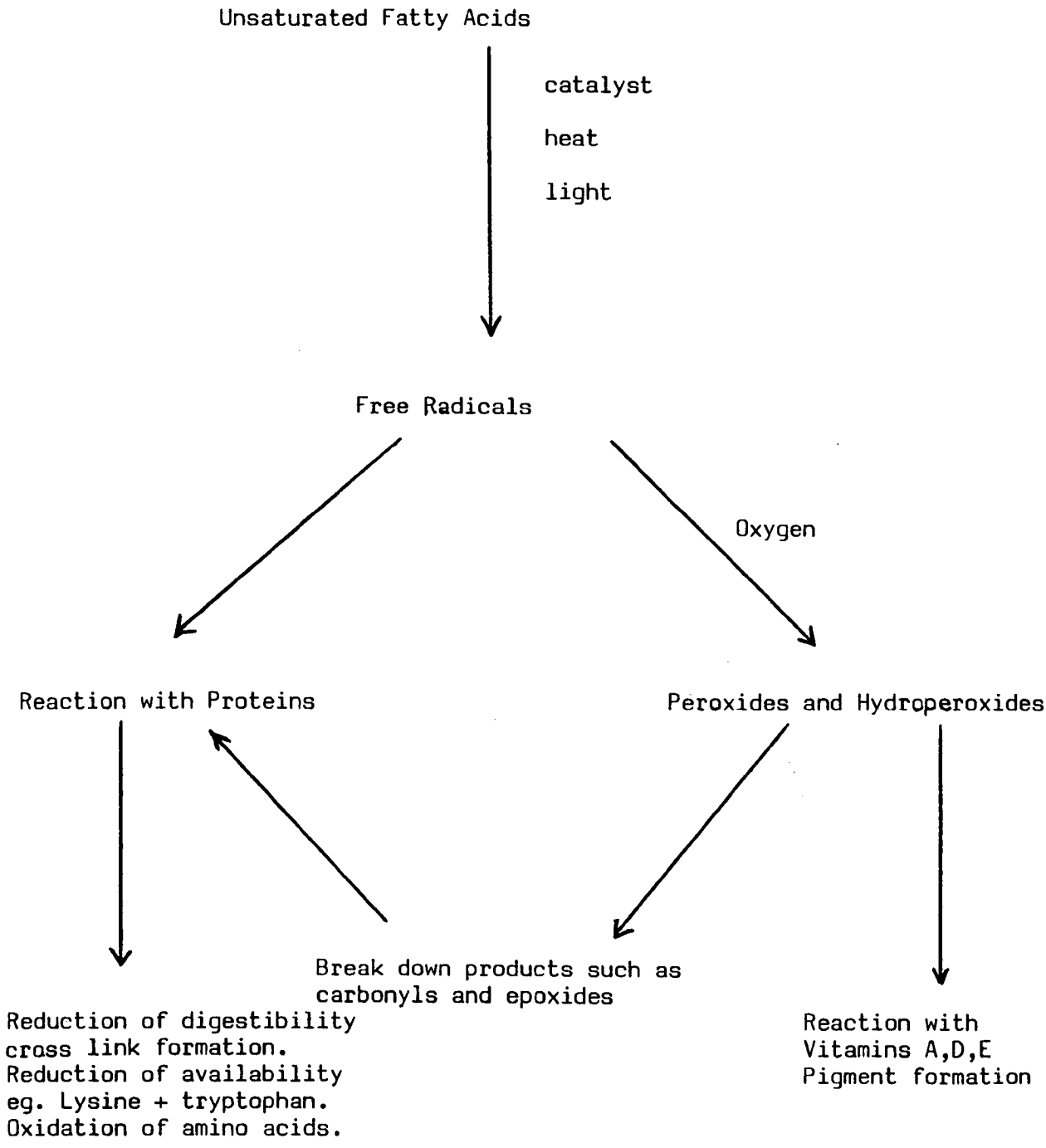


Figure 2.11 Simplified scheme for the reactions of lipids with other food components.

either during enzymic browning reactions or by alkaline treatments. It has been suggested they react with lysine, methionine, cysteine and tryptophan reducing their availability (Cheftel, 1979).

Reports of damage to proteins by nitrites, sulphites and chlorinated molecules have been reviewed by Cheftel, (1979). However, they are of secondary importance in thermally processed foods when compared to the damaging Maillard reactions.

It can be seen from the different mechanisms of protein and vitamin damage that the type of nutrient damaged and the extent of the nutritional damage is dependent on the type of treatment applied to the food and environmental factors such as pH, temperature, presence of other nutrients, etc. These factors will be discussed in the next section within the specific context of canning and pouch processing.

SECTION III

EFFECTS OF FOOD PROCESSING, BLANCHING AND STERILISATION

Heat treatment of foods is one of the most common methods of food preservation. It is well known that thermal processing of foods has beneficial and detrimental effects, which have been extensively reviewed by Bender, (1978); Benterud, (1971); Chichester, (1973); Fennema, (1976); Harris and Karmas, (1977); Harris and Von Loesecke, (1973); Karel et al., (1975); Paul, (1977); Priestley, (1979); Ratnatunga et al., (1978) and Tannenbaum, (1979).

Some of the most important effects are: inactivation of enzymes and microorganisms (which may damage nutritional and other quality factors); improvement of nutritional value

by liberating nutrients present as unusable complexes; damage to heat sensitive nutrients such as vitamins.

One of the most important methods of food preservation is the thermal sterilisation of foods in hermetically sealed containers. This method of preservation normally includes blanching as a pre-sterilisation treatment. The nutritional effects of blanching and heat sterilisation will be discussed in the following sections.

2.5 Blanching

In the preservation of tissue systems (vegetables and fruits) by canning, freezing or dehydration, blanching is a basic step. Blanching prior to heat sterilisation has several different functions (Lee, 1958 and Lund, 1976), the most important can be

- Removal of foreign materials.
- Wilting of the tissue to facilitate uniform packaging.
- Expulsion of air and gases which may create excessive pressure in the sealed container.
- Increase of the food temperature before sealing.
- Inactivation of enzymes.
- Bacterial load reduction.

Blanching normally consists of subjecting the food to a moderately high temperature for a short period of time. There are several methods of accomplishing blanching reported in the literature, which include: Hot water immersion, steam blanching, individual quick blanch, microwave blanch, electronic blanching, and superheated steam blanch.

The two commonest methods of blanching are immersion in hot water or treatment with steam. The main factors

affecting nutrient degradation in these methods are thermal effect, leaching and oxidative losses. The main difference between the two methods with respect to nutrient retention is the extent of leaching. Lund (1975) stated that the main factors affecting hot water blanching would be those factors affecting mass transfer, mainly: surface area, concentration of solutes in the hot water, and agitation of the water, since the loss of water soluble nutrients increases with contact time. Therefore, steam blanching is believed to produce lower blanching losses than immersion in hot water blanching.

Steam and water blanching of green peppers retained 72% and 67% of their vitamin C respectively (Matthews and Hall, 1978).

Recently, a new method of steam blanching has been reported, (Lazar et al., 1971), which is called "individual quick blanch", (IQB). This IQB method is claimed to solve the overblanching problems of steam and hot water immersion blanchings by ensuring uniform heat-treatment. As the samples are rapidly heated in a thin layer, the blanching time is reduced, thus improving nutrient retention. Nevertheless, it is an experimental technique not a commercially adopted practice.

Microwave blanching has only been applied to fruits and vegetables on a small scale due to its high cost. Dietrich et al., (1965) reported vitamin C losses in brussels sprouts using steam blanching, hot water immersion and microwave treatment. They concluded that microwave blanching resulted in a better ascorbic acid retention.

Electronic blanching and superheated steam blanching

have restricted use on a commercial scale and no information on nutrient damage by these methods is available.

As nutrient damage due to blanching depends mainly on the food product, nutrient in question and method of blanching, the nutrient losses of vitamins (C and B_1) and proteins (Lysine) during blanching will be individually mentioned, with particular reference to the two most common methods of blanching, steam and hot water immersion.

2.5.1 Vitamin C

Several factors are known to affect the loss of vitamin C during the initial stages of blanching, presence of solids in the blanch water, product to water ratio, size of vegetable and damage caused to vegetables prior to blanching.

Temperature and Time

The vitamin C losses by thermal destruction are expected to be caused by parallel aerobic and anaerobic oxidation reactions, (section 2.3.1). However, as reported by several authors, the rate of aerobic destruction is very much greater than the rate of anaerobic destruction. The rate of the aerobic reaction is greatly increased when metals ions such as copper or iron are present, (Labuza, 1972, Lin and Agalloco, 1979, Lee, 1958 andWaletzko and Labuza, 1976). Therefore as blanching is usually performed in the presence of oxygen, most of the thermal losses of vitamin C would be by aerobic oxidation.

Hendshall (1974) reported that HTST blanching increased ascorbic acid retention. However, Lund (1975) considers

that from the standpoint of thermal degradation of nutrients, there would not appear to be a significant advantage in using HTST blanching, particularly when blanching for the freezing industry is considered. He suggested that special care should be taken with inactivation of some heat resistant enzymes such as peroxidase.

However, leaching losses of vitamin C are reported to be the prime cause of loss when high water temperatures for blanching by immersion or steam blanching are used, (Birch, 1974; Lee, 1958, and Selman et al., 1982).

Birch et al., (1974), Boushell and Potter, (1980) and Selman et al., (1982) reported that the main losses of ascorbic acid in peas were by leaching with only negligible oxidation losses. They also reported that losses by blanching increased with time and temperature of blanching. Selman et al., (1982) reported that a substantial amount of vitamin C, (28%) was leached into the brine after blanching at 97° C for 2 min., with negligible losses due to oxidation of vitamin C. Lathrop and Leung, (1980) reported also greater losses due to leaching (16.2%) than due to thermal destruction (12.5%) after blanching at 100° C for 5 min. They also reported better AA retention in steam blanching than in water immersion.

Boushell and Potter (1980) proposed that some other subtle changes may occur in potato during high temperature blanching such as partial denaturation of cellular membranes which may affect the diffusion rates of cellular components, including vitamin C. This effect may contribute to the increase of leaching AA losses with the increase of temperature.

From these data it can be concluded that leaching

rather than destruction is largely responsible for vitamin C losses during both water and steam blanching.

Enzymic activity

It is well known that blanching of fruits and vegetables improves vitamin C retention during storage and further processing by inactivation of enzymes such as ascorbic acid oxidase and peroxidases, (Birch, 1974 and Selman, 1982). Guerrant et al., (1947) reported that increasing temperature and using the same time for blanching of several vegetables produced a reduction of thiamine and ascorbic acidlosses. Later, Birch et al., (1974) when studying the effect of blanching on vitamin C in peas found a similar effect. They reported that high blanching temperature (97 °C and 85 °C) produced negligible vitamin C oxidation, the sum of the leached vitamin (28%) and the retained (70%) virtually equalled the initial vitamin C concentration. However at low blanching temperatures greater vitamin C losses were encountered and a large proportion of ascorbic acid was oxidised. From these findings they concluded that vitamin C losses due to enzymic action, both prior to or during blanching were significant. Selman (1978), suggested that the relatively greater loss due to enzymic oxidation might be expected at lower temperatures as an effect of slower heat penetration rate, which permitted an increase in the period during which enzyme activity could occur. Recently, Selman and Rolfe, (1982,) reported the confirmation of Birch's findings when working with peas. Furthermore, they reported that inactivation of enzymes was achieved at 70 °C and blanching above this temperature produced no losses of AA except by leaching.

This data suggests that the use of high temperatures (above 70 °C) in order to achieve adequate enzyme inactivation will result in reduced vitamin C destruction.

Presence of solids

Salib (1980a and 1980b) reported that retention of ascorbic acid is increased when the pH of the blanching solution was slightly acidic (pH = 6). Also the addition of 2% sodium chloride to the blanching water has been claimed to reduce the loss of vitamin C (Bender, 1978). Guerrant et al., (1974) reported that the proportion of AA lost tended to decrease with repeated use of the same blanch water and Henshall (1974) suggested that in the continuous blanching procedures used in industry, the blanching water becomes a nearly isotonic solution, with the subsequent improvement in ascorbic acid retention by reduction of leaching. Supporting evidence was found by Lathrop and Leung (1980), who reported that the leaching rate in water blanched peas appeared to be controlled by diffusion. They reported that leaching rate was independent of agitation rates.

Product to water ratio

Benterud (1971) reported that the ratio of vegetable to water affects the vitamin C retention during boiling. Higher losses were obtained when the ratio was increased. This effect can also be assumed true for blanching.

Product size

Lund (1975) suggested that an extended surface per unit of volume would increase the leaching losses of water

soluble nutrients. However, Selman (1978) discussed that in a large size food particle the heat penetration rate my be reduced and the possibility of enzymic damage may also be increased in this way.

Damage to food

It is generally accepted that rough handling of vegetables and fruits causes cellular damage which favourably affects the ascorbic acid-enzyme interactions. Also that leaching losses increase due to the loss of cellular fluids. Lee et al., (1976) reported that under the same blanching conditions, whole vegetables retained more vitamin C than cut vegetables. They suggested that enzymic activity was responsible for the increase in vitamin C losses, due to cellular damage.

Some of the literature reports on vitamin C losses during water immersion blanching are presented in Table 2.1 to illustrate the range of losses reported.

From these reports it can be concluded that vitamin C retention during blanching can be generally improved by following the recommendations given below.

1. Rapid achievement of enzyme inactivation.
2. Using moderately high temperatures for short periods of time.
3. Avoiding presence of metals and sugars in the blanching water.
4. Using blanching water slightly acid and isotonic solute concentrations preferably with AA and salt.
5. Reducing damage to food prior to blanching and ratio of vegetable to blanching water.

Table 2.1 Examples of the reported losses of ascorbic due to leaching and processing.

Author	Food	Blanching treatments temperature °C	time min	Ascorbic Acid in vegetable	in blanch water
Lathrop and Leung, 1980	peas	100	1	90.8	3.1
Lathrop and Leung, 1980	peas	100	3	73.0	8.5
Lathrop and Leung, 1980	peas	100	5	68.3	16.2
Guerrant, 1947	peas	87.8	6	77.0	23.9
Birch, 1974	peas	70	2	74.0	15.0
Selman and Rolfe, 1982	peas	97	1	72.0	28.0
Boushell and Potter, 1980	potatoes	97	1	≈72.0	28.0
Boushell and Potter, 1980	potatoes	25	7.5	95.0	n.g.
Boushell and Potter, 1980	potatoes	40	7.5	91.0	n.g.
Boushell and Potter, 1980	potatoes	60	7.5	72.0	n.g.
Boushell and Potter, 1980	potatoes	80	7.5	65.0	n.g.
Lee, et al, 1976	peas	85	3	86.5–89.5	n.g.
Lee, et al, 1976	green beans	77	0.75	83.9	n.g.
Salib, 1980	vine leaves	100	2	71.0	n.g.
Salib, 1980	artechokes	100	2	95.7	n.g.
Salib, 1980	kidney beans	100	2	93.6	n.g.

n.g. not given

2.5.2 Vitamin B_1

The same factors that affect vitamin C retention during blanching are important for vitamin B_1 losses during blanching.

Temperature and time

As vitamin B_1 is not easily oxidised, the main losses of thiamine during blanching would be by leaching, and a negligible amount by thermal destruction. Feaster (1960), after an extensive review of the published data, concluded that changes in composition accompanying the blanch of tissue systems are caused presumably by leaching of water soluble nutrients, along with some oxidation of ascorbic acid. Also that thermal destruction of thiamine during blanching was negligible. Lee (1958) in his review concluded the same.

Leaching losses of thiamine are reported to increase with temperature and time of blanching (Guerrant et al., 1974; Lee, 1958; Bender, 1978). However, the main sources of vitamins are cereals and meats which are not submitted to blanching procedures. One exception to this statement is potatoes, which according to Oguntona and Bender (1976) provides the 10% of the calculated average intake of thiamine in the British diet. Salib et al., (1980) reported losses of 16%, 4.2%, 9.9% and 9.3% during blanching for 2 min. at 100 °C of vine leaves, artichoke, potatoes, unpeeled and peeled, respectively. These findings agree with Farrer, (1955,) Benterund, (1971) and Feaster, (1960,) who stated that losses during blanching of thiamine by leaching was of the order of 10% to 20%.

Enzymic activity

Archer and Tannenbaum (1979), and Porzio et al., (1973), reported antithiamine activity in fish, pork and beef products. Bender (1978) mentioned 3,4-dihydroxycinnamic acid in fern as antithiamine but some antithiamine factors have not yet been identified. Chan and Hilker (1976) studying plant products reported that thiamine is degraded by polyphenol derivatives by a phenolic oxidative destruction catalysed by poly phenol oxidase (PPO). They concluded that deactivation of PPO would be one of the effective methods to preserve thiamine in foods. However, no reports on PPO damage to thiamine during the initial stages of blanching or thermal processing have been found in the literature. Nevertheless, Chan and Hilker (1976) suggest that the two unidentified fluorescent compounds observed by Dwivedi and Arnold (1972) when heating thiamine containing samples might have the same or similar structure as the decomposition compounds they obtained.

Presence of solids

It has been reported by Salib (1980a and 1980b) that retention of thiamine during blanching in slightly acidic (pH 6) water was increased when compared with blanching under the same conditions in distilled water.

Yagi and Itokawa, (1979) reported that the presence of residual chlorine in tap water produced the cleavage of thiamine into hydroxymethyl pyrimidine and the thiazole moiety, when boiling rice. It seems reasonable to assume that these effects may be present in blanching in tapwater to a similar degree since the time and temperature studied include the

range of temperatures and times involved in blanching, i.e., 100 °C for approximate times of 1 min., 3 min., 5 min. and 10 min.

Oguntona and Bender (1976) reported that the presence of sulphite in the blanching water from sulphite-treated potatoes increased the thiamine losses up to 40%. Consideration of product to water ratio, product size and damage to food particles prior to blanching are the same as for vitamin C, since both vitamins are water soluble.

In resume, the same general recommendations given to improve vitamin C retention are true for vitamin B_1, the only difference being that especial care should be taken to avoid the presence of nucleophiles such as sulphite and chloride in the blanch water. It also can be concluded that losses of thiamine during blanching seem to be less important than vitamin C losses.

2.5.3 Proteins

During blanching, according to Bender (1978), there can also be small losses of soluble protein leaching into the processing water. This effect is negligible, particularly since it mostly affects vegetables, which are not an important source of proteins, (Heinz, 1959, and Bender, 1966, 1978).

2.6 Thermal Sterilisation Processes and Storage

One of the most important methods of food preservation is the heat treatment of foods in hermetically sealed containers. This process aims at the destruction of microbiological life and inhibition of enzymic activity, with the least possible effect on the nutritive value of the food in order to increase

the food product shelf life (see section 2.2).

Unfortunately, the application of sufficient heat to destroy food spoilage microorganisms and inactivate enzymes also results in some damage to heat sensitive nutrients. Furthermore, since many particulate foods are packaged in syrup or brine, losses of water soluble nutrients may also occur as these liquids are rarely consumed.

This "thermal damage" to heat sensitive nutrients may also be influenced by several factors, depending on the nutrient damage mechanisms, such as:

- Presence of residual oxygen in the package, such as in the head space of cans, or dissolved and trapped in the food itself. This may affect nutrients which are easily oxidised such as vitamin C.

- The pH of the food product, in most cases will be slightly acidic (low acid foods) or neutral, since natural alkaline foods are rare. Strongly acidic foods are more frequent and include products such as berries, pickles, certain citrus fruit products, and fermented foods.

- Other compounds present in the food such as food components (sugars) and contaminants or additives (SO_2, metals) will depend for their importance on the nutrient being damaged.

The effect of thermal sterilisation and storage on the nutritive value of foods have been reviewed by several authors, (American Medical Association, 1974a, 1974b; Bender, 1966, 1978; Fennema, 1976; Cheftel, 1979b; Chichester, 1973; Harris and Karmas, 1975; Harris and Von Loesecke, 1960; Hollingsworth, 1972; Institute of Food Technologists, 1974;

Kramer, 1977; Lee, 1977; Lund, 1977, 1979, and Tannenbaum, 1979).

Here only the changes relating to vitamin C, vitamin B_1 and damage to lysine (proteins) will be mentioned.

2.6.1 Vitamin C

Considerable information has been published on the effect of commercial canning on vitamin C content.

Most authors agree that leaching during commercial processing (blanching and thermal sterilisation) is the main cause of vitamin C loss. However, during the heat sterilisation process, significant losses result from the chemical degradation of vitamin C which is greatly influenced by the residual oxygen present in the container.

According to Heid, (1960), after an extensive review of the literature, vitamin C losses during canning vary between 65% to no losses at all. Later Benterund (1971) presented a summary of data published on vitamin C losses during canning. He showed that in 90% of the cases, 90% of the vitamin was retained in canned high acid citrus fruits and 25% to 75% was retained in canned vegetables. He suggested that the wide variation in vegetables vitamin C retention reflected the varying conditions of blanching. Nevertheless, he concluded that it would be possible to keep vitamin C losses within 10 - 20% by careful processing.

Nagy (1980) in an extensive review on citrus fruit products, also reported that in single strength juices the retention of vitamin C during canning ranged from 89% to 100% with an average retention of 97%.

Ratnatunga et al., (1978) stated that vitamin C retention

during canning in citrus fruit juices was greater than in vegetables and suggested that this effect may be due to the retardation of oxidation of ascorbic acid in many natural fruit juices due to both the presence of substances like pectin, thiamine and β-carotene and due to the slightly more acid pH of the fruit juices.

The effect of temperature on vitamin C destruction has been already mentioned (section 2.2.2). As expected most of the reports (Kramer, 1977) on storage of canned foods agree that higher temperatures lower the retention. Nagy and Smoot (1977; 1979) reported a change in reaction kinetics at 28 °C. They concluded that storage temperatures above 28 °C for citrus fruit juice the rate of destruction was faster than at temperatures below 28 °C. Saguy et al., (1978), reported a similar effect with a slope change between 27°C and 37°C.

The reduction of residual oxygen by deaeration has been reported to increase vitamin C retention during thermal treatment and storage by Henshall, (1974), Mannheim and Passy, (1979) and Nagy, 1980. However there are several reports showing that vitamin C is retained better in plain cans than in lacquered ones, More et al., (1951). Bender, (1978), Henshall, (1974), and Nagy, (1980) agree in suggesting that the residual oxygen in plain cans is normally used up in the electrochemical process of corrosion with the tin and thus disappears more rapidly than in lacquered cans.

Another processing variable that can influence the vitamin C retention is the presence of sulphite from sulphur dioxide treated vegetables such as potatoes (Oguntona and Bender, 1976) or fruits, (Shaw et al., 1970), which enhances

vitamin C retention both during storage and processing.

One of the best methods of studying nutrient retention is through kinetic studies (Harris and Karmas, 1975 and Lund, 1976)

There is some evidence which suggests that the anaerobic destruction of ascorbic acid in food systems, follows a first order kinetics which is dependent on temperature as reported by Saguy et al., (1978) during heat processing of grapefruit juice; Lathrop and Leung, (1980) for canned peas and Rao et al., (1981) during processing of canned peas also. Nagy and Smoot (1977) reported departures from a pure first order reaction at high storage temperatures in canned orange juice. Lee et al., (1977) also reported a non first order reaction when small concentrations of oxygen are present during storage which agrees with Herrmann et al., (1978) who stated that ascorbic acid degradation during production and storage of fruit nectar and baby food presents two phases. In the first phase an oxidising degradation takes place as a second order reaction parallel to a non oxidising degradation as a first order reaction. At the second phase there is only a non oxidising ascorbic acid degradation.

Several authors have reported kinetic parameters such as $D_{121.1}$, Z, E_a and K_{121} but there is poor agreement in the results, as shown in Table 2.2. The marked differences of E_a indicate that kinetic studies should be conducted for different food systems.

2.6.2 Vitamin B_1

Farrer (1955), reported that conventional canning methods caused an average 40% loss in thiamine. The main

Table 2.2 Examples of reported kinetic parameters for ascorbic ac. thermal destruction

Year	Author	Ea Kcal/mol	K_{121} min^{-1}	Q_{10}	D_{250} min	$Z_{°F}$	T range °C	Food Product
1979	Nagy and Smoot	4.58	-	2.7	-	-	4.50	grapefruit juice
1977	Nagy and Smoot	12.8	-	2.2	-	-	4-28	orange juice
1977	Nagy and Smoot	24.5	-	3.7	-	-	28-50	orange juice
1977	Lee, et al	3.3	-	-	-	-	10-37.8	tomato juice
1978	Saguy, et al	5	-	-	-	-	61-96	grapefruit juice (11° BX)
1978	Saguy, et al	11.3	-	-	-	-	61-96	grapefruit juice (62° BX)
1980	Lathrop and Leung	41.0	0.75×10^{-3}	-	-	-	110-132	canned peas
1981	Rao, et al	13.1	9.26×10^{-3}	-	246	91	210-260°F	canned peas

mechanisms of thiamine damage are leaching and thermal destruction.

Since thiamine is also water soluble it can be leached out during processing and storage in the same way as vitamin C.

Thiamine, unlike vitamin C, is not affected by the presence of residual oxygen in the package since thiamine destruction is thermal not oxidative (Mulley et al., 1975a; Farrer, 1955; Williams and Spies, 1938).

According to Farrer (1955) the most important factors in the thermal destruction of thiamine were duration of heat, temperature and pH, as discussed previously.

Bendix et al., (1951), in a study of thiamine stability in peas, corn, lima beans and tomato juice during thermal processing found that thiamine destruction in peas over the temperature range 104.5° to 132.5°C followed first order reaction. This observation was not applicable to all the other vegetables. Later, Farrer in his review of the thermal destruction of thiamine in foods showed that the loss of vitamin B_1 could be generally described as a first order equation. More recently, Mulley et al., (1975a, 1975c) showed that thiamine destruction in food systems satisfied the Arrhenius equation. They calculated $D_{121.1}$- values for the chemical breakdown of thiamine in pH 6 phosphate buffer solution (D = 156.8 min.), pea puree (D = 246.9 min.), beef puree (D = 254.2 min.), and peas in brine puree (D = 226.7 min.), with Z values ranging from 25°C to 27.2° C. Teixeira et al., (1975a) also reported $D_{121.1}$- values for pork puree (178.6 - 165.6 min.) and pea puree (165.6 min.) and Z-value of 25° C. These values show the temperature dependence of thiamine destruction.

Felliciotti and Esselen, (1957), reported that thiamine in foods was more stable to thermal destruction than thiamine in aqueous or buffered solutions. Some authors tried to explain these findings by suggesting that co-carboxylase was more stable than free thiamine, however most researchers agree that co-carboxylase is less stable than the free form. Mulley et al., (1975b) presented evidence that co-carboxylase is a good deal less stable than thiamine. So the increased stability of thiamine in foods may be due to the acidic pH of the foods and the protective action of some of the food components.

According to Kramer, (1977) losses of thiamine during storage can be significant, averaging from 25% for 12 months at 27 °C to 25% for 12 months at 37° C. Everson et al., (1964) stated that in three food sterilised products thiamine was destroyed during storage independently of type of container and was influenced slightly by storage temperature. The method of processing did not affect the amounts of thiamine loss during storage. Farrer (1955) approached the problem by calculating the rate constant and could predict, through the Arrhenius equation, the life of thiamine during storage. Teixeira (1975b) also predicted thiamine losses during storage and processing.

2.6.3 Proteins

The extent of damage to proteins during heat sterilisation will depend on the composition of the food, temperature and time of process, pH value, moisture content and presence of other substances, as described in the possible mechanisms

of protein damage mentioned in section 2.4.

Damage to proteins with low carbohydrate content like fish and meat, may undergo protein-protein damage. Beuk et al., (1948 and 1949) recovered all the amino acids with the exception of cysteine after autoclaving for 24 hours at 112 °C but found unavailability in all of them. However, Wheeler and Morgan, (1958) reported no loss of nutritive value after heating pork at 120°C for 1 hour, but some damage was observed after two hours. It has been generally accepted that meat canning produces little damage to protein digestibility and nutritive value. According to Cheftel (1979) canning of meat reduces the protein nutritive value by less than 10% of the original value. As already mentioned the amino acids that are the most affected in this type of damage are cystein, lysine, methionine, tryptophan, serine, threonine, arginine andleucine (Beuk et al., 1948, 1949; Donoso et al., 1962; Bjarnason and Carpenter, 1970; and Miller et al., 1965a, 1965b, 1965c).

Also it has been mentioned that alkaline natural foods are scarce, therefore during heat sterilisation protein damage of the alkaline type was not thought to occur. Recently Sternberg and Kim, (1977), Aymard (1979), and Raunio et al., (1978), found residues of lysinoalanine in processed foods, that have never been submitted to alkaline treatment. Although the amounts were very small their significance is increased since lysinoalanine is known to reduce protein digestibility (Provansal, et al., 1975, and Tanaka et al., 1974) and to produce nephrocytomegalia in rat kidney, (Woodward and Short, 1973). The toxic effect of lysinoalanine has not

been proved in humans and there is evidence that other animal species (such as dogs, monkeys and hamsters) are not affected by this compound, (De Groot et al.). The main amino acids damaged by the formation of lysinoalanine are cysteine and lysine.

Lipid-protein interactions are unlikely to occur to an appreciable level in thermo sterilised food since oxygen is present only in limited amounts. Szebiotko et al., (1979) reported that during the induction period of fat oxidation, changes in protein quality by lipid-protein interaction are insignificant even for the availability of the particularly susceptible lysine. They stated that protein-oxidised fat interaction occurs mainly during the phase of maximum accumulation of peroxides. According to Dugan, (1979), at this point the organoleptic quality of the food is already damaged by the production of off-rancidity-flavour. Consequently the food product may not be consumed. Yanagita and Sugano (1975) reported that the main losses of casein-lipid interaction were lysine methionine, arginine and histidine and lysine availability. Cuq et al., (1974) also mentioned methionine availability losses.

In the presence of reducing sugars nutritional changes by Maillard reactions appear to be the major cause of protein damage. Maillard reactions can take place during heating or long term storage of foods even at low temperatures such as room temperature, i.e., 20 ^{o}C, Lea and Hannan, (1949). As already mentioned the Maillard reaction is greatly affected by temperature, pH and moisture content.

Maillard reactions have relatively high energies of

activation, between 41.8 - 167.2 KJ/mole, (Labuza and Saltmarch, 1982; Tsao et al., 1978; Thompson and Wolf, 1979; and Wolf et al., 1978). Thus when foods containing reducing sugars are moderately heated, Maillard reactions occur in preference to any other type of damage. Tsao et al., (1978) reported that the thermal damage to available lysine can be described as a monomolecular reaction in fortified rice meal processed at temperatures 115° - 185°C for 7.5, 15, 30, 60 and 120 min. The effect of temperature on the reaction rate constant followed the Arrhenius equation with an E_a = 52.25 KJ/mole. Labuza and Saltmarch, (1982), reported activation energies between 20 and 104.5 Kcal/mole for loss of lysine availability in whey powders during storage at 25, 35 and 45°C.

From the nutritional point of view, Maillard reactions damage mainly lysine, and to a lesser extent other types of basic amino acids such as tryptophan, methionine, cysteine and threonine. The loss of nutritive value of lysine occurs with the formation of the Amadori compound (Finot and Mauron, 1969; and Mauron, 1970). Adrian, (1974), added premelanoidins (intermediate stage of Maillard reactions) to rat diets and reported that low amounts of premelanoidins increased the appetite and growth of rats but great amounts provoked a decrease in growth. Tanaka et al., (1974) reported reduction of the nutritional value of egg albumin, damaged by Maillard reactions and concluded that reduction in digestibility was an important factor to consider in Maillard damaged proteins.

Losses of available lysine in canned products vary between 0% and 20%. Alho et al., (1978) reported that a

sterilisation process at 121° C for 50 and 60 min. in baby canned foods produced an approximate 15% decrease in lysine availability. Grandborg (197) reported that increasing $F_{115^{\circ}C}^{10^{\circ}C}$ value increases the amino acid destruction and that the greatest destruction (40%) was encountered between $F_{115^{\circ}C}$-values of 0 to 4.0 in canned soya protein-dextrose mixture. Bender and Husani (1976) reported losses in the nutritional value of commercial canned meat products such as stewed steak with onions and in beef autoclaved for 1 hour at 115°C.

2.6.4 Pouch Processing

It has been suggested that two of the major advantages in pouch processing are the improved quality and nutritional value of the food. Quality has been proved better by organoleptic tests. Platt, (1979), tested eight different main dishes against cans and found no difference between the sensory rating, however, Schultz (1978) reported better taste panel ratings for 22 pouch processed items including main dishes, vegetables and fruits; Tung et al., (1974, 1975) reported better quality for pouch sweet corn than for the canned products during sensory evaluation and colour measurements. Lyon and Klose (1981) using a trained panel, reported that poulty meat was better in cans than in pouches and Lazar and Hudson (1976) reported the same for apple slices.

However in the evaluation of nutrients only a few reports are available. Thorne (1976) reported better vitamin C retention after processing for pouch processed potatoes than canned potatoes and mentioned that the main cause of loss was the leaching of vitamin C to the canned liquid

(pouches were packaged without brine), but canning processing retained more vitamin (21 mg per 100 g). Since pouches can be packaged without brine or syrup or with limited amounts Herrmann (1977) leaching of water soluble nutrients is expected to be greatly reduced. Chen and George (1981) when working with canned and pouch processed green beans, reported reduced leaching in pouches (only 10% of the amount retained), while 40% of the ascorbic acid retained was leached to the brine in cans. This was probably due to the reduced amount of brine used for the pouch processed green beans. However, they also reported better overall ascorbic acid retention in canned green beans than in the pouch product. This is contrary to expectations since thermal destruction of the vitamin should be reduced by the shorter processing time. Chen and George's (1981) work is open to criticism in this respect since they admitted poor control during pouch processing. It was done in a normal pressure cooker which was held at processing temperature 30% less time than the canning retort, but they allowed the pressure cooker to stand for 30 min. without cooling to slowly reduce the pressure thus avoiding pouch bursting. So their work does not provide conclusive evidence about the effect of thermal sterilisation in both pouches and cans.

Storage tests were reported by Salunkhe et al., (1978) in eight foods packaged in pouches. They reported minimal losses during storage at 4.4°C and the losses increased slightly when products were stored at 21°C and increased even more for storage at 37.8 C. The losses of ascorbic acid and thiamine were considerable at 37.8°C storage temperature 20% - 50%

of the initial content. Further evidence that low storage temperatures improve the nutrient retention was provided by Kramer (1979) when he statistically analysed the results of Salunke et al., (1979).

The general factors affecting vitamins C and B_1 and lysine (pH, temperature, time, etc.) would be expected to be the same as for canned products.

SECTION IV

ANALYSIS OF NUTRIENTS

There are several types of methods for the analysis of nutrients, animal, microbiological and chemical. Each group of analysis has certain advantages or disadvantages. Biological (animal and microbiological) assays are normally time consuming and expensive, and some of them are variable since individual organisms vary in their response. Chemical analysis can be carried out relatively more rapidly and economically than biological methods, consequently, chemical methods are preferred when large batches of routine assay are required. Unfortunately there are cases when no reliable chemical method is available and biological methods are the only alternative. Biological methods are very useful when new chemical methods are developed, for comparative studies in the evaluation of the chemical methods.

The nutrients used in this project were vitamin C and B_1 and available lysine so the techniques employed for their analysis will be briefly discussed. No attempt will be made to cover all the methods available, only the techniques used and some others considered important for this work.

2.7 Vitamin C

A simple and highly specific chemical method for vitamin C determination has not yet been obtained. This has resulted in a great number of different methods for its determination. Therefore, only the two official A.O.A.C. methods for vitamin C determination: the 2,6 dichlorophenol indophenol titrimetric method and fluorimetric procedure will be discussed. The titrimetric procedure is based on the oxidation-reduction properties of ascorbic acid and it is known to evaluate only the reduced form of the vitamin. The second method is based on the reaction of the dehydroascorbic acid with O-phenylediamine to form a stable quinoxaline which is determined fluorimetrically. This method evaluates both oxidised and reduced forms of the vitamin.

2.7.1 Vitamin C Extraction

Due to the instability of vitamin C in aqueous solutions special care must be taken in the extraction procedures. The extraction of vitamin C from biological tissues presents two main problems: the destruction of enzymic activity present in cut or bruised samples, and the incorporation of oxygen into the extraction media by grinding, blending or homogenizing of the food.

The first problem is only important in raw samples and to a limited extent in blanched samples since thermally sterilised samples are normally free of enzyme activity. So to avoid enzyme damage a general recommendation is produced that samples must be handled very rapidly and stabilised in a proper extraction medium. To solve the second problem

some authors (Freed, 1966) have suggested the use of inert gases such as nitrogen during the blanching procedure.

Ponting (1943), after comparing a great number of potential extracts, concluded that metaphosphoric acid and oxalic acid were the most suitable. Fujita and Iwatake (1935) preferred the use of metaphosphoric acid and Yager (1948, from Freed 1966) recommended the use of metaphosphoric acid and EDTA. As mentioned by Oliver (1972) when summarising the findings of his review, metaphosphoric acid is the most satisfactory extractant for many authors. However, as pointed out by Freed (1966), due to its high cost either combinations of metaphosphoric acid and other acids, or different acids (such as oxalic acid) have been preferred. Large ratios of extract solution-sample have been preferred (Pelletier, 1967; Pelletier and Brassard, 1977) between 7:1 to 4:1. The concentrations of metaphosphoric acid preferred for foods has been 5 - 6%.

An extraction medium of 50% ethanol - 50% metaphosphoric acid solution has been reported by Garcia et al., (1975) as a suitable extraction medium for multiple analysis of vitamins.

Blending or homogenising should preferably be determined by the investigator since long times of blending can reduce the amount of ascorbic acid present by oxidation as reported by Roe et al., (1948) and small blending times may not extract the total amount of vitamin, especially in uncooked tissues.

2.7.2 2,6-Dichlorophenolindo phenol Titration

The use of 2-6, dichlorophenol indophenol as the basic

indicator in the vitamin C estimation has been justified in the case of fruits and vegetables and their products by several authors and some of them have compared the determination with biological methods confirming its accuracy (Tilmans et al., 1932).

There are many substances which are known to interfere with the ascorbic acid determination, most of them are substances capable of reducing the dye such as sulphite, sulphides, thiols, reductones, etc. The normal procedure set the conditions of the assay so that the interfering substances are inhibited or allowed for by difference.

Reductones produced by heat treatments of foods are an important source of interference. Pelletier (1967) proposed the use of formaldehyde in a blank correction for reductones based on Lugg's (1942) findings. Lugg (1942) reported that formaldehyde condenses with ascorbic acid at pH 3.5 rapidly but in negligible amounts with reductones produced by heat. Sulphites, thiols and sulphides which are also interfering substances are removed by condensation with formaldehyde at pH 1.5 and 0.6 (Lugg, 1942) before titration of ascorbic acid.

Hall and Deutsch (1965) proposed important modifications to improve the titrimetric method and to widen its applicability. They suggested preliminary tests to determine the presence of interfering substances and the acidity of the sample in extraction solution, in order to select the appropriate extraction solution for the sample. This was done by either addition of H_2SO_4 to lower the pH of the solution or by the use of acetic acid-metaphosphoric acid solution when large amounts of reducing sugars were present.

One of the limitations of this method is that it only can be used with slightly coloured, or colourless, extraction solutions since the end point of titration is characterised by the formation of a faint pink colour. Removal of pigments have been tried by chromatographic methods by some authors like Wollenweber (1969).

Several authors have suggested purification procedures to eliminate interfering substances in the extract like thin layer chromatography (Pelletier, 1965), column chromatography (Pelletier, 1975) and high-pressure-liquid chromatography (Sood et al., 1976). Some authors have used automation procedures to improve the method, like Hiromi et al., (1970) who used a stopped-flow apparatus.

Another important limitation of this method is that dehydroascorbic acid is not included in the determination. Some authors have suggested reduction with H_2S (Tilmans et al., 1932) and homocysteine (Hughes, 1956), but some others have reported that the levels of dehydroascorbic acid in raw products (Roe and Oesterling, 1944) and canned foods is very low, (below 15%) (Burger et al., 1956). Egberg et al., (1973) mentioned that dehydroascorbic acid undergoes such facile destruction to biologically inactive substances that its nutritional value may be subject to doubt.

In spite of all its limitations, this method, due to its simplicity and reproducibility, is widely used in research laboratories and in quality control laboratories where large numbers of samples are analysed. The simplified scheme of the titration is shown in Figure 2.12.

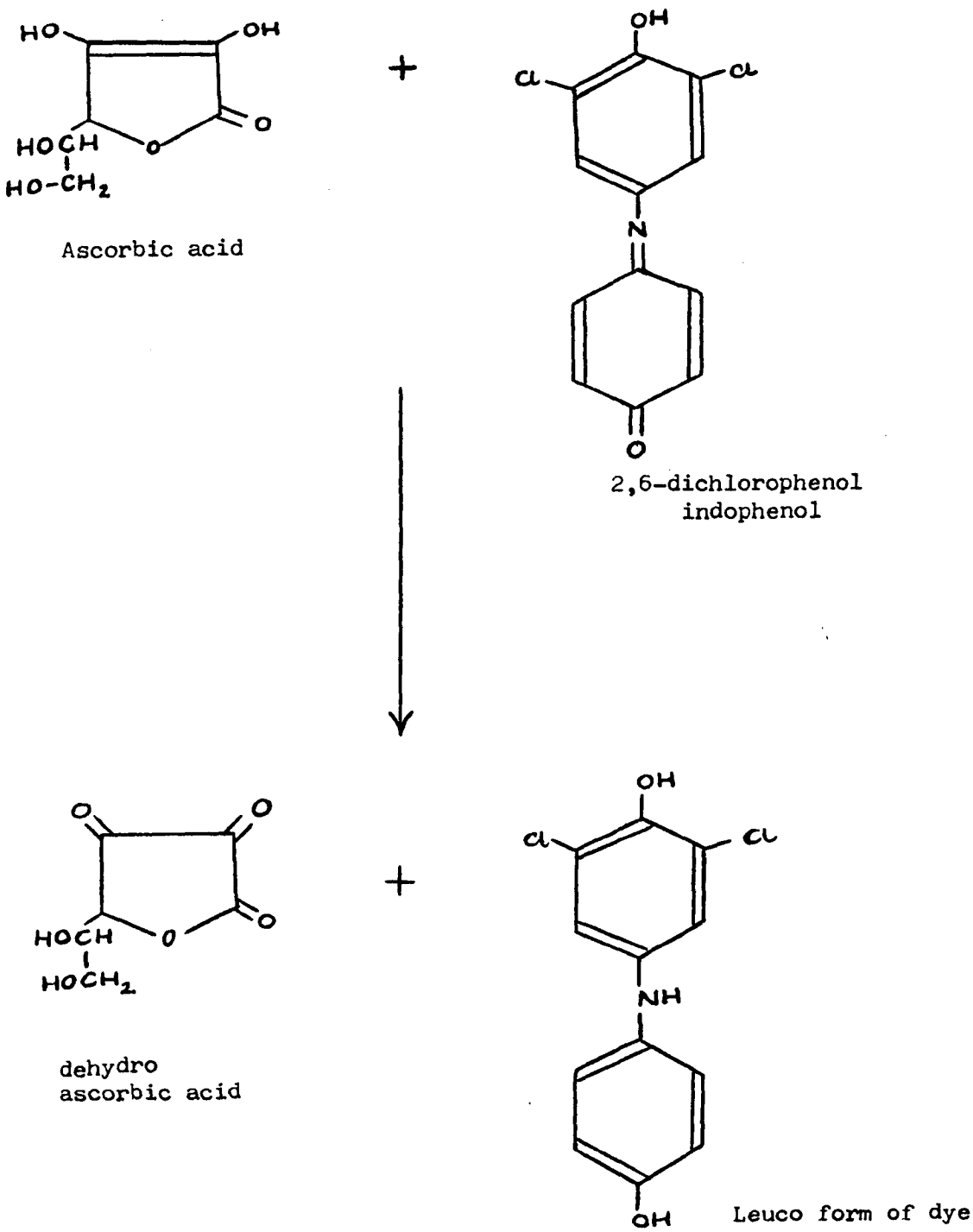


Figure 2.12 Simplified Scheme of 2,6-Dichlorophenolindophenol Visual Titration.

2.7.3 Fluorimetric Procedure

This method was proposed by Deutsch and Weeks in 1965, and later adopted as the official method of analysis by the A.O.A.C., in 1966. This method is based on the reaction of dehydroascorbic acid with O-phenylenediamine (OPDA) to form a fluorescent quinoxaline 3-(1,2-dehydroxyethyl) furo(3,4-b)quinoxaline-1-one as shown in Figure 2.13.

This method uses the formation of a boric acid - dehydro-ascorbic acid complex to prevent the development of the fluorescent dehydroascorbic acid quinoxaline to obtain a blank. This blank provides a means of differentiating between the fluorescence from the vitamin derivative and that from possible interfering substances.

In their work Deutch and Weeks (1965) tested a variety of substances that could interfere with the method. These substances were similar in structure to ascorbic acid. They found that pyruvic acid reacted to form a fluorescent compound but not to form a boric acid complex. Based on these findings, they concluded that the method was very specific for ascorbic acid determination and had the added advantage of including both the reduced and oxidised form of the vitamin.

Ascorbic acid was included in the analysis by mild oxidation with Norit, (acid washed activated carbon), to dehydroascorbic acid and determined as such (Deutch and Weeks, 1965).

The extraction solutions used were the same as for the 2,6 dichlorophenol indophenol (DCP) titrimetric method so the same considerations apply.

This method was later (Deutsch, 1967) tested in a collaborative study where variations in the concentration

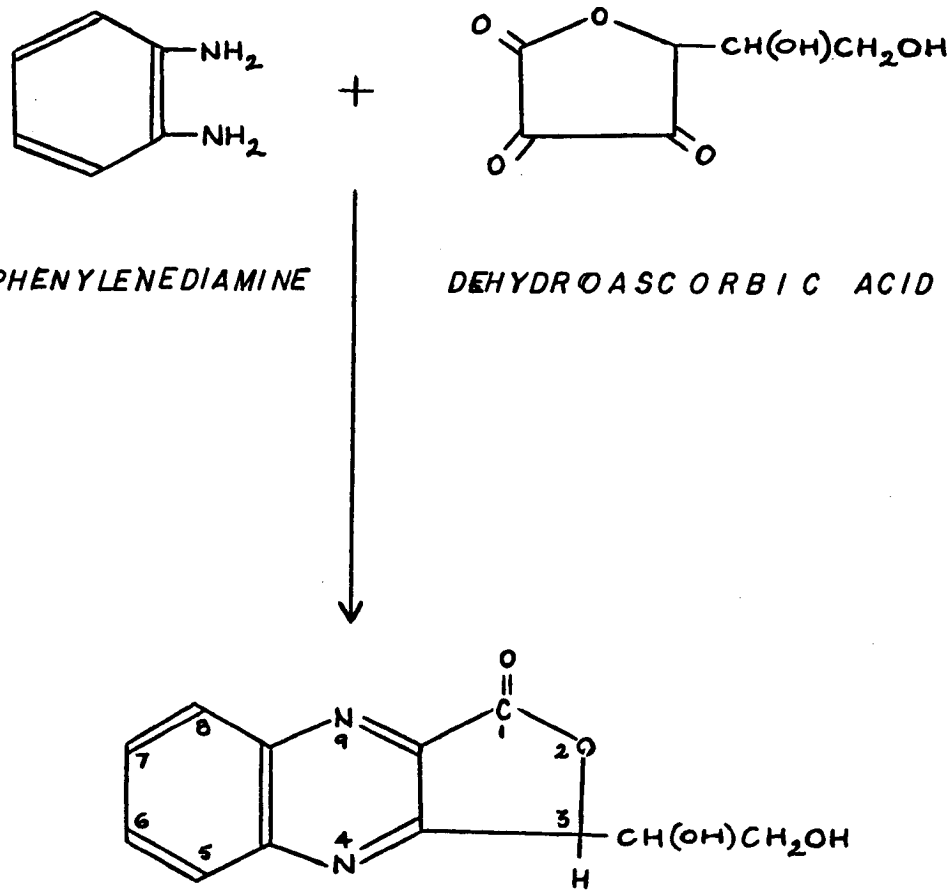


FIGURE 2.13

Simplified Scheme of O - Phebylene-diamine Vitamin C determination

of OPDA solution, extracting solution, boric acid solution, sodium acetate solutions; weights of norit, mixing times and exposure to light were tested. They found that in general the fluorimetric procedure was less susceptible to interference than the DCP visual titration method and the 2,4-dinitrophenyl hydrazine (DNPH) method. The results of the three methods were in very good agreement.

Various studies to compare different methods for ascorbic acid determination have been carried out. Deutsch (1967) reported that the reproducibilities were DCP-titrimetric method OPDA - fluorimetric method DNPH - method in a collaborative study. Dunmire et al., (1979) reported that recovery values of added vitamin C were 100.6% for both DCP-titrimetric procedure and OPDA-fluorimetric method.

Sood et al., (1976) used High pressure liquid chromatography to reduce interference of the method and Kamangar et al., (1977) included extraction with chloroform just before the fluorimetric measurement to reduce interference.

Several automated and semiautomated methods have been reported. Roy et al., (1976) reported an automated method using N-bromosuccinamide instead of norit for the oxidation of ascorbic acid. He claimed improved sensitivity and specificity. Egberg et al., (1977), automated the OPDA method using norit for the oxidation. Comparison of this method with DCP-titrimetric procedure and OPDA-fluorimetric procedure were in very close agreement.

Dunmiere et al., (1979) compared these automated methods (Roy, 1976 and Egberg, 1977) with the DCP-titrimetric method and the OPDA-fluorimetric method in various canned

and frozen foods. He reported good correlation between the four methods and after recovery tests he reported 97.8% for Egberg's, 99.3% for Roy's and 100.6% for the A.O.A.C. methods.

One disadvantage of the OPDA fluorimetric procedure was thought to be the inclusion in the analysis of diketo gulonic acid since this product is not of nutritional significance. However, Egberg et al., (1977) using the calcium salt of the diketogulonic acid found no fluorescence development. He concluded that 2,3 diketo-L-gulonic acid does not interfere with the OPDA method.

Recently Augustin, (1981) reported unrealistically high values of vitamin C determination by both OPDA-method and DCP-method in deep fat fried products. They developed a modified procedure for the high liquid pressure chromatography technique reported by Sood (1976). So further studies are needed on this particular subject.

Therefore, it can be concluded that both A.O.A.C. procedures are highly specific, accurate and reproducible when the necessary precautions are taken.

2.8 Vitamin B_1

Thiamine is usually determined by the thiochrome method which is the official A.O.A.C. (1975) method of analysis. There are also several microbiological and animal assays available, from these the microbiological techniques are preferred and comparison between the methods available have been reported by Balke (1977), Bell (1974), Voigt et al., (1978a and 1979). But the most widely used method for the determination of thiamine, particularly in foodstuffs, is the fluorimetric thiochrome method.

Thichrome fluorimetric procedure was proposed by Jansen in 1936, since then several improvementns and modifications have been reported. An excellent review on the developments until 1965 was presented by Freed. (1966)

The thiochrome procedure is based on the oxidation of thiamine to thiochrome which is determined fluorimetrically. The reaction is simplified in Figure 2.14.

2.8.1 Extraction

Since the vitamin is present in foods in several forms (Lamden, 1972; Hennessy and Cercedo, 1939; Ochoa and Peters, 1938) the extraction procedure must account for all of them. Acid extraction in a boiling water bath has been widely recommended for both free thiamine and bound thiamine extraction, but pyrophosphate thiochrome is insoluble in isobutanol (extraction media) so an enzymatic hydrolysis is carried out with phosphatase containing preparations, (Hennessy and Cercedo, 1939). Andrews and Nordgren (1941) recommended the determination of optimal time for digestion when working with new materials.

2.8.2 Purification

Hennessy (1941) first suggested the use of Decalso as a medium for the purification of thiamine. Freed suggested that although 1 μg of Decalso will absorb 10 μg of pure thiamine, 10 μg should be considered as the upper limit since other substances such as amino acids greatly reduce the capacity of the column.

However, several authors have claimed that thiamine

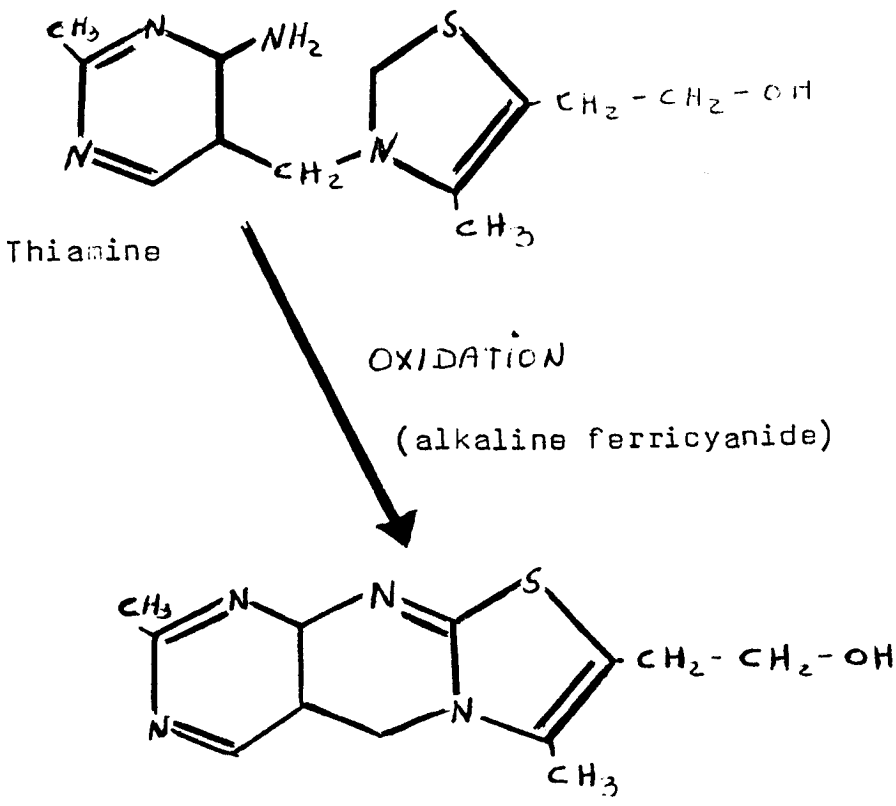


FIGURE 2.14 Simplified Scheme of Thiochrome Fluorimetric procedure

could be determined directly on the crude extracts (Glick, 1944; Rice and Beuk, 1945; Ministry of Food, 1942). Hennessy and Cercedo (1939) proposed a series of preliminary tests to decide if the purification procedure was necessary for the food material being subjected to analysis, such as the relation of blank fluorescence to total fluorescence detected.

2.8.3 Conversion to Thiochrome

Thiamine is oxidised in alkaline medium by ferricyanide and thus converted to thiochrome. When irradiated with light in the near ultraviolet region of the spectrum, thiochrome shows a strong blue-violet fluorescence, the intensity of which is proportional to thiochrome formed. This, in turn is proportional to the amount of thiamine from which it was originated. According to Freed (1966) some controversy in the optimal conditions reported for the conversion to thichrome exists in the literature, therefore he recommends standardised conditions for reproducible results. The reaction is shown in Figure 2.14.

2.8.4 Separation of Thiochrome

Thiochrome is highly soluble in isobutanol and increases its fluorescence in this medium. Hennessy (1942) recommended the use of constant volumes of isobutanol for the extraction and anhydrous Na_2SO_4 is used to eliminate residual water

2.8.5 Measurement of Thiochrome

Precautions against the excessive exposure of thiochrome to ultraviolet light and day light (Hennessy, 1942) should

be taken, since thiochrome is destroyed by light. So readings should be carried out as soon as possible.

Two types of blanks are used in the measurement of thiochrome, a blank carried through the procedure omitting only the ferricyanide is preferred by most authors. However, the rapid blank obtained by quenching of thiochrome fluorescence by strong acids may be useful for some cereal and urine determinations (Edwin et al., 1975). Consequently both types of blanks would be useful at the beginning of the thiamine assays for any batch of samples.

An internal standard is recommended for each set of measurements in combination with a standard curve in the range of concentration measured for a more reproducible determination of thiamine.

Several new improved semi-automated or automated methods have been reported in the literature (Ang and Mosley, 1980; Kirk, 1977; Kaman et al., 1980; Pelletier and Madere, 1975; and Toma and Tabeknia, 1979). All these methods are also based in the conversion to thiochrome by oxidation of thiamine. The levels of thiamine determination had been lowered from 0.2 µg/ml of the A.O.A.C. method to 0.02 µg/ml by some of these improved methods, (Dougherty and Cobb, 1971).

2.9 Available Lysine

As mentioned before the protein quality of foods depends not only on the content of amino acids but also their physiological availability. It has been mentioned that lysine is one of the most sensitive amino acids to damage during

processing and storage conditions, and that although not destroyed, it becomes unavailable for animal utilisation.

It has been demonstrated by Finot and Mauron (1967), and Mauron, (1970) that the Amadori compound of lysine (ξ -N-(deoxy-1-D-fructosyl)-L-lysine) has no physiological activity as a source of lysine for rats. Finot et al., (1977) reported that Schiff bases of ξ-aminolysine with aromatic aldehydes are available as sources of lysine. Both Schiff's base and Amadori compounds liberate lysine during acid hydrolysis. Therefore, a measurement of lysine content after acid hydrolysis will not be a useful indication of the amino acid nutritional availability. This point has been made repeatedly by numerous authors, (Knipfel, 1981). These reports prompted studies into ways of determining the physiologically availability of lysine in proteins. True physiological availability can only be measured by biological animal growth assays. Unfortunately, biological tests are time consuming, expensive and imprecise because of individual variations between organisms. Some other methods like digestibilities in vivo and in vitro and microbiological assay have also been found useful for the determination of available lysine, however, they are slow and sometimes expensive for routine analysis.

Several authors have developed chemical methods for the determination of available lysine. They are based on the capacity of lysine units with a free ξ-amino group to react with a chemical compound, and the assumption is that a lysine molecule with a free ξ-amino group is biologically available. This is not entirely true, since there have been

some reports (Hurrell et al., 1976) that glutamyl-lysine can be utilised as a source of lysine by rats, even when the ξ-amino group of lysine is bound to the glutamyl residue. Another factor not accounted for by the chemical methods is the loss in digestibility due to hinderance of the enzyme activity, or due to the anti-nutritional effect of some compounds present in the food, such as premelanoidins (Adrian, 1974). Nevertheless, if chemical methods can be correlated with biological assays to measure the significance of the results they are valuable tools in quality control of large batches of samples, because of their simplicity. Chemical methods therefore measure the "reactive lysine" to a specific chemical and not the true nutritional availability. In the present work available lysine will be used when referring to the true physiological availability of lysine.

A variety of chemical methods have been developed for available lysine measurement. These methods have been extensively reviewed by several authors (Anderson, 1980; Carpenter, 1973; Cheftel, 1979; Hurrell and Carpenter, 1981; Mauron, 1981; and Morrison and McLaughlan, 1972). One of the most widely used methods for the measurement of available lysine is reaction with Fluoro 2,4-dinitrobenzene (FDNB) and its several modifications. This method will be briefly discussed below.

2.9.1 Fluoro 2,4-dinitrobenzene (FDNB) methods

The use of FDNB for available lysine determination was first reported by Carpenter and Ellinger in 1955, and employs Sanger's reagent (Sanger, 1945). It is based on the reaction of FDNB with free amino groups in proteins to

form (DNP) amino acids, as shown in Figure 2.15.

After hydrolysis, the DNP-amino acids are released and measured spectrophotometrically. There are several modifications to the method, the two more important are the FDNB direct method and the FDNB indirect method.

(a) FDNB Direct Method

This method was developed by Carpenter (1960). He reported that the procedure was useful for the estimation of available lysine in a wide range of proteins.

The stability of the DNP-amino acids to acid hydrolysis after 16 hours in HCl at 105° C was reported by Porter and Sanger (1947). They found that ξ-DNP-lysine was one of the most stable amino acids. This was later confirmed by Mathenson, (1968). After hydrolysis, the procedure involved extraction with ether. Carpenter (1960) reported that most of the amino acids were removed by the ether extraction but that in addition to ξ-DNP-lysine, the aqueous residue may contain also, O-DNP tyrosine, (im-DNP) histidine, α-DNP arginine, δ-DNP-ornithine and ξ-DNP-hydroxylysine. Also the procedure does not measure terminal lysine, since it becomes α, ξ-DNP-lysine, which is ether soluble. O-DNP-tyrosine and (im-DNP)-histidine were not coloured but absorbed at 360 nm, so by measuring the absorbance at 435 nm their interference could be eliminated. Unfortunately, as reported by the same author, vegetable materials produced coloured interfering compounds during the assay procedure.

A modification using methoxycarbonyl chloride (MCC) which reacts with ξ-DNP-lysine to form an ether soluble derivative

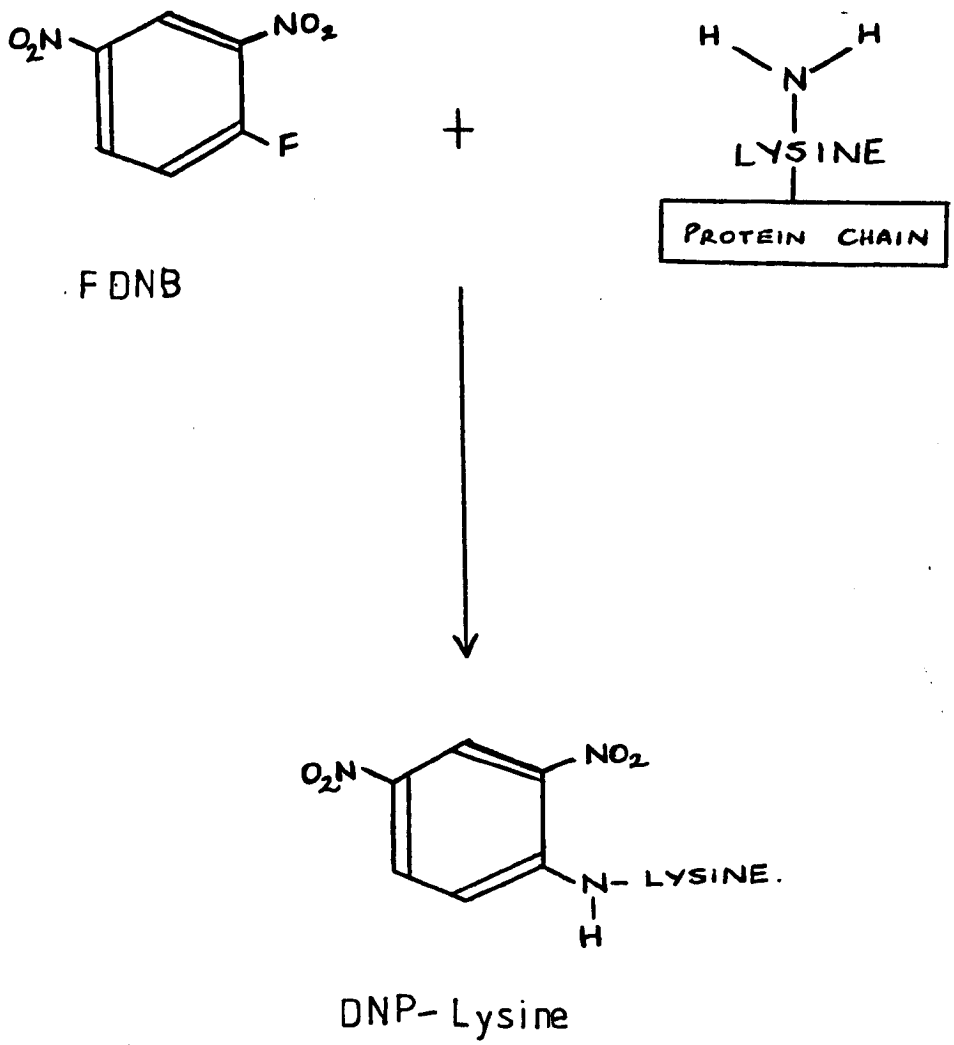


Figure 2.15 Simplified Scheme of 1-Fluro-2,4-Dinitrobenzene Available Lysine Determination

without changing colour intensity was proposed by Bruno and Carpenter (1959). This procedure eliminated interference from α-DNP-arginine, which is measured by the blank. However, Carpenter et al., (1959) reported that (im-DNP)-histidine reacted with MCC to form a coloured product. This coloured product increased the blank reading and consequently reduced the lysine value. However, as the product of the histidine and MCC reaction is ether soluble the problem was overcome by measuring the absorbance of the MCC-treated hydrolysate after ether extraction and untreated hydrolysis after extraction. By difference the ξ-DNP-lysine was obtained.

After this modification, the procedure still measures in addition to ξ-DNP-lysine, ξ-DNP-hydroxylysine and δ-DNP-ornithine. However, for this study both are of negligible importance since hydroxylysine is only present in appreciable concentrations in animal connective tissues and ornithine is not present in naturally occurring proteins although it is known to be formed during alkaline treatment of foods.

Other sources of interference are the formation of soluble coloured humin and charred materials during hydrolysis of vegetable materials, which absorb the DNP-lysine. Booth (1971) suggested several modifications to the procedure, such as hot filtration of the hydrolysate, followed by thorough washings. He also suggested second digestions for vegetable materials to improve ξ-DNP-lysine recovery.

Handwerek et al., (1966); Blom et al., (1967); and Fraenkel and Conrat, (1944), reported that DNP-amino acids were destroyed during hydrolysis in the presence of reducing agents, carbohydrates and sugars. Structural carbohydrates (Booth, 1971) and starch (Mathenson, 1968b and El-Nockrashy,

1965) were reported to be more damaging than sugars.

Blom et al., (1967) and Ruderus and Kihlberg, (1970), reported that the presence of dinitrophenol protected the DNP-amino acids during hydrolysis when carbohydrates were present. Dinitrophenol is one of the decomposition products of FDNB during acid hydrolysis and as reported by Handwerek et al., (1966) the unreacted excess of FDNB was in enough quantity for the protection of DNP-amino acids during the acid hydrolysis.

Several attempts to overcome the error caused by the damaging presence of carbohydrates during hydrolysis have been reported. Lyman and Thomas (1965), claimed that the addition of thiodiglycollic acid reduced the destruction of ξ-DNP-lysine due to carbohydrates. They suggested that this compound reacted with the carbohydrate decomposition products in competition with lysine. El-Nockrashy (1965) suggested that, as starch was not inactivated by contact with FDNB, an enzymic hydrolysis of starch before the protein reaction with FDNB, would eliminate the damaging effect of the carbonyl groups on the nitro groups of both FDNB and DNP-amino acids.

Some other authors proposed purification procedures before the spectrophotometric measurment of ξ -DNP-lysine such as paper chromatographic separation (Baliga et al., 1959) and high performance liquid chromatography (Peterson and Warthesen, 1979). A novel procedure was described by Selim (1965) who proposed the quantitative conversion of amino acids to their copper salts before FDNB treatment. He claimed that only lysine gives a coloured DNP-derivative.

However, the modifications introduced by Booth (1971) are the most widely used. He improved the ε-DNP-lysine recovery by using longer and more complete DNP-protein hydrolysis, by using hot filtration followed by thorough washing of the hydrolysates and by the use of recovery factors. The recovery factors were obtained by the addition of ε-DNP-lysine standard solution before hydrolysis. Some of the recovery factors recommended by Booth (1971) are 1.05 for carbohydrate free samples, 1.09 for soluble albumins, 1.14 for cereals. However, other workers (Blom et al., 1967 and Ruderus and Kihlberg, 1970) reported that pure ε-DNP-lysine was more sensitive to damage during hydrolysis than ε-DNP-lysine in a protein. Ruderus and Kihlberg (1970) used casein to determine the recovery factors and reported more reproducible results.

(b) FDNB Indirect Procedure

Roach et al., (1967) proposed the "difference method" or "indirect procedure" as an attempt to avoid the problem of incomplete recovery of -DNP-lysine due to carbohydrates in the FDNB-direct determination (this method is also known as the Silcock available lysine test).

The determination of available lysine was obtained as the difference between the total lysine liberated by acid hydrolysis and the lysine liberated after hydrolysis of the DNP-protein, thus including the terminal lysine. Although the method gives very good results with carbohydrate free materials, (Blom et al., 1967 and Couch, 1975), when it is applied to materials damaged by initial stage Maillard

reactions, this method is reported to overestimate nutritionally available lysine (Hurrell and Carpenter, 1974). However reasonable agreement with the direct method has been obtained (Mauron, 1981). They also reported that for intermediate and advance Maillard damage both methods were equally sensitive.

After a collaborative study on the performance of FDNB indirect procedure, Cough (1975) concluded that the FDNB indirect method was satisfactory for the determination of available lysine in foods and feeds. He recommended the adoption of the method as official first action. The recommendation was later approved. (A.O.A.C., 1975).

The FDNB direct method and the FDNB indirect methods are probably the most widely used for food studies. There are, however, several other procedures.

2.9.2 Other Methods

(a) 2,4,6 trinitrobenzene-1-sulphonic acid method

This method was introduced by Kakade and Liener (1969) as an alternative to the FDNB methods. It uses the reagent (2,4,6 trinitrobenzene-1-sulphonic acid or TNBS) used by Okuyana and Satake (1960a and 1960b) for the determination of primary amino groups. They studied the properties of the 2,4,6-trinitro-phenylated (TNP) amino acids and their spectrophotometric determination.

Habeeb (1966) describes the use of TNBS for the determination of free amino groups in serum albumin. Freedman and Radda (1968) studied the kinetics of reactin between the ξ and α amino groups with the TNBS.

Their method is very rapid when compared to the existing methods for available lysine determination since it does not require long acid hydrolysis. Ecklund (1976) reported that only 20 min. of digestion time at 110° C were needed in his proposed improved TNBS method. However, according to Hurrell and Carpenter (1974) TNP-lysine is more sensitive than DNP-lysine to destruction during hydrolysis in the presence of carbohydrate. The conditions of TNP-protein hydrolysis were also investigated by Tella and Ashton (1978), who reported that the optimum time and temperature for the reaction were 37 °C for 2 hours and also 2 hours for the optimum hydrolysis time. Methods to improve the assay in the presence of carbohydrate were reported by Hall et al., (1975 and 1979), who suggested the reaction with hypochlorite to "bleach" the interfering colour formed due to charring of carbohydrates. However, Hurrell and Carpenter (1974) and Holguin and Nakai (1980) reported poor reproducibility with the TNBS method and Carpenter, 1973; Hurrell and Carpenter (1974, 1981) and Mauron (1981) reported poor sensitivity to early Maillard damage. Nevertheless, several workers have been trying to improve the accuracy and overall performance of the method because it offers a simpler and more rapid method of available lysine determination than the accepted methods.

(b) α-Methyisourea method

This method is based on the reaction of O-methylisourea (MIU) with the free amino groups to form homoarginine, (Bujard and Mauron, 1964) which can be measured by ion-exchange

chromatography or by gas chromatography (Nair et al., 1978). According to Mauron (1981), Hurrell and Carpenter (1974), it is very specific and sensitive to all types of Maillard damage. The main drawback of this method is that it is very time consuming.

(c) Borohydrate method

This reaction was first proposed by Thomas (1970). The borohydrate stabilises the sugar-amino acid bond so that under acid hydrolysis conditions, only available lysine is liberated for later measurement after chromatographic separation. This method, according to Hurrell and Carpenter (1974) does not appear to be affected by the presence of carbohydrates.

2.9.2 Dye binding methods

Dye binding procedures have been used successfully for the determination of protein content of foods by several authors. The dyes used include bromophenol blue by Florea (1978); amido black, by Ashworth and Chaundry (1962) and Kroger and Weaver (1979); acid orange G by Bunyan (1959), Ashworth (1966, et al., 1960) and Udy (1956a, 1956b); and acid orange 12 by Ashworth (1971) and Udy (1971). All these authors claimed good agreement between dye bound and percentage protein present in samples. Some of the early work on the binding reaction between dyes and proteins were studied by these authors. Bunyan and Price (1960) reported that a correlation between acid orange G absorption and N.P.U. existed in the same way as with the available

lysine value determined by FDNB direct procedure. These findings were later corroborated by the findings of Mossberg (1965) and Moran et al., (1963). They reported that samples heated at different times had a dye binding capacity (DBC) that correlated with the growth of chicks fed by the meals.

Fraenkel-Conrat and Cooper (1944) described the use of acid orange G for the determination of basic groups in proteins. They suggested that the dye binding capacity (DBC) of a protein was dependent on an electrovalent attraction between negatively charged basic imidazol, guanidine and amino groups present in acid solution; probably generated from the lysine, histidine and arginine and terminal groups of proteins. A simplified diagram is presented in Figure 2.16 of the mechanism of binding.

Mossberg (1970) described a procedure for the determination of basic amino acids with acid orange-12 (AO-12) as a measure of protein quality. Hurrell and Carpenter (1976, 1975) based on this method, proposed a method for the determination of available lysine. This method is based on the propionylation of the free ξ-amino groups of lysine thus making them unavailable for AO-12 binding. The difference between the DBC measured after treatment with propionic anhydride and the DBC of the untreated sample gives the value of DBC-reactive lysine, an estimate of the available lysine present in foods.

Extensive studies on the conditions of optimum binding of proteins with acid orange G (Gullord, 1974) and acid orange 12 (Walker, 1979b) have been reported. From these studies it was concluded that time of shaking of the dye for the binding reaction and amount of sample/volume of dye used needs to be established before the method is applied

to a particular foodstuff.

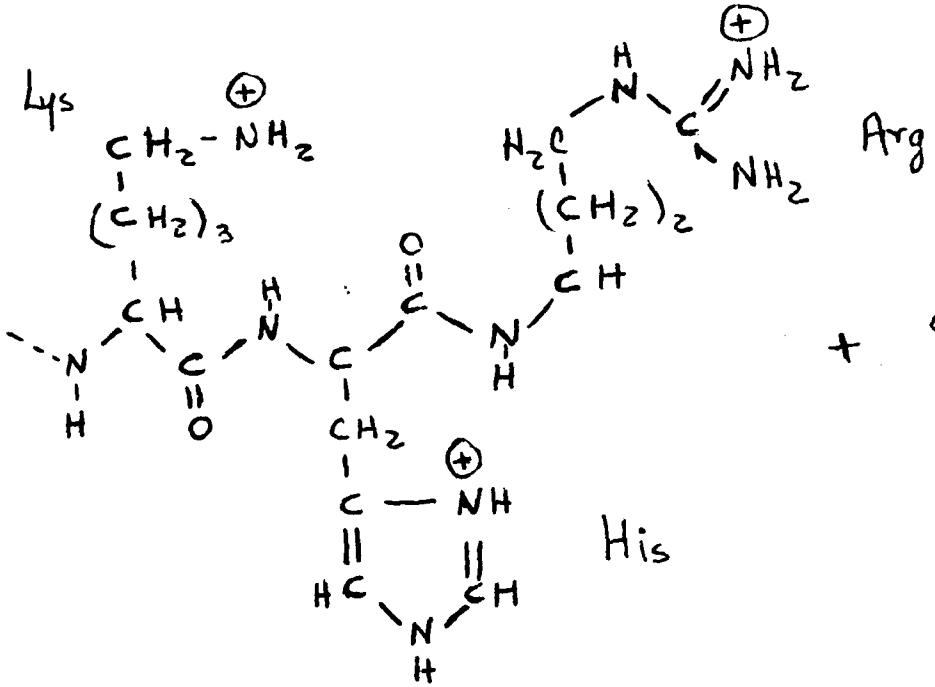
Hurrell et al., (1979) compared the FDNB direct method and the AO-12 dye binding procedure with several proteins including undamaged early Maillard and advance Maillard damage. They reported close agreement for all samples. However, for the samples with early Maillard damage, lysine was reduced by an increase in the HAR-value, contrary to expectations. They suggested as an explanation that AO-12 binds with the deoxyketosyl compound, which is positively charged, thus increasing the HAR-value. They proposed a scheme showing four basic groups (Histidine, Arginine, Lysine and deoxyketosyl compound) binding AO-12. When propionylation occurred three basic groups (Histidine, Arginine and deoxyketosyl compound) were left to react with the dye. So the available lysine value was nevertheless obtained, showing good agreement with the FDNB-available lysine.

Further support for the method came from Almas and Bender (1980) who reported that the DBC method when applied to legumes agreed extremely well with the FDNB direct method. Also Walker (1979), reported good correlation between the two assay procedures when using leaf-protein.

Hurrell and Carpenter (1974) measured available lysine using different methods in a comparative study. They selected early Maillard damaged and advance Maillard damaged albumin. The methods compared with FDNB direct and indirect procedures, MIU, TNBS and borohydrate methods. They reported that to detect early Maillard damage MIU, borohydrate and FDNB direct methods were most sensitive, with TNBS methods grossly overestimating available lysine. For advanced Maillard

damage, all methods were adequate and the same was true for protein-protein damage with the exception of the borohydrate procedure which largely overestimated available lysine.

Mauron (1980) reported a comparative study, including AO-12, borohydrate, MIU and FDNB direct methods. He reported that for early Maillard damage, all methods were adequate, with close agreement between them. Therefore, it can be concluded that the selection of the analytical method depends on the type of damage present in the sample and the level of accuracy required.



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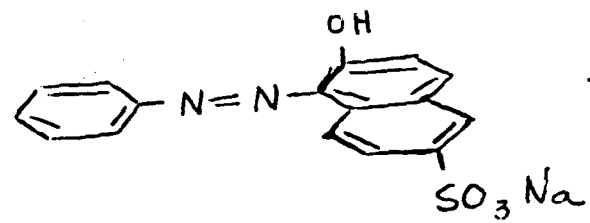
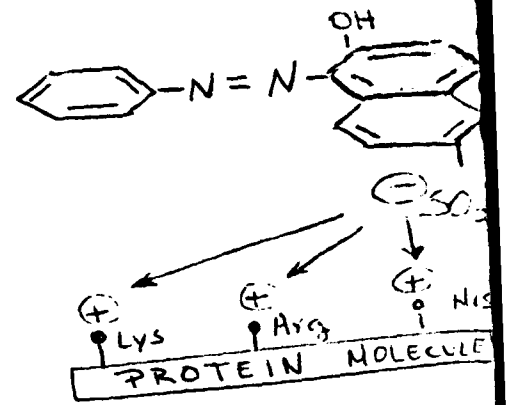


AO-12

HARL-value.



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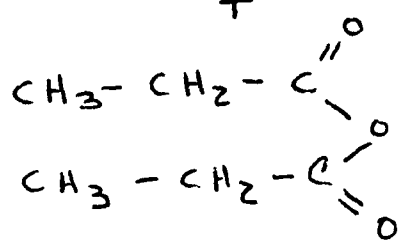

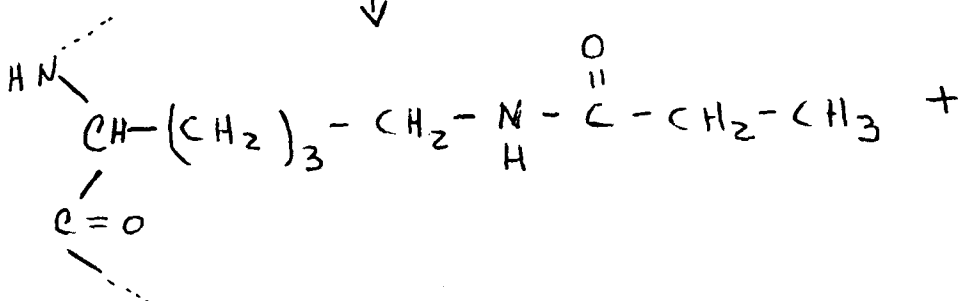

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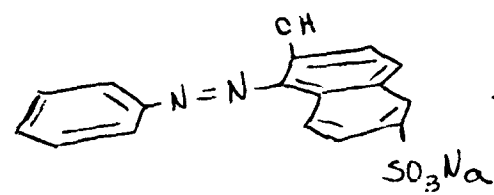
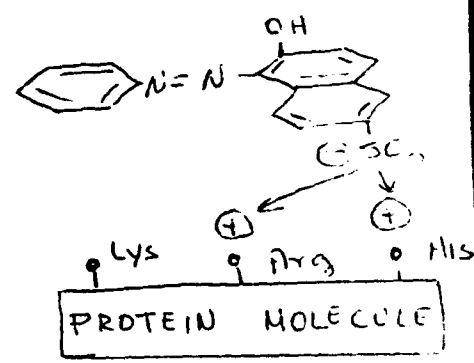


HAR-value



CHAPTER 3

EQUIPMENT FOR MONITORING AND CONTROL OF THE STERILIZATION PROCESS

Before the food processing could take place it was necessary to implement several systems. These included temperature measurement, process monitoring and process control. The implementation and assembly procedures of those systems are described in this chapter together with the characteristics and specifications of the equipment used for food processing.

3.1 Process Equipment

3.1.1 Containers Used

Four types of containers were used for packaging. A-1 cans with their equivalent in pouch - "A-1 pouches", and A-10 cans with their equivalent in pouch - "A-10 pouches". All packaging materials were purchased from Metal Box Ltd. Table 3.1 gives specifications and inner dimensions for the containers used, which are shown in Fig. 3.1.

3.1.2 Sealing Machines

Both size of pouches were sealed using a pouch vacuum heat sealer. A-1 cans were vacuum sealed in a MB1 vacuum closing unit and A-10 cans were sealed with a MB10 closing unit, whose features are described below.

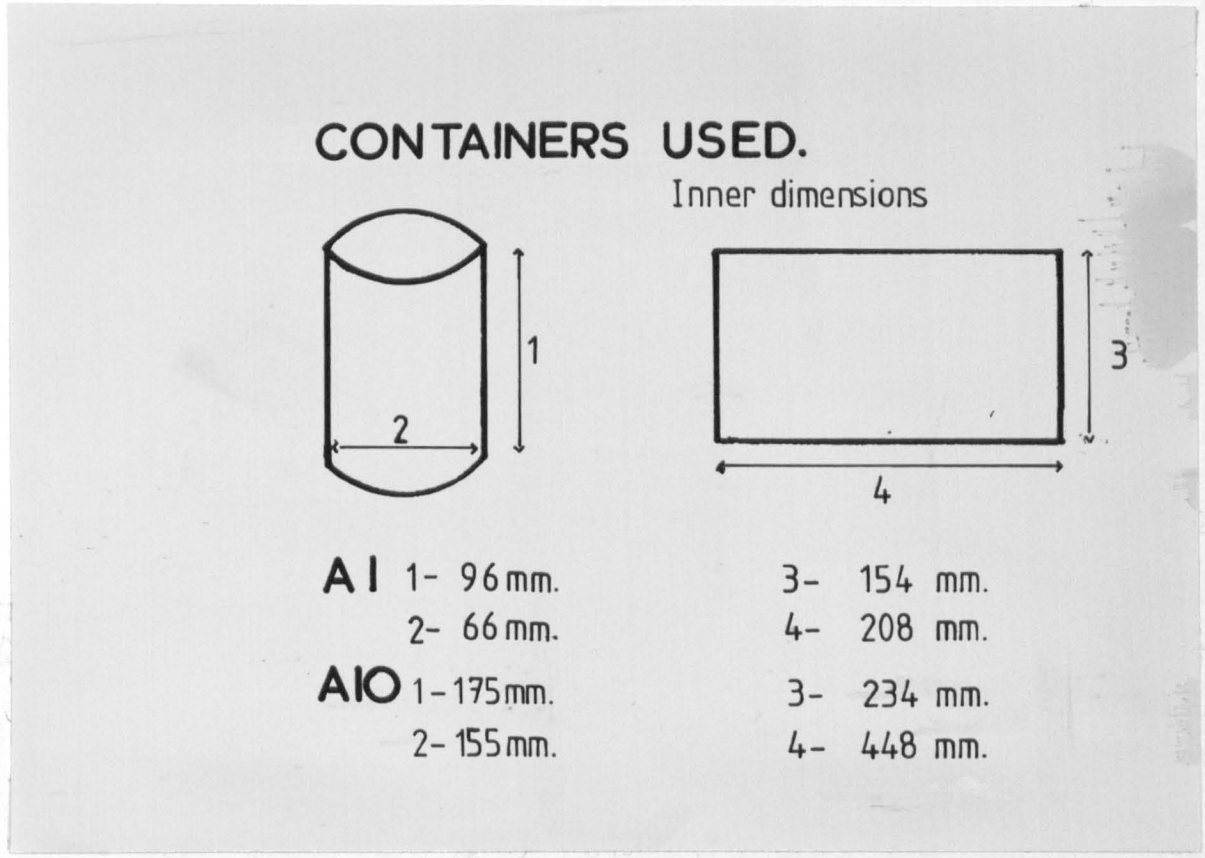


Figure 3.1. Containers used - inner dimensions and relative proportions.

Package	Specifications	Inner Dimensions in Millimetres
A-1 can	Steel, tin plated wall thickness of 0.22 mm	96 mm x 66 mm
A-10 can	Steel, meat lacquered wall thickness of 0.26 mm	175 mm x 155 mm
A-1 pouch	Laminates: Polyester Aluminium foil H.D. 37 c. film wall thickness of 70 microns	208 mm x 154 mm
A-10 pouch	As for A-1 pouch	448 mm x 234 mm

Table 3.1 Containers used for packaging

a. The pouch vacuum heat sealer was a Multivac vacuum closing unit type A69, which consisted of a vacuum pump, vacuum chamber with an electrical impulse bar for sealing and controls for vacuum and electrical impulse intensity. The unit was set to maximum vacuum power and six seconds impulse for all cases.

b. MBI closing unit for A-1 cans consisted of a vacuum pump and a vacuum chamber with a double seam unit. The equipment was set at working pressure of 137 KPa below atmospheric pressure.

c. The MB10 closing unit for A-10 cans consisted only of a double seam unit. Consequently, exhausting was carried out by heating the can contents up to 90°C in a boiling water bath immediately before seaming.

3.1.3 Retorts Used

Three types of retorts were used for processing, a static canning retort, a rotating canning retort and a pouch water retort. The main characteristics of these are described briefly below.

a. The static canning retort, manufactured for John Fraser and Sons Ltd., was a Millwall vertical retort model of 50.8 cm diameter and 61 cm of nominal internal depth, suitable for a working pressure of up to 344.7 KPa. The cover was activated by a pneumatic cylinder and secured by swing bolts and bow nuts. The main body contains two wire baskets with capacity for 108 A-1 cans. The retort is fitted with two brass cases for thermometers calibrated from 40° C to 150° C, and a packaging gland on the side wall for the introduction of thermocouples. Steam, compressed air, water, vent and drain valves with automatic and manual controls are fitted as shown in Fig. 3.2.

b. The rotating canning retort was also a Millwall model rotary sterilizer with variable speed. It was also manufactured by John Fraser and Sons Ltd. This machine has a capacity of 25 A-1 cans and a working pressure limit of 241.3 KPa. Cold water, steam, compressed air, vent and drain valves were operated manually. Fig. 3.3 shows a general view of

Figure 3.2. Static canning retort. (1) Steam input, (2) compression gland for thermocouple insertion, (3) compressed air line, (4) cold water line, (5) vent and drain.

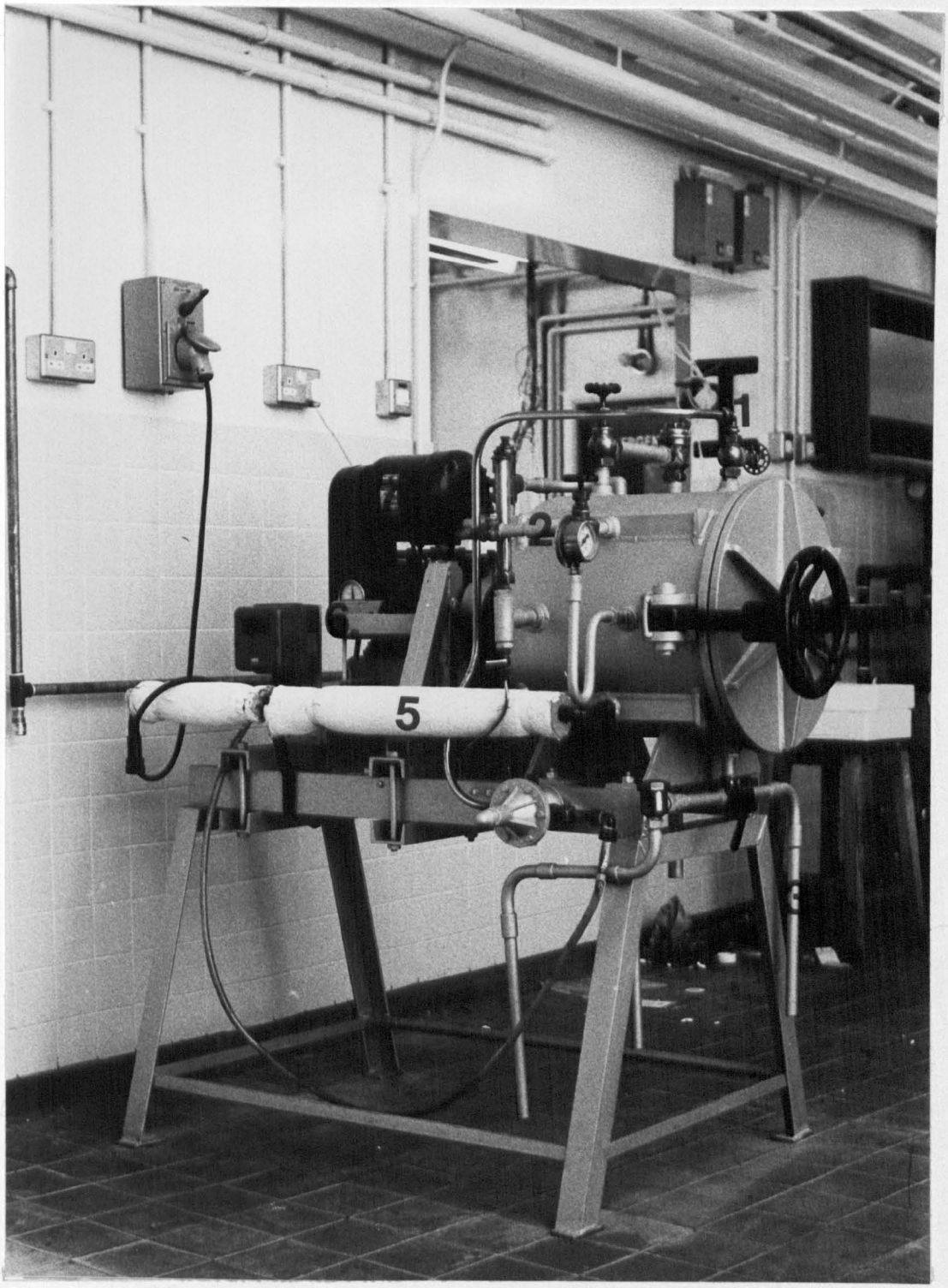


Figure 3.3. Rotating canning retort. (1) Compressed air imput, (2) cold water line, (3) drain, (5) steam line, (6) slip ring contact for Ellab thermocouple, (6) rotation motor and (7) cold water input.

this retort.

c. The pouch water retort was a Millwall laboratory retort, designed to sterilize flexible retortable pouches. It consists of a cylindrical water tank and a cubic processing tank. The cylindrical container acts as storage and preheating tank for the water used during process. It is located above the processing tank, to which it is connected through a manually controlled drain system. The processing tank is connected to steam and compressed air lines through pneumatic automatic valves used to control the process, and with manually controlled by-pass valves. Drain and vent systems are also fitted with manual and automatic controlled valves. Two pumps are installed. One assists in pumping the cold water from the mains to the processing and heating tanks, while the second pump circulates the water during heating and cooling cycles in the processing tank. The working pressure limit is 3.8 bars and the nominal capacity is for 30 A-1 pouches. Fig. 3.4 shows a general view of this retort.

3.2 Thermocouples and Monitoring Devices

It was required that all temperature measurements should be carried out using the same thermocouple-potentiometer-recorder system to reduce variability during process monitoring between

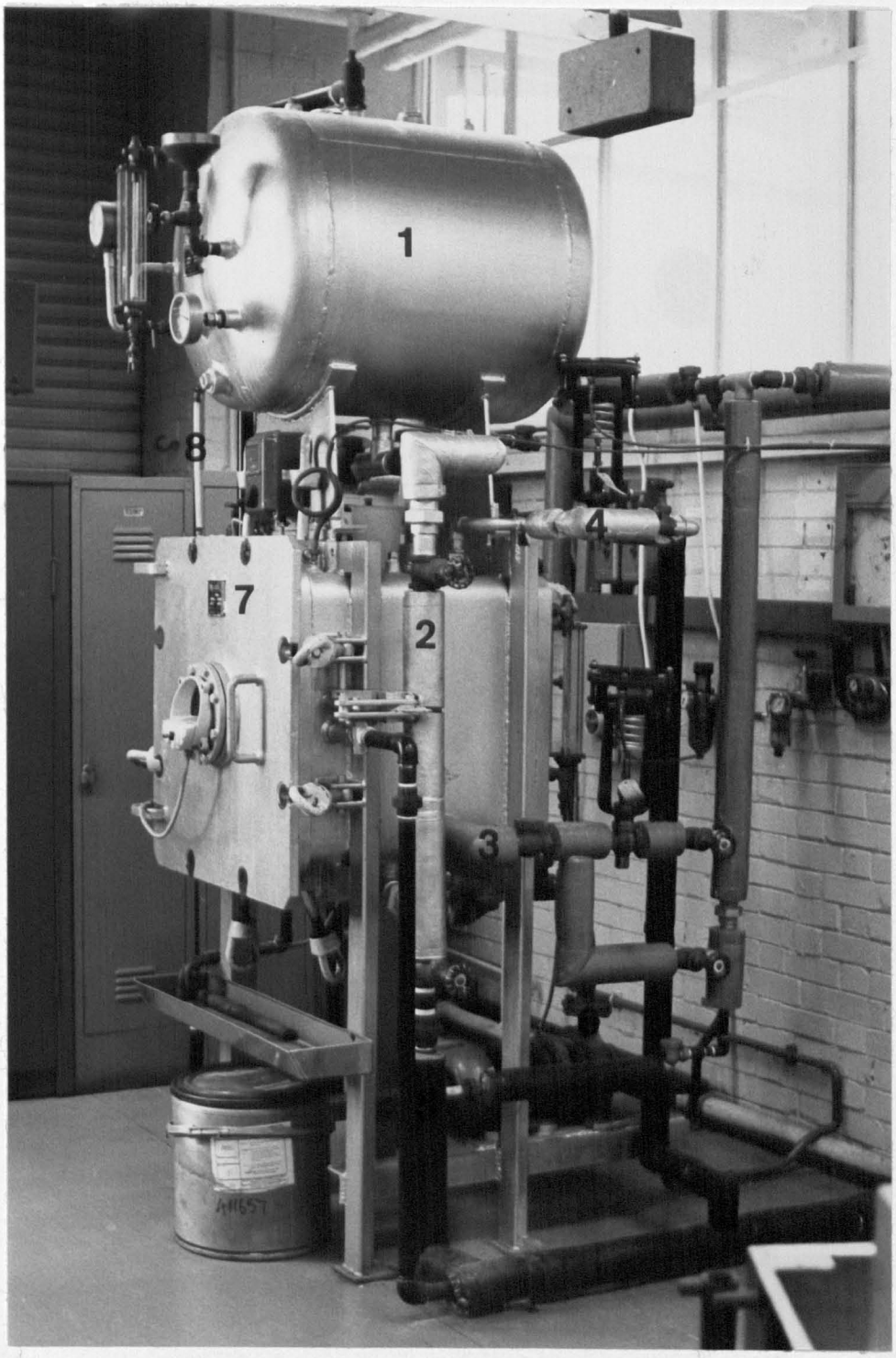


Figure 3.4. Pouch water retort. (1) Hot water tank, (2) hot water input, (3) steam line, (4) vent, (5) drain, (6) cold water line and pump, (7) processing tank and (8) compressed air line.

types of package. Consequently, the traditional C-1 moulded Ecklund canning thermocouples were unsatisfactory and an alternative was sought.

In the static canning retort and for the pouch water retort all temperature measurements were carried out using P.T.F.E. insulated copper-constantan, twisted wire thermocouples of 0.315 mm diameter. These thermocouples were passed through the container walls via specially designed brass glands. Fig. 3.5 shows a diagramatic representation of the arrangement. The thermocouple tips were placed in the centre of the container with the help of nylon supporting rigs. This same basic system was used for both cans and pouches.

For the can processed in the rotating retort a different system was used. It consisted of a commercial Ellab copper-constantan thermocouple, type DC 19 fitted with a slipring contact for use in rotating autoclaves.

The thermocouples were connected to a chart recorder, except the process control thermocouple (see section 3.1.6). The recorder used was a multipoint modular Philips chart recorder, model PM 8235, calibrated to be used with the copper-constantan thermocouples.

3.3 Pouch Thermocouple Assembly

In the preparation of the monitoring device assembly each thermocouple circuit was checked using an Avo-meter before it was placed in the brass gland. The thermocouple was then passed through the brass gland via a perforation in the middle

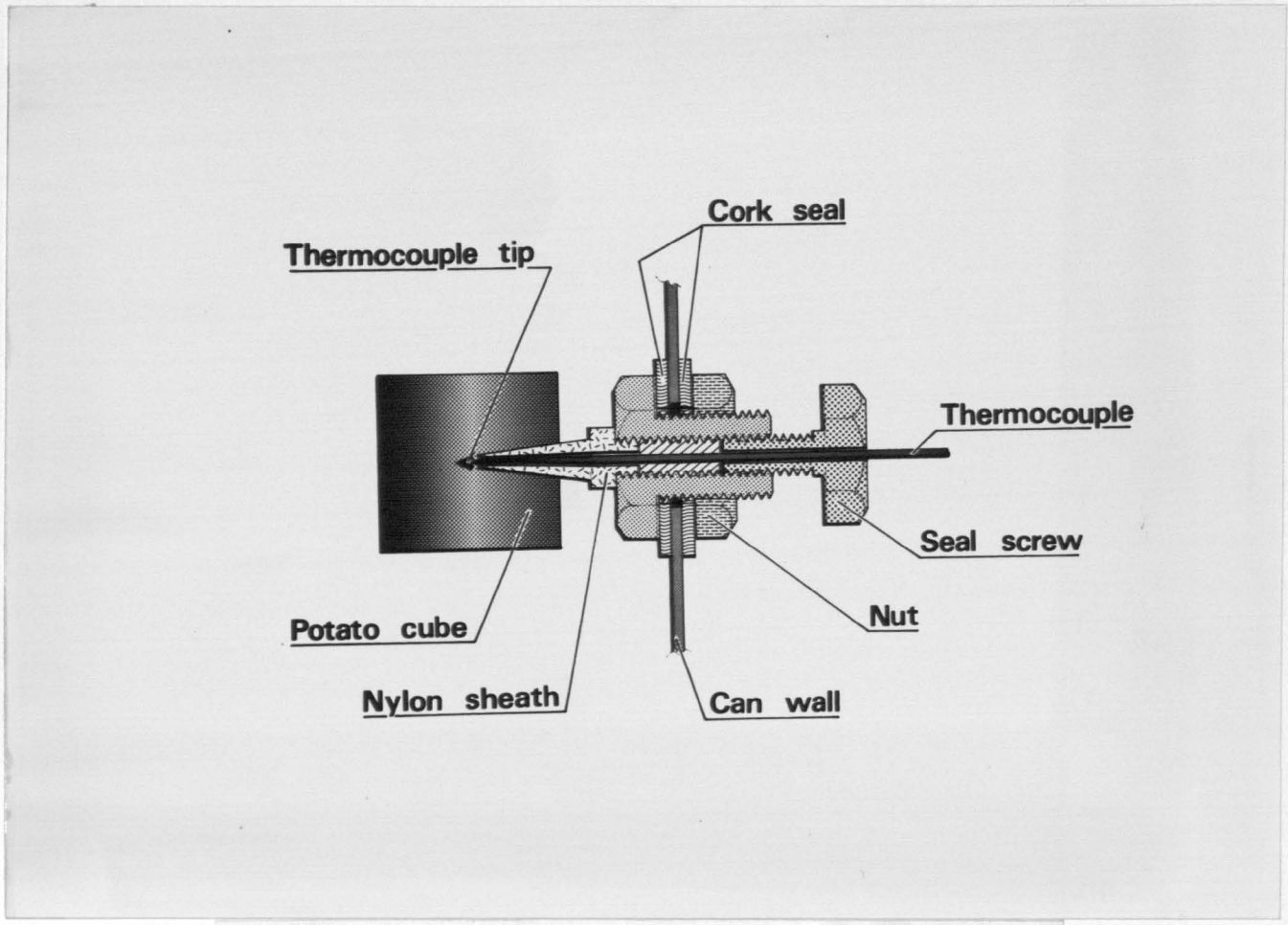


Figure 3.5. Details of the Brass Gland used for thermocouple insertion.

of the main gland body, using PVC tape and a perforated screw for sealing. For a perfect seal, a liquid silicone rubber product stable at processing temperatures (Silastic 9161 RTV supplied by Dow Corning, Seneffe, Belgium) was mixed with 4% (w/w) catalyst and immediately applied to seal the thermocouple into the glands. This was found to be essential to avoid any leakage during or after processing as air leaking into the package seriously affected the heat transfer rate. Before the use of this product was adopted a large number of failures due to leakage were obtained. Once prepared, the thermocouple assembly was left to set overnight.

To mount the thermocouple assembly to the package itself, a hole was punched in the pouch wall at one of the top corners, in order to reduce any interference with the heat transfer in the centre of the pouch. The brass gland with the thermocouple assembly was then passed through the pouch wall and securely fitted using cork washers on both sides of the pouch wall. This arrangement was found to be satisfactory to avoid air leakages.

Inside the package, the thermocouple was secured to the nylon supporting rig with PVC tape. Contact between the food and the thermocouple tip was achieved in one of two ways, depending on the nature of the food. For products containing large particles, (potatoes, brussels sprouts and pork casserole) the thermocouple tip was inserted into the centre of an appropriate food particle (see section 4.3) which was secured to the nylon rig with cotton thread. For the fourth product processed, rice pudding, the thermocouple tip was located at the

geometrical centre of the container via the nylon supporting rig (Fig. 3.6). The thermocouple fixed to the nylon rig was carefully placed in the centre of the pouch.

The theromocouple leads were fitted with Comark connectors which were small enough to pass through the compression gland at the rear of the retort, thus avoiding connections inside the retort.

It was considered important to have all the thermocouple connections outside the retort because the temperature, pressure and circulation of the hot water developed during the process could cause fluctuations in the temperature readings or loss of the connection. These problems were observed in early experiments with the pouch water retort and with the static retort using Ecklund thermocouples.

3.4 Can Thermocouple Assembly for the Static Retort

The brass gland carrying the thermocouple was fitted to the can wall using two cork washers, via a hole in the centre of the can wall. The thermocouple was passed through a nylon supporting rig, which was pressure inserted in the brass gland. The position of the thermocouple was adjusted leaving only the tip visible, protruding from the end of the nylon supporting rig, as shown in Fig. 3.7.

The rubber silicone product was applied to the outside of the can to seal both the thermocouple into the brass gland and the gland to the can wall. Then, the assembly was left to set overnight.

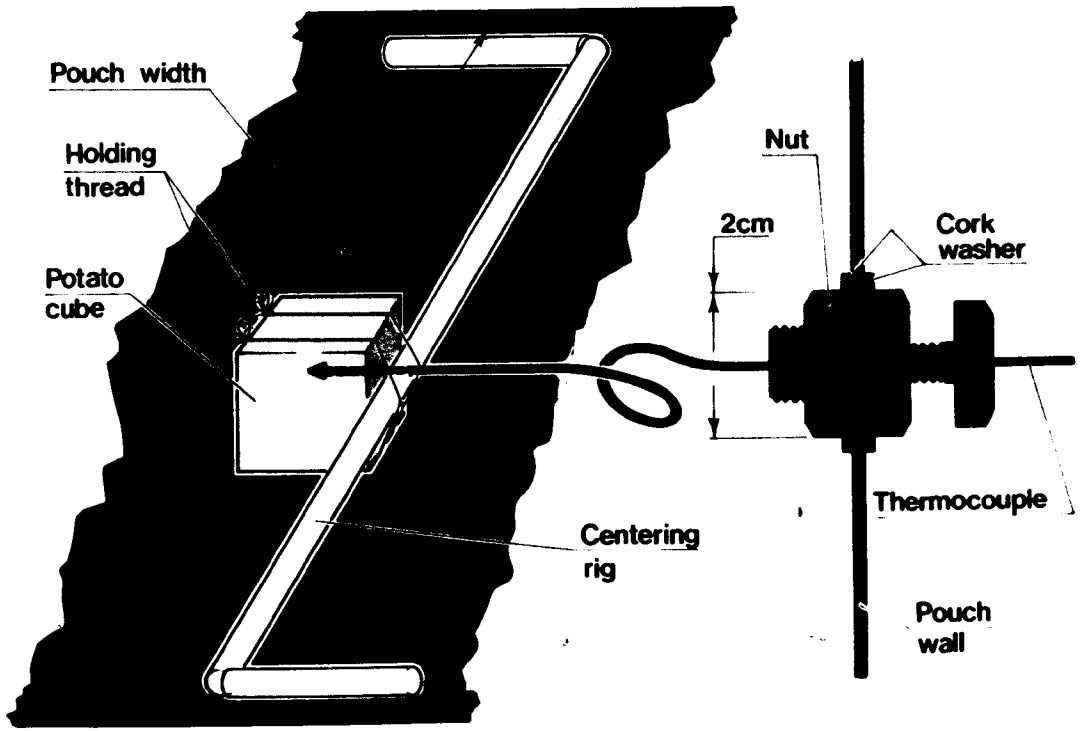


Figure 3.6. Pouch thermocouple assembly.

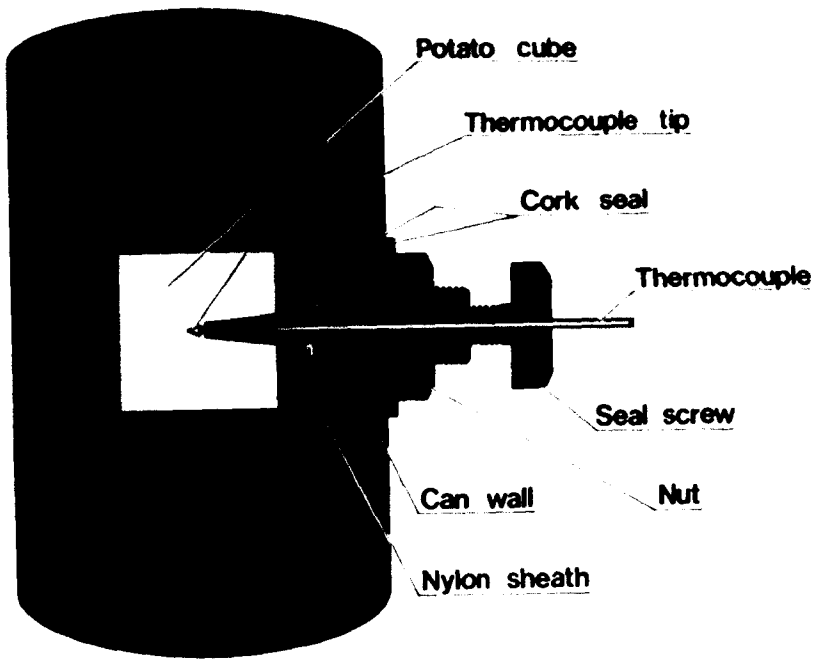


Figure 3.7. Can thermocouple assembly for the static retort.

Contact between the food and the thermocouple tip was achieved as for the pouch thermocouple assembly, but the nylon supporting rig was inserted in the piece of food instead of using cotton thread to hold it in position. The cans were then carefully filled with the rest of the food product and seamed.

The thermocouple connections were located outside the retort as described in section 3.1.3.

3.5 Can Thermocouple Assembly for the Rotating Retort

The commercial Ellab copper-constantan DC 19 thermocouple system used was designed to be used in rotating autoclaves.

A hole was perforated in the centre of the can wall to screw in the packaging gland using a rubber washer. The thermocouple nylon case whose length was half of the can diameter, was in turn screwed into the packaging gland. The thermocouple itself was screwed to its working position in the seamed can and connected, through a compression gland at the rear of the retort, to a slipring contact.

With this system, only one thermocouple was used at a time, thus causing obvious limitations in process monitoring of rice pudding (the only product which required the rotating retort).

3.6 Process Control Equipment

The equipment used for process control (Fig. 3.8) was based on a system developed by O'Sullivan and Novais (Novais,

Figure 3.8. Process control equipment.

1981).

A Commodore 32K PET model 2001 computer was interfaced to one thermocouple using a Comark (type 5335) digital thermometer as an analogue to digital converter. The computer was programmed to use the thermocouple readings to calculate the F_o - value achieved at several time intervals, and to predict the remaining process time required. This enabled the operator to be prepared to initiate the cooling cycle. Thus, with a minimum amount of preliminary work, the target F_o - value could be achieved, with a considerable degree of precision and reproducibility (section 2.1).

The computer was equipped with a tractor-printer which enabled a print out giving temperature, time and F_o - value to be obtained.

CHAPTER 4

PRELIMINARY STUDIES

This chapter deals with all the preliminary work carried out, which was essential to lay the foundations for the central research work. Before food sample production and evaluation could take place, it was necessary to assess the monitoring and process control systems implemented, to set up and evaluate the analytical techniques used, and to develop adequate food formulations for the food sample production.

4.1 Thermocouple Evaluation

Accurate measurement is essential during the sterilization of foods in hermetically sealed containers since precise knowledge of time-temperature cycles is necessary for the sterilization process evaluation, (Baselt and Ball, 1939).

In measuring the temperature of any object or material, by the repeated temperature measurements, it is possible to obtain the reproducibility or precision of the measurement. But this does not show the relationship between the temperature value measured and the true temperature. This relationship is the accuracy of the measurement. No matter how precisely a temperature measurement is made, there are always basic minimum deviations in any temperature determination. If the measurement is made very carefully and there are no gross experimental errors, the deviation of the temperature measurement

will not be greater than a quantity called "the uncertainty of the measurement", which is known to depend upon several factors, including range of temperature measured, type of measuring instrument and the state of its calbiration (Gray and Finch, 1973).

In the present work, a significant number of the temperature measurements were carried out using copper-constantan thermocouples and the location of the hot junction was always the geometrical centre of the container. The thermocouples were used following a standard procedure (described in section 3.2-3.6), so that experimental variation was minimised. Consequently the main cause of deviation in the temperature measurement was considered to be the "uncertainty" of the measurement inherent in the measuring instruments.

The temperature range and types of measuring instrument used in this work have been described in Chapter 3. The accuracies claimed by the manufacturers are; for the Philips chart recorder ±0.35% of the temperature measured; ± 0.2% in the temperature reading for the digital thermometer; for the twisted wire copper-constantan thermocouples ±0.25% of the temperature reading and 1°C for the Ellab DC9 thermocouple.

(i) Experimental Procedure

The accuracy of the value recorded by the thermocouples was investigated utilising the thermal arrests at the freezing point of urea and the melting point of ice. These calibration experiments were carried out in two stages:

(a) Using the Philips chart recorder described in section 3.2 as a monitoring device, eleven thermocouples and a mercury in glass thermometer were tied together and immersed in a boiling tube containing Analar grade urea crystals. The tube was heated in an oil bath up to 150°C, after which the melted urea was left to freeze. The temperature readings of the eleven thermocouples were then recorded and the mercury in glass thermometer readings taken.

A similar procedure was followed for pure water. In this case a 2 litre glass beaker was filled with crushed ice, and the eleven thermocouples and the mercury thermometer were immersed in the melting ice. The thermocouple readings were recorded and the thermometer readings taken.

(b) Using the twelve channel digital thermometer device used for process control (section 3.6), eleven thermocouples were connected to the digital thermometer device. The thermocouples were bound together with a mercury in glass thermometer and immersed in urea or ice, as described above. The temperature readings were recorded at various times by changing the channel selector.

All data obtained were then compared against the reported freezing point of urea, which is between 130°C and 132°C (depending on purity), and the melting point of ice, which is 0.01°C in standard condition.

(ii) Results

When the Philips chart recorder was employed, the sensitivity of the instrument was such that it did not detect differences between the thermocouple readings, as shown in Figure 4.1.

When the digital thermometer was used, small variations between the thermocouples readings were detected. This was because the instrument sensitivity was higher. These temperature values ranged between 130.28°C to 131.36°C for the freezing point of urea and -0.26°C to 0.350°C for the melting point of ice. This is clearly shown in Table 4.1. This table gives the mean value of 10 readings for each thermocouple.

Thermocouple No.	READINGS IN DEGREES CENTIGRADE	
	Urea Freezing Point	Water Melting Point
1	130.92	-0.10
2	130.55	0.02
3	130.98	-0.18
4	130.93	-0.36
5	130.82	0.02
6	130.58	0.06
7	130.28	0.13
8	131.36	0.35
9	130.53	0.20
10	130.76	0.03
11	130.96	0.08
Thermometer	130.50	0.00

Table 4.1 Mean values of the temperature readings obtained in in the calibration of the thermocouples used

4.2 Comparison of Manual and Computerized Process Evaluation

In the evaluation of heat treatments applied to the samples throughout the present work, the parameter used was the F_o - value (section 2.1.2).

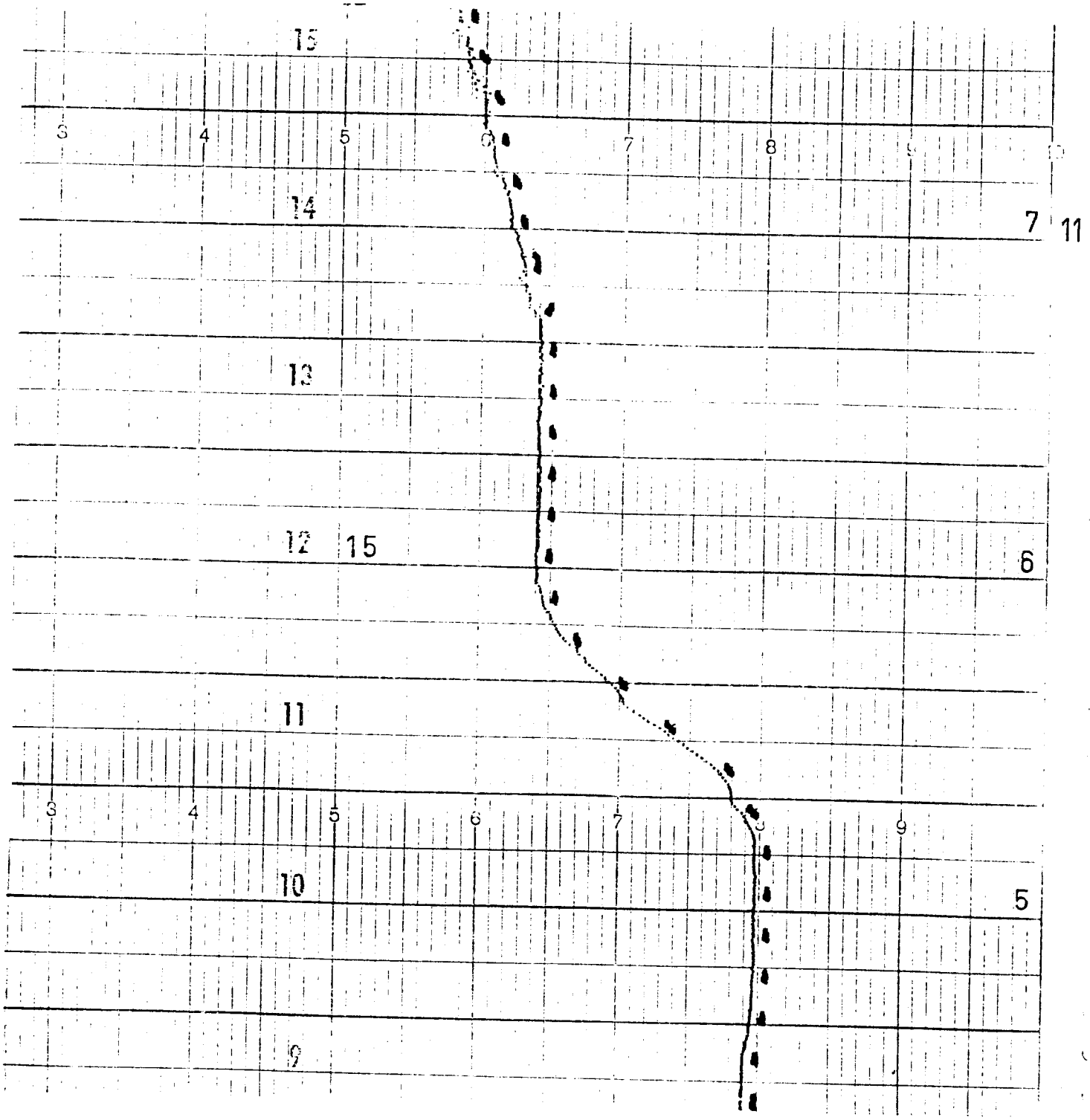


FIGURE 4.1. Philips chart recorder temperature scan in Urea Thermal Arrests

(i) Experimental Procedure

Since the process monitoring method utilized both a time-temperature file in the computer (for only one thermocouple) and chart recorder plots (for up to twelve thermocouples), it was necessary to establish whether the results of data processed from the two types of records were significantly different. This was achieved using two methods.

Method (a). For the time-temperature relationship stored in the computer, the lethal rates were obtained from tables (National Canners Association, 1968 and Stumbo, 1965) for each temperature reading. Using Simpson's numerical approach, the lethal rate time curve was integrated manually. The integrated value obtained was then compared with the real time computer evaluation (described in section 3.1.6).

Method (b). From the chart recorder plots the temperature-time relationships were read and the F_o -values calculated, both manually and by the simulated time option in the computer program. Both (a) and (b) were carried out with a range of food types requiring different F_o - levels for sterilisation.

(ii) Results and Discussion

Table 4.2 gives the results obtained using the real time computer evaluation and the manual calculation for time intervals of one and 0.5 minutes from the temperature-time relationships of the computer print out. These results

Sample	F_o - values, minutes		
	Computerized Procedure	Manual Procedure	
	Real Time	1 Minute Intervals	0.5 Minute Intervals
BC-1	22.83	22.46	22.57
BC-2	22.48	22.16	22.20
BC-3	20.57	20.11	20.14
BC-4	20.96	20.57	20.68
$\bar{X}_{BC}$	21.71	21.33	21.40
PC-1	10.34	10.10	10.15
PC-2	9.88	9.65	9.70
PC-3	10.30	10.07	10.11
$\bar{X}_{PC}$	10.32	10.08	10.13
RP-1	6.72	6.56	5.59
RP-2	6.32	6.18	6.19
RP-3	5.92	5.78	5.80
$\bar{X}_{RP}$	6.60	6.19	5.80
RS-1	4.70	4.59	4.68
RS-2	4.74	4.74	4.79
$\bar{X}_{RS}$	4.72	4.67	4.74
BS-1	3.34	3.29	3.30
BS-2	3.07	3.00	3.02
$\bar{X}_{BS}$	3.21	3.15	3.16

Table 4.2 F_o - values obtained from the real time computer evaluation and manual calculations. Temperature time relationship of the computer print-out

show that most of the F_o - values obtained using the manual calculation with one minute time intervals are slightly lower than their corresponding F_o - values obtained from the on-line computerized procedure. The mean percentage deviation was 2.10. Also, it can be seen that this difference is reduced when a smaller time interval - 0.5 minutes - was used for the manual calculation. The mean percentage deviation obtained for this case was 1.58.

Analysis of variance was applied to data from Table 4.2 grouped by food type and with similar sterilization treatments. Results are given in Table 4.3, where it can be seen that no significant differences were found between the F_o - values obtained with the two methods of calculation. This was so both for 95.0% and 99.0% confidence levels.

Table 4.4 shows the results obtained from the chart recorder plots and using both the manual and the simulated time computerized calculations. Both calculation methods used temperature readings taken at one minute time intervals. The standard deviation obtained for the difference between the manual and computerized calculations is 0.31%. The maximum difference found when calculating the F_o - values with both procedures was of 1.719%, obtained with sample BS-2.

The results of the analysis of variance applied to the data from Table 4.4 are given in Table 4.5. For this statistical analysis the results were also grouped according to their type of food and sterilization treatment. The results of the analysis of variance for the F_o - values show that no

Source of Variation	Degrees of Freedom	Sum of Squares	Mean of Square	Mean Square Ratio		
				Calculated	Tabular	Confidence Level
BC samples (Beef Casserole) -						
Between methods of calculation	2	0.331	0.165	0.13	3.63	95.0%
Residual	9	11.876	1.32	-	6.42	99.0%
Total	11	12.206				
PC samples (Pork Casserole) -						
Between methods of calculation	2	0.09	0.046	0.74	5.14	95.0%
Residual	6	0.37	0.063	-	10.90	99.0%
Total	8	0.047				
RP samples (Rice Pudding) -						
Between methods of calculation	2	0.189	0.095	0.69	5.14	95.0%
Residual	6	0.818	0.136	-	10.90	99.0%
Total	8	1.007				
RS samples (Rice Pudding) -						
Between methods of calculation	2	0.005	0.002	0.42	5.41	95.0%
Residual	3	0.017	0.006	-	12.10	99.0%
Total	5	0.022				
BS samples (Brussels Sprouts) -						
Between methods of calculation	2	0.004	0.002	0.015	5.41	95.0%
Residual	3	0.117	0.039	-	12.10	99.0%
Total	5	0.122				

Table 4.3 Analysis of variance of data of Table 4.2

Sample	F_o - values, minutes		
	Computerized Method One Minute Intervals	Manual Procedure One Minute Intervals	Manual Result Expressed as % of Computerized Method
BC -1	23.13	22.96	99.26
BC -2	22.94	22.82	99.47
BC -7	22.57	22.61	100.17
IBC-8	21.54	21.63	100.41
IBC-5	21.18	20.88	98.58
IBC-4	21.11	21.41	101.42
BC -10	16.64	16.56	99.51
BC -11	17.04	17.11	100.41
PC -1	10.88	10.93	100.46
PC -2	10.96	10.87	99.13
PC -3	10.93	11.03	100.91
RP -1	6.78	6.83	100.73
RP -2	6.51	6.58	101.07
IRP-3	6.11	6.06	99.18
IRP-4	6.09	6.16	101.14
IRP-5	5.98	6.03	100.83
IRP-6	5.02	4.96	98.80
RS -1	4.85	4.91	101.23
BS -1	3.83	3.77	98.43
BS -2	3.49	3.43	98.28
$\bar{X}$			100.640
			0.30

Table 4.4 **F_o - values obtained from chart recorder plots. Data processed both manually and by computer**

significant differences were found between the two methods of calculation.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean of Square	Mean Square Ratio		
				Calculated	Tabular	Confidence Level
BC samples (Beef Casserole) -						
Between calculations	1	0.01	0.01	0.19	7.71	95.0%
Residual	4	0.224	0.056	-	21.2	99.0%
Total	5	0.235				
IBC samples (Beef Casserole) -						
Between calculations	1	0.199	0.199	3.62	7.71	95.0%
Residual	4	0.219	0.055		21.2	99.0%
Total	5	0.418				
PC samples (Pork Casserole) -						
Between calculations	1	0.001	0.001	0.12	7.71	95.0%
Residual	4	0.017	0.004		21.2	99.0%
Total	5	0.018				
RP samples (Rice Pudding) -						
Between calculations	1	0.004	0.004	0.11	1 85	95.0%
Residual	2	0.068	0.034		98.5	99.0%
Total	3	0.071				
IRP samples (Rice Pudding) -						
Between calculations	1	0.001	0.001	0.17	7.71	95.0%
Residual	4	0.19	0.0055		21.2	99.0%
Total	5	0.02				

Table 4.5 Analysis of variance for data from Table 4.4

As the F_o - values obtained using both calculation techniques - computerized and manual - were found to be not significantly different, the computerized calculation was used for all subsequent work.

4.3 Cold Point Determination in the Pouch Water Retort

It is generally agreed that for heat penetration measurements during thermal sterilization processes, the thermocouples used should be inserted in the slowest heating position of the containers. Also, these containers should be placed in the coldest area in the retort. (Bigelow, 1920, Brennan and Butters, 1979; Charm, 1971, Desrosier, 1970; National Canners Association, 1976).

For canning retorts the literature is extensive and the slowest heating points are known to depend on the operational procedure, particularly venting and can loading in the retorts (section 2.1.2). For the present work, the canning retorts were adequately vented and operated. Also, the process was carried out using only steam as heating medium, so no cold air zones were expected in the retorts. Consequently, the coldest area was suspected to be in the centre of the can load distribution. Following that criterion, the centre of the can load distribution was the place selected for temperature monitoring, process control and heat penetration studies. This was so for both the static and the rotating canning retorts.

For pouch water retorts, only limited information on thermal behaviour was available. Therefore, heat distribution tests were carried out to determine the cold spot.

(i) Experimental Procedure

The experimental procedure involved the selection of a slow heating food and the use of thermocouples in every possible pouch position. Potatoes were used for several reasons, namely: low cost; large standard size pieces could be obtained; they could be kept in good conditions in cold storage for a reasonable period of time and they can be considered a reasonably homogeneous material. A 2.54 cm per side cubic piece of potato was selected to conduct the heat penetration studies. Each thermocouple assembly was prepared as described in sections 3.3 and 3.4. The package was filled with a cubic piece of potato and 300 cm^3 of simulated gravy. The simulated gravy was prepared using 325 grams of H.P.C. modified starch, 150 grams of white flour, 10 grams of colour and 6675 cm^3 of water.

The number of thermocouples that could be monitored at one time was limited to twelve by the multipoint chart recorder. An extra thermocouple, placed near the thermometer pocket, was used for process control and was connected to the computer. Consequently, thirteen thermocouples were used for every run, twelve in pouches and one in the heating medium. The heating medium temperature was compared against a mercury in glass thermometer reading.

The 30 A-1 pouches were divided between five horizontally positioned wire shelves, each one holding six pouches./ (Figure 4.2) Twelve runs were carried out, producing four readings for each one of the thirty possible pouch positions in the retort. It was observed that the load of the retort affected some variables such as the come up time of the

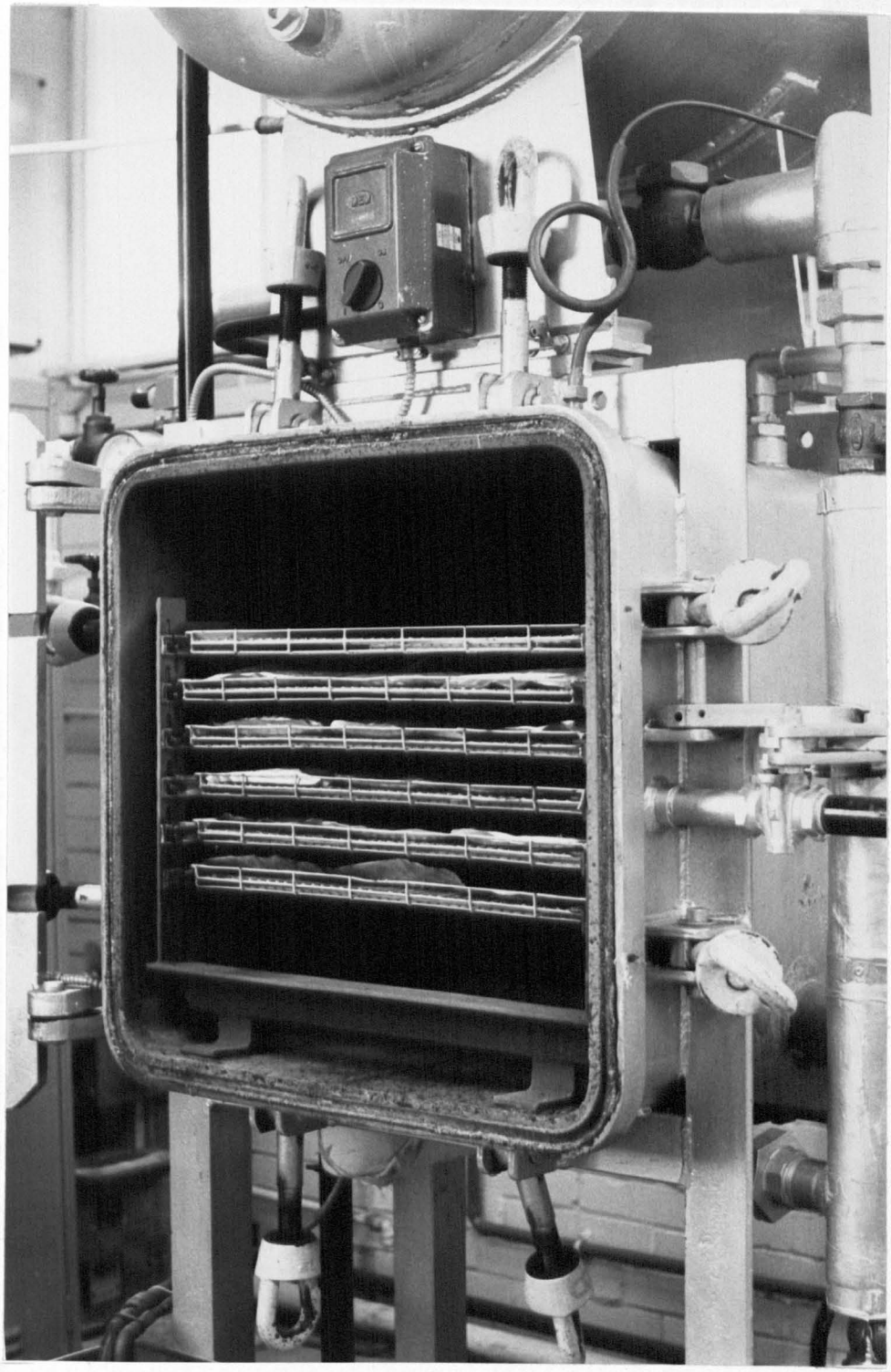


Figure 4.2. Processing tank of the pouch water retort showing the pouch restraining shelves.

retort, heat transfer rate of the packages, etc. Therefore, to avoid these variations during the studies, dummy pouches were filled with water and used to produce a full load of the retort each time. The pouch used for process control was always in the same position inside the retort.

Results and Discussion

The four temperature-time curves obtained for each of the thirty pouch positions were plotted on semilogarithmic paper and the f_h-value was evaluated for each curve. Examples of the semilogarithmic heat penetration curves found are shown in Figure 4.3 (68% of curves), Figure 4.4 (16.8% of curves) and Figure 4.5 (14.2% of curves). While the apparent break in linearity in Figures 4.4 and 4.5 could reflect a change in the heat transfer characteristics, it was equally likely to have been due to instrumental inaccuracies, as at higher temperatures, f_h-values become very sensitive to small variations. Therefore for the analysis of the results, the f_h-values obtained from the initial linear sections were used. These values are reported on Table 4.6 and were used to detect variation in the heating rate due to pouch position.

Variation between retort runs was evident from the wide range of f_h-values obtained for each position. For statistical analysis, this source of variation was reduced by adjusting the f_h-value means using regression analysis and applying analysis of variance to the modified model (non-orthogonal design). The adjusted means obtained and the analysis of variance results are given in Table 4.7 which

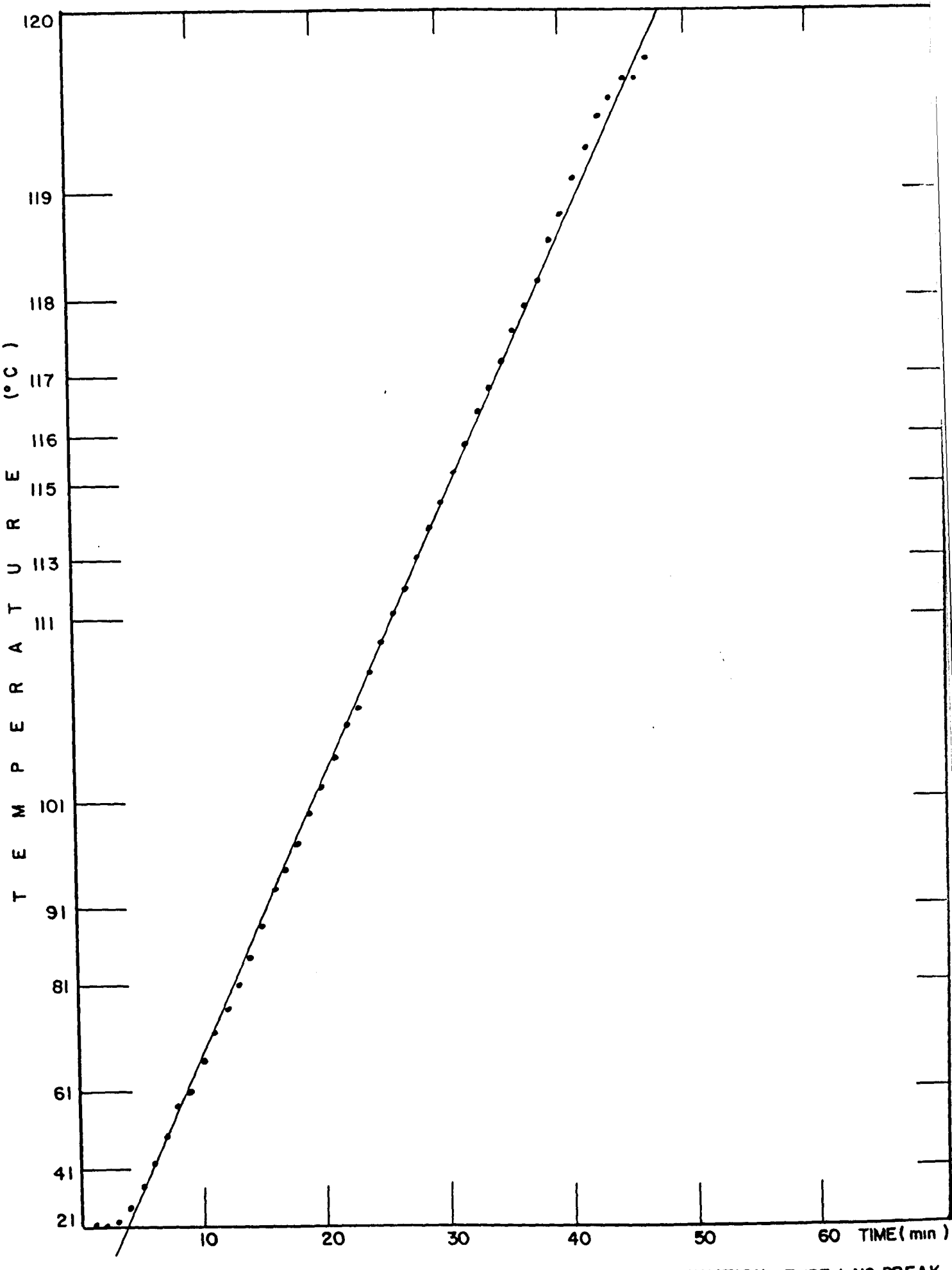


FIG. 4.3 HEAT PENETRATION CURVE . COLD POINT DETERMINATION. TYPE I NO BREAK

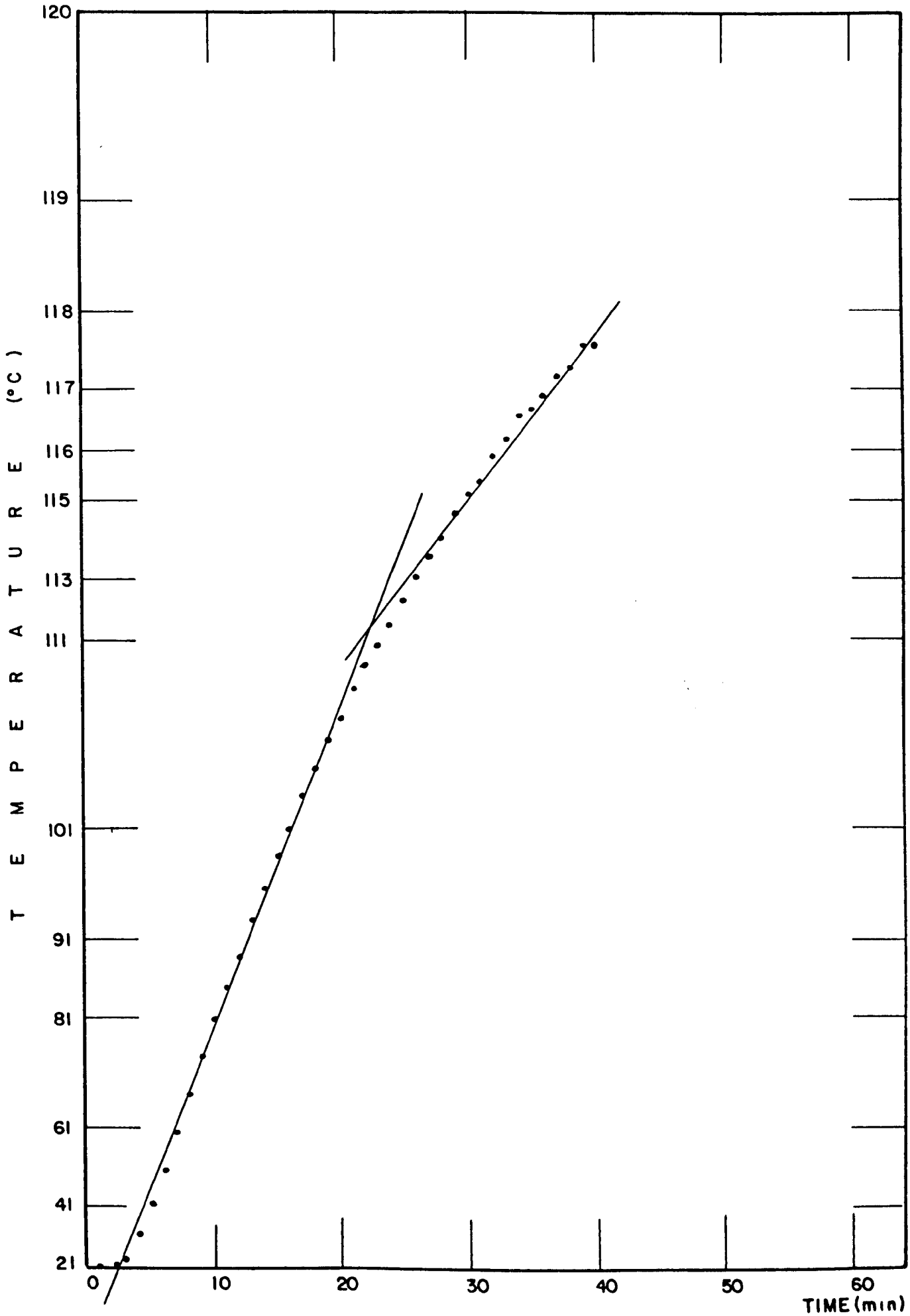


FIG. 4.4 HEAT PENETRATION CURVE. COLD POINT DETERMINATION. TYPE 3 BROKEN CURVE.

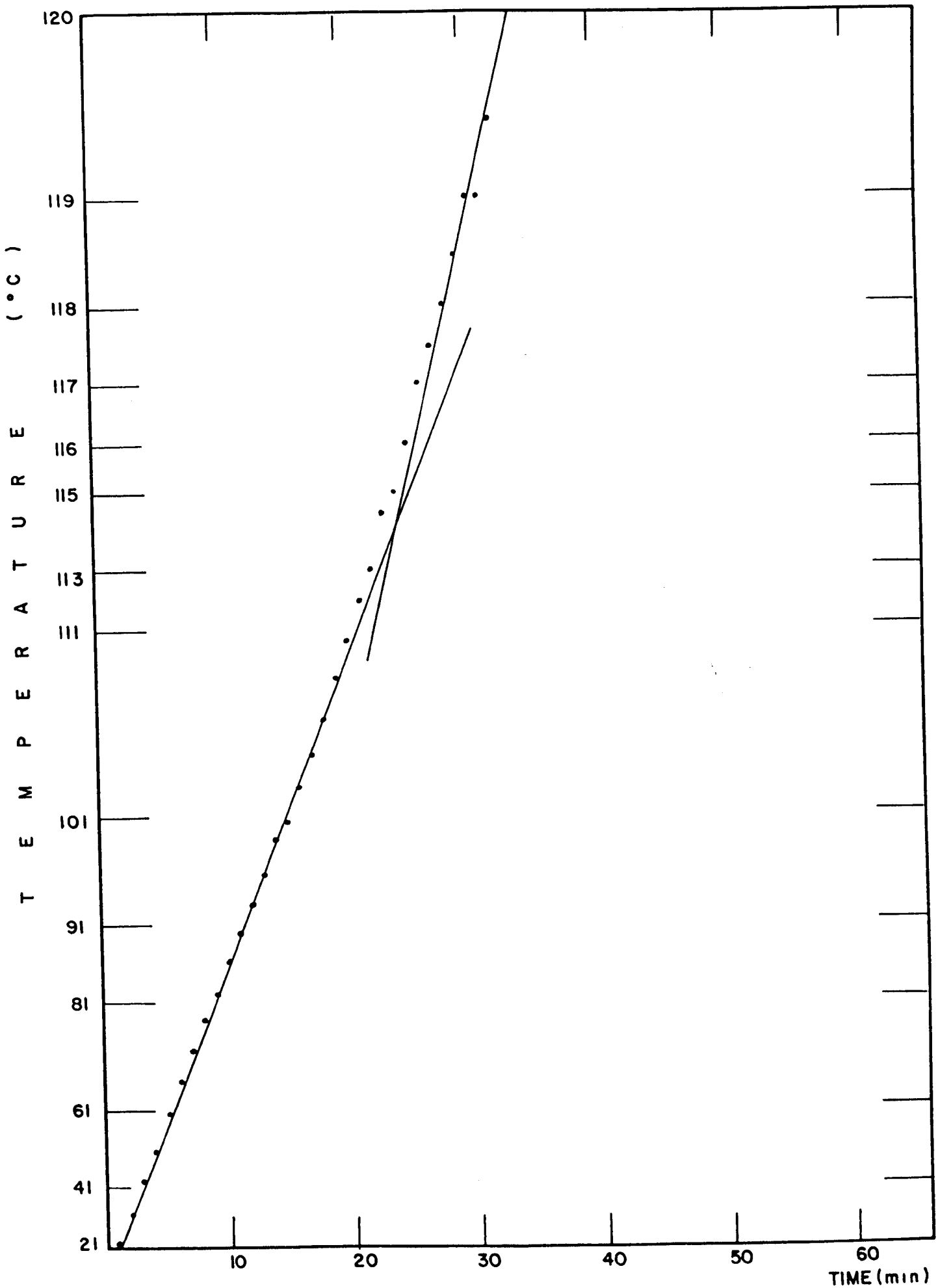


FIG. 4.5 HEAT PENETRATION CURVE. COLD POINT DETERMINATION. TYPE 2 BROKEN CURVE.

Pouch Position	f_h-values in Minutes				Shelf Number
1	24.0	19.8	15.0	14.8	1
2	26.4	15.0	15.5	14.8	
3	24.0	14.8	16.0	13.8	(bottom)
4	20.0	20.0	13.6	15.8	
5	23.2	17.0	15.5	14.8	
6	24.0	16.5	14.5	14.8	
7	22.5	18.0	15.8	14.4	
8	26.0	15.8	15.8	-	2
9	24.0	15.2	18.0	19.0	
10	23.6	21.2	18.0	16.6	
11	19.0	18.8	16.0	18.0	
12	19.8	13.8	16.0	18.0	
13	30.0	16.8	15.6	21.5	
	22.8	22.0	23.2	20.4	3
	25.2	22.0	18.8	21.0	
14	28.4	20.8	24.4	21.4	(middle)
15	20.0	28.0	21.0	17.8	
16	20.4	22.8	21.0	-	
17	20.4	22.8	21.4	17.0	
18	20.0	21.0	19.4	19.0	
19	14.8	14.6	20.5	19.0	
20	15.8	12.0	18.4	14.0	4
21	11.8	19.0	15.8	16.0	
22	14.6	13.1	17.0	14.0	
23	18.8	18.8	19.0	16.0	
24	14.6	11.7	16.0	-	
25	14.2	12.2	21.2	15.0	
26	13.2	12.6	18.0	14.8	5
27	15.2	8.8	14.6	14.4	
28	12.8	11.8	18.0	14.0	(top)
29	09.6	18.4	13.0	13.0	
30	13.4	11.2	20.0	14.0	

Table 4.6 f_h-values obtained for each pouch position in the pouch water retort

	Sum of Squares	Degrees of Freedom	Mean of Squares	Mean Square Ratio Calculated	Tabular 99.0%
Positions unadjusted	930.305	29	-	-	-
Runs adjusted	877.580	11	79.780	27.90	2.50
Residual	240.166	84	2.859		
TOTAL	2048.052	124			

	Sum of Squares	Degrees of Freedom	Mean of Squares	Mean Square Ratio Calculated	Tabular 99.0%
Positions adjusted	434.061	29	14.968	5.24	2.03
Runs unadjusted	1373.824	11	-	-	-
Residual	240.166	84	2.859		
TOTAL	2048.052	124			

Shelf Position	Pouch position (n) and adjusted means of the f_h-values of Table 4.6		
Shelf 5 or top shelf	(25) 17.7 (26) 16.7	(27) 15.3 (28) 16.2	(29) 15.4 (30) 16.7
Shelf 4	(19) 18.9 (20) 16.5	(21) 16.1 (22) 16.9	(23) 17.9 (24) 17.5
Shelf 3 or middle shelf	(13) 21.5 (14) 21.3	(15) 21.7 (16) 20.6	(17) 20.4 (18) 19.8
Shelf 2	(7) 17.8 (8) 17.2	(9) 16.7 (10) 17.6	(11) 17.7 (12) 16.6
Shelf 1 or bottom shelf	(1) 16.4 (2) 15.9	(3) 15.5 (4) 15.4	(5) 15.6 (6) 15.5

Table 4.7 Analysis of variance for data of Table 4.6 and adjusted means obtained for each pouch position

shows highly significant differences between pouch positions and between runs at the 99.0% level of confidence. The adjusted means are presented in Table 4.7. The least significant differences (LSD) were calculated, based on the student's (t) distribution, and used to find the significantly different means. The results show that the middle shelf pouches (positions 13 to 18) heated significantly more slowly than those on the top and bottom shelves, with positions 15 and 13 (back left-hand corner) having the larger f_h-value means, although there is not a statistically detectable difference between positions on the middle shelf. In all subsequent studies the pouch in the left-hand corner of the middle shelf, (position 13), was used to carry the process control thermocouple, (see Figure 4.6). Pouches in positions 14 and 15 were used for carrying the monitoring thermocouples (connected to the chart recorder) whose main function was to provide an alternative thermocouple for process control in case of unexpected failures.

4.4 Investigation of the Between Package Variation of the Sterilization Process within a Retort Load

These experiments were designed to determine the variation in the heat treatment received by the food due to the package's position inside the retort. They were carried out for the static canning retort and for the pouch water retort. The heat treatments applied and the products used were designed to be similar to those required for sample production.

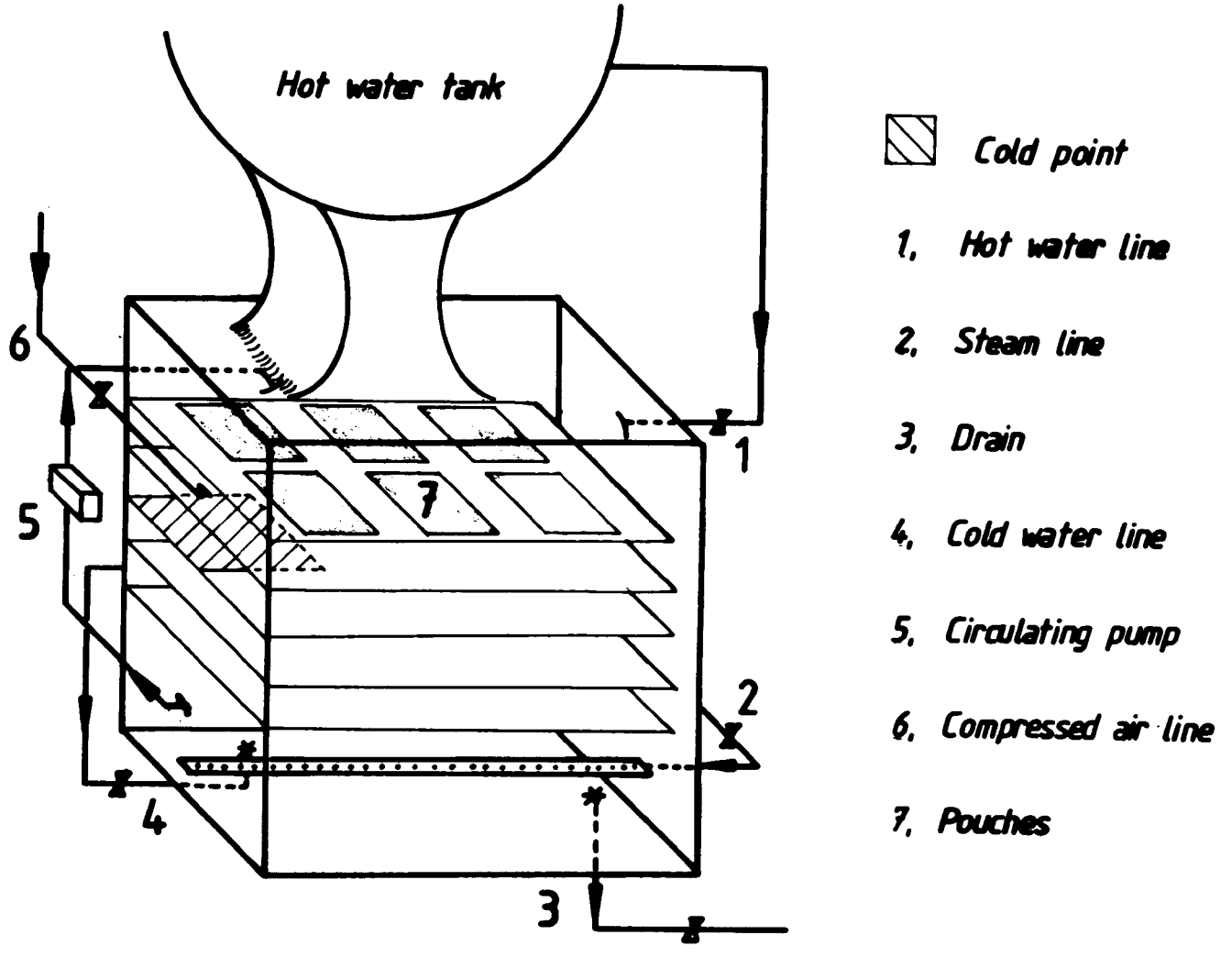


Figure 46. Schematic representation of the pouch water retort showing the cold zone.

4.4.1 Pouch Water Retort

All process monitoring and control was carried out as described in Chapter 3. The food selected for these experiments was potatoes in gravy, to simulate a mainly conduction heated pack such as pork casserole; and potatoes in brine to simulate a pack heated by both convection and conduction.

(i) Experimental Procedure

In both cases, 130 grams of small pieces of potatoes were weighed. For each pack, one 2.54 cm per side cubic piece of potato was used for thermocouple insertion. 150 cm^3 of water or 180 grams of gravy were used to fill the package. The simulated gravy was prepared as described (section 4.3).

As described in section 4.3, 12 thermocouples were placed in pouches and a thermocouple in the heating medium. This arrangement was used for each run, with dummy pouches to complete the retort load. In these experiments the dummy pouches were commercial products, braised liver with vegetables produced by Howerd's Haute Cuisine with an average weight of 250 grams.

Target F_o -values of 10 minutes for potatoes in gravy, and 5 minutes for potatoes in brine were used. Ten retort runs were caried out for each type of food, giving three values for each position in the retort. As already mentioned (section 4.3), for these tests the pouch carrying the process control thermocouple was always located in the cold spot of the retort. A

second pouch with a thermocouple was always positioned next to the process control pouch. This second pouch was used as an internal standard for the temperature monitoring system. The objective of this second control was to avoid comparisons between temperature measurements obtained from two different types of equipment - process control equipment and chart recorder - and thus reducing experimental variations.

(ii) Results and Discussion

The mean total F_o-values obtained (including both heating and cooling cycle effects) for each pouch position using potatoes in gravy are illustrated in Figure 4.7 in the form of a histogram to indicate their position in the retort. It shows that the lower sterilization treatments were received by the pouches processed in the middle shelf and that the samples located on the bottom and top shelves during processing recieved the most severe sterilization treatments. The F_o-values were arranged as shown in Table 4.8 to search for significant differences between pouch position on the shelves and between shelves, using analysis of variance.

The differences between sterilization treatments received by pouches in different shelves are highly significant as shown by the high mean square ratio (m.s.r.) obtained, 35.95. The

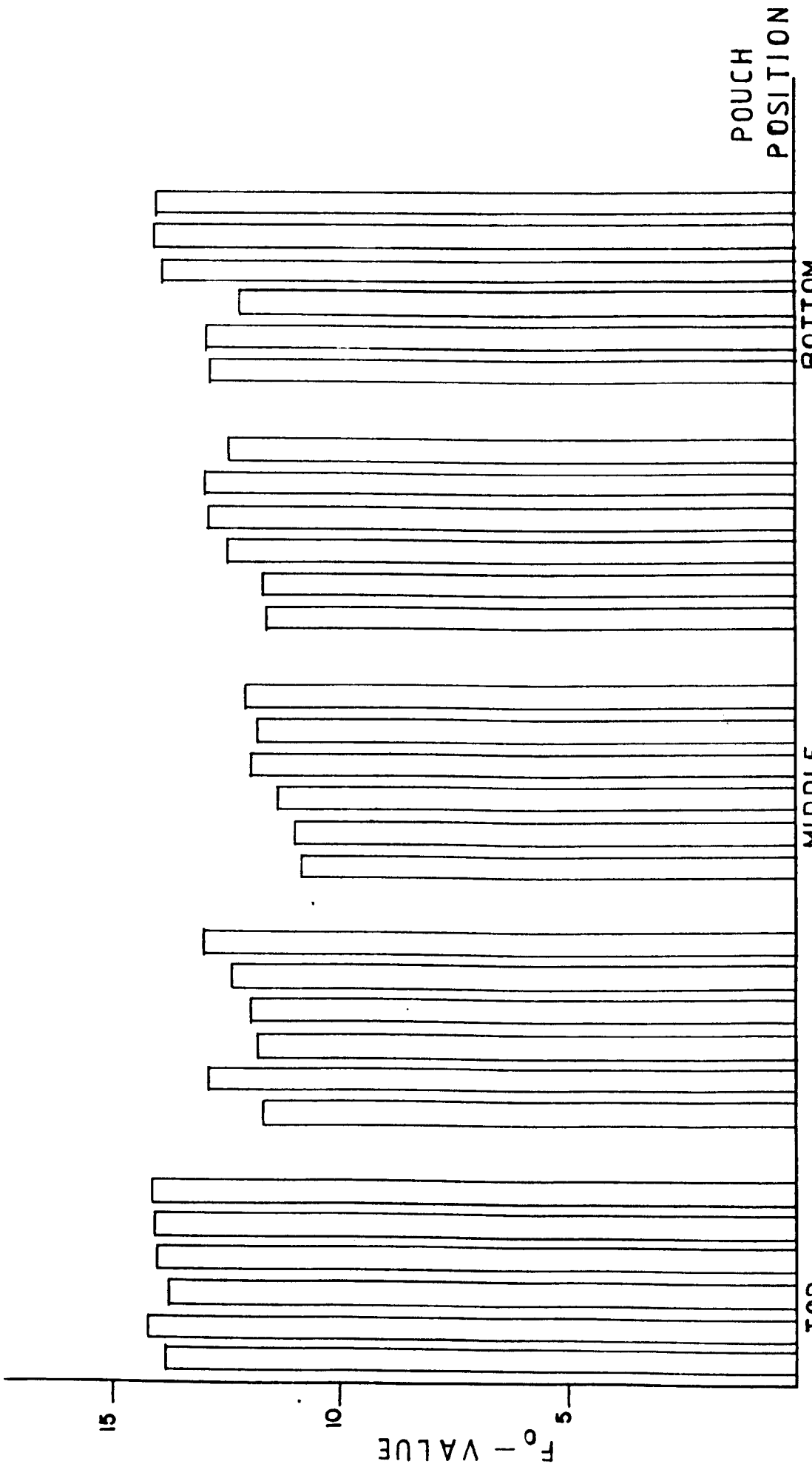


FIG. 4.7 POUCH WATER RETORT – EFFECT OF POUCH POSITION ON HEAT TREATMENT RECEIVED USING POTATOES IN GRAVY.

differences between pouch position on the same shelf are also significantly different, but to a lower degree than the differences between shelves, as shown by the m.s.r. obtained, 4.87. To determine which mean scores are significantly different at 99.0% confidence level, the least significant difference (L.S.D.) was calculated. The values obtained were ±0.73 for between positions on the shelves and ±0.82 for between shelves. The mean F_o -values of the samples were sought using the L.S.D. values obtained. Thus, in Table 4.8 any two means underscored by the same line are not significantly different.

The mildest heat treatment, which was received by the pouch in the back left hand corner of the middle shelf was significantly different from similar positions on other shelves, a result anticipated from previous studies on heating rates (section 4.3). However, pouches on the left zone of the retort on all shelves received a milder total heat treatment than pouches in other positions on the same shelf. No such significant differences were obtained for the heating rates studies.

The mean standard deviation for the results presented in Table 4.8 is 1.04 min. at 121°1.C with a coefficient of variation of 8.2%.

The mean F_o -values obtained for each position in the pouch water retort with potatoes in brine are presented in Figure 4.8 where the same behaviour as for the potatoes in gravy is observed. The analysis of variance for these F_o-values is shown in Table 4.9. When comparing the values for the mean squares ratios calculated with the tabular values at 99.0% level of confidence, significant differences were found between the

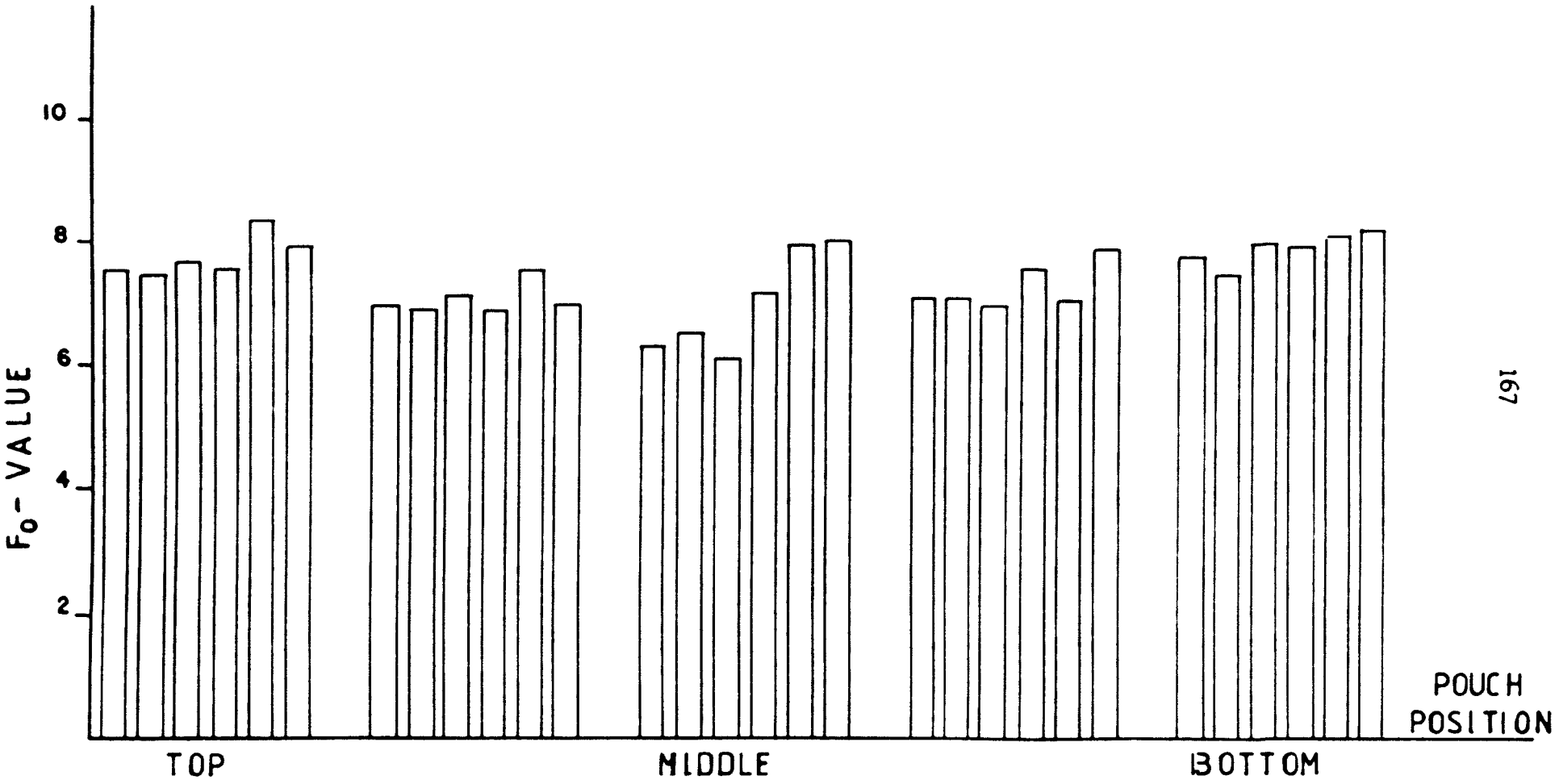


FIG. 4.8 POUCH WATER RETORT - EFFECT OF POUCH POSITION ON HEAT TREATMENT RECEIVED USING POTATOES IN BRINE .

Shelf	F_o-values in Minutes						$\bar{B}_i$
	Pouch Position						
	1	2	3	4	5	6	
1	13.84	14.21	13.82	14.07	14.11	14.16	14.035
2	11.74	12.78	11.78	11.91	12.29	12.92	12.2367
3	10.78	10.91	11.29	11.99	11.84	12.09	11.4833
4	11.69	11.84	12.48	12.81	12.93	12.45	12.3666
5	12.84	12.89	12.21	13.88	14.12	14.01	13.325
$\bar{X}_i$	12.178	12.526	12.316	12.912	13.058	13.126	12.6893

ANALYSIS OF VARIANCE TABLE

Source of Variation	Degrees of Freedom	Sum of Squares	Mean of Squares	Mean Square Calculated	Ratio Tabular 99.0%
Between position on shelf	5	4.0181	0.8036	4.87	4.10
Between shelves	4	23.74	5.94	35.95	4.43
Residual	20	3.3021	0.1651	-	-
Total	29	31.0645			

LEAST SIGNIFICANT DIFFERENCES:

Sample means comparison

Shelf 3	Shelf 2	Shelf 4	Shelf 5	Shelf 1
11.48	12.24	12.37	13.32	14.04

Position 1	Position 3	Position 2	Position 4	Position 5	Position 6
12.18	12.53	12.32	12.91	13.06	13.13

Table 4.8 Pouch water retort - effect of pouch position on heat treatment received with potatoes in gravy. Analysis of variance for data corresponding to Figure 4.7

Shelf	F_o-values in minutes for each pouch position (n) in the retort						B_i
	(1)	(2)	(3)	(4)	(5)	(6)	
1	7.51	7.44	7.63	7.54	8.33	7.90	7.72
	(7)	(8)	(9)	(10)	(11)	(12)	
2	6.93	6.89	7.07	6.85	7.49	6.95	7.03
	(13)	(14)	(15)	(16)	(17)	(18)	
3	6.26	6.53	6.08	7.16	7.87	7.91	6.96
	(19)	(20)	(21)	(22)	(23)	(24)	
4	7.08	7.02	6.93	7.58	7.03	7.83	7.24
	(25)	(26)	(27)	(28)	(29)	(30)	
5	7.66	7.44	7.95	7.94	8.02	8.16	7.86
$\bar{X}_i$	7.088	7.064	7.132	7.414	7.748	7.75	7.366

ANALYSIS OF VARIANCE (Table 4.8)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean of Squares	Mean Square Ratio Calculated	Mean Square Ratio Tabular 99.0%
Between vertical positions	5	2.595	0.519	4.15	4.10
Between shelves	4	3.962	0.99	7.93	4.43
Residual	20	2.498	0.125	-	-
Total	29	9.054	-	-	-

LEAST SIGNIFICANT DIFFERENCES:

Shelf 3	Shelf 2	Shelf 4	Shelf 1	Shelf 5
6.968	7.03	7.245	7.725	7.862

Position 2	Position 1	Position 3	Position 4	Position 5	Position 6
7.064	7.088	7.132	7.414	7.748	7.75

Table 4.9 Pouch water retort - effect of pouch position on heat treatment received using potatoes in brine. Analysis of variance of data from Figure 4.8

sterilization treatments received by both the pouches on different shelves and between pouch positions on each shelf. The L.S.D. values calculated to seek for significant differences are ± 0.63 (between shelves) and ± 0.57 (between pouch positions on the shelves). These differences can be seen in Table 4.9, where the mean F_o -values which are not significantly different are underlined.

The mean standard deviation found for potatoes in brine is 0.56 F_o -value units which gives a coefficient of variation of 7.60%. It is interesting to note that the coefficients of variation for the F_o-values of potatoes both in gravy and in brine are very similar, 8.2% and 7.6%.

The results presented above correlate very well with the findings of the cold point determination (section 4.3) as it was expected, although the variability in F_o -values (with coefficients of variation of 8.2% and 7.6%) was smaller than the variability in f_h -values, with a coefficient of variation of 15.03%. This could be partially explained by the moderating effect of the cooling cycle. There is some evidence that the samples presenting the fastest heat penetration rates, also show the fastest cooling penetration rates. Therefore the smaller F_o -value obtained during the slow heating of a large f_h -value sample is compensated during the comparably slow cooling phase.

4.4.2 Static Canning Retort

(i) Experimental Procedure

The foods selected for these experiments were also potatoes

in gravy and in brine. Food preparation, process control and monitoring systems were as described in section 4.4.1 and Chapter 3 respectively.

The retort had two wire baskets in which to place the cans, although only the top basket was used during processing. The cans were placed in three concentric circles in the basket. For the tests, 12 cans fitted with thermocouples (1 per circle) were placed on perpendicular radii while one thermocouple was placed in the centre of the can load to monitor the heating medium temperature. Eighteen dummy cans filled with rice pudding were used to simulate a full load of 30 cans. This was not the maximum number of cans that could be placed in the retort, but as with the case of the pouch retort, it was the maximum load used at any one time.

(ii) Results and Discussion

The variation in sterilization treatments received by each can according to its position in the static canning retort, using both potatoes in gravy and potatoes in brine, is presented in Figure 4.9. There it is clearly shown that the heat treatment variations are very small in both cases. The mean F_o-values obtained are given in Table 4.11 where the standard deviation and overall mean F_o-values are also included. The coefficients of variation obtained are 1.39% for potatoes in gravy and 3.63% for potatoes in brine.

These results show that the variability on sterilization treatments received by samples processed in the pouch water

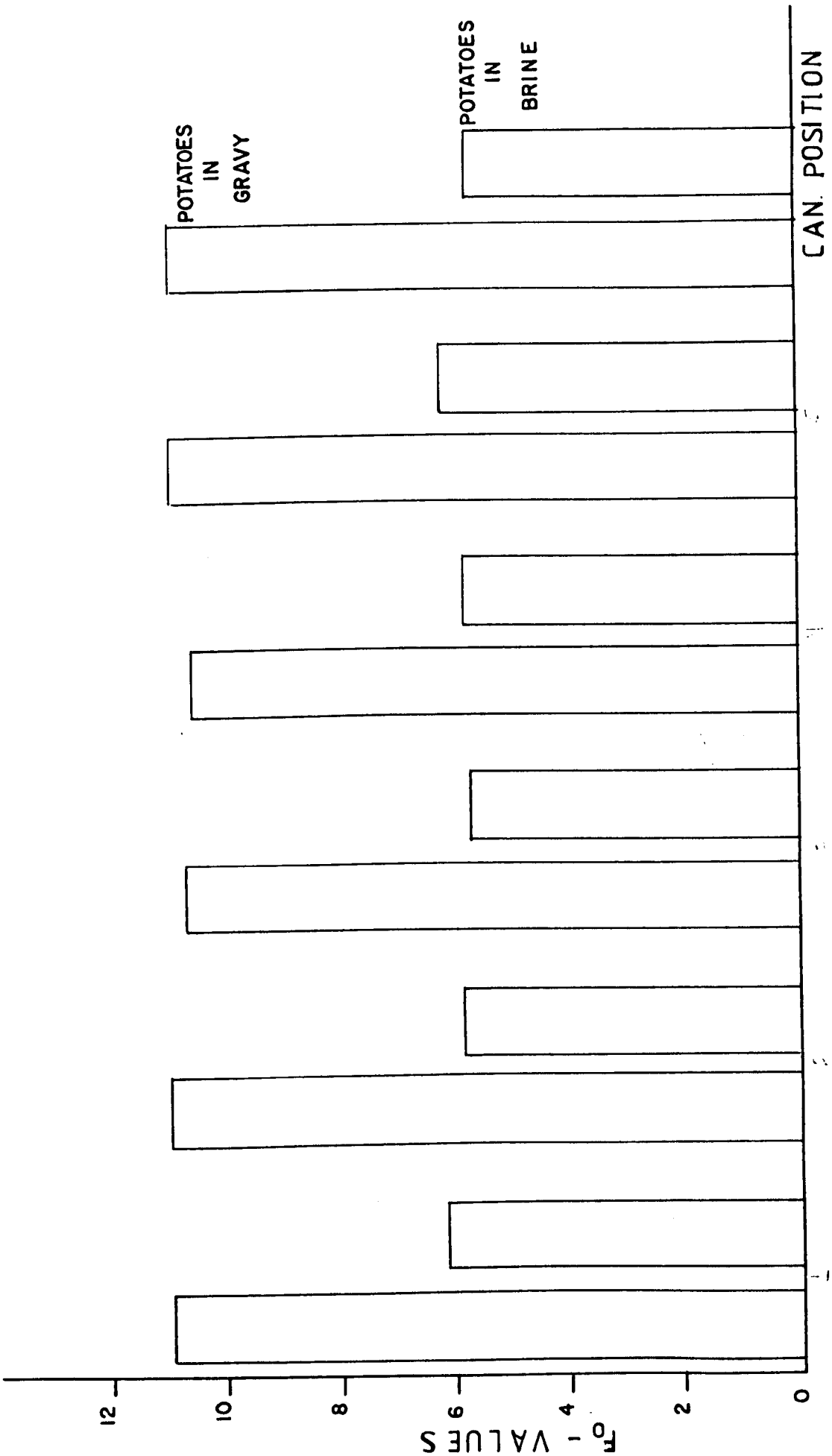


FIG. 4.9 STATIC CANNING RETORT – EFFECT OF CAN POSITION ON HEAT TREATMENT RECEIVED

Potatoes in Gravy F_o-value in Minutes		Potatoes in Brine F_o-value in Minutes	
Position	F_o-value	Position	F_o-value
1	10.82	1	6.08
2	10.93	2	5.76
3	10.61	3	5.65
4	10.57	4	5.74
5	10.84	5	6.18
6	10.89	6	5.77
X_i	10.777		5.863
σ	0.15		0.213

Table 4.10 **Static canning retort - effect of can position on heat treatment received**

retort is greater than the variability found for samples processed in the static canning retort, as can be seen from the coefficients of variation, 8.2%, 7.60% and 1.39%, 3.63% respectively. Although all the factors affecting the variation in sterilization treatments cannot be properly controlled and are not well understood, in these studies the food material, type of package, packaging conditions and temperature and time of process were carefully controlled. The variability reported here however for the pouch water retort is very large. Therefore problems inherent to the pouch water retort design were concluded to be the cause of this large variation. Some of these problems include inadequate circulation of the cooling and heating mediums and the positioning of the cold water, hot water and steam inlets. Another important factor is the

difficulty in controlling pressure in a manual water retort which in pouch processing may affect the dimensions of the pouch thus affecting the heat transfer rate.

4.5 Comparison of Heating Rates in Pork Casserole Ingredients

In the pork casserole formulation chosen, several solid ingredients were present. Consequently, to select the food in which to insert in the monitoring thermocouple, several tests were carried out. The aim was to determine the slowest for heat transfer under food processing conditions. For these tests three of the pork casserole ingredients were investigated, namely carrots, meat and potatoes. This was because these ingredients were the largest solid particles present.

(i) Experimental Procedure

The pork casserole product was prepared and weighed according to the formulation selected (Appendix 4) and one piece of each tested ingredient (carrot, meat and potato) was saved for thermocouple insertion.

The thermocouples for temperature monitoring and process control were always located in the centre of the package, inserted in a piece of food which was always of the upper size limit of the solid particles in the container.

The tests were conducted in pouches and the thermocouples were assembled as described in section 3.3 but instead of one thermocouple per package, three thermocouples per container were used. Three brass glands for thermocouple insertion were located on the pouch walls as

far away as possible from the centre of the pack, i.e. the top middle position and the top corners of the pouch.

The three ingredients were placed on the central axis of the pouch width, fixed to the nylon supporting rig with cotton thread (see section 3.3 and Figure 3.6). The food particles' positions inside the pouches were interchanged to cover all possible combinations and to avoid bias in the heat transfer rate due to their location.

Six packages were prepared, two for each possible combination, and then processed in two retort runs. This was because three was the maximum number of packages that could be monitored during any one run due to the limitations of the temperature recording system.

The target F_o-value for the test packages was 15 minutes. The temperature-time charts from the Philips chart recorder were used to obtain the heat transfer data required to compare ingredient heating rates.

Results and Discussion

(ii) Plots on semilogarithmic paper were prepared to obtain the individual f_h-values, which are given in Table 4.11. In this table two f_h-values are reported for those set of data that presented broken type semilogarithmic heating curves.

In this case of carrots all the heat penetration curves show a break. In some plots (4 cases) the heat penetration later increases and sometimes (2 cases) falls. In potato and meat this break does not always occur but when it

does it is always associated with a faster heat penetration towards the end of the process. It is not clear why this break occurs, although it is known that during processing a considerable amount of chemical and physical change takes place. Some of these changes may affect the thermal properties of the food such as changes in the porosity of the food product, changes in moisture content due to water transfer between the liquid medium (gravy) and the solid and the considerable degree of softening of food texture, etc. It was considered that softening of the food texture - particularly in the small carrot pieces used - would allow some movement of the thermocouples in spite of the experimental technique used to minimize movement. Such movement could cause an apparent decrease or increase in the heating rate depending on the nature of the movement. If such movement did occur it was not detected by the after processing inspection of the tested packages and therefore it was very small. Consequently thermocouple movement seems a more reasonable explanation for broken heat penetration curves than a sudden change in the thermal diffusivity of the product or a change in the heat transfer characteristics of the surrounding gravy. The f_h-values obtained for each ingredient before the break in the heating curves (f_{h_1}) were statistically tested for significant differences. The analysis of variance results are shown in Table 4.12, where it can be seen that there are significant differences between food f_h-values at the

Pouch Number	f_h-values (in minutes)					
	Potato		Meat		Carrot	
	f_{h_1}	f_{h_2}	f_{h_1}	f_{h_2}	f_{h_1}	f_{h_2}
1	26.0	15.0	23.2	9.0	17.6	12.0
2	22.0	-	18.8	11.2	14.0	6.4
3	18.4	-	19.0	11.2	18.0	13.0
4	20.8	-	21.0	-	11.0	22.0
5	21.2	-	18.0	-	20.0	11.0
6	19.0	-	15.0	-	12.0	19.0
$\bar{X}$	21.23		19.17		15.43	
σ						

Table 4.11 f_h-values obtained for pork casserole ingredients

Source of Variation	Sun of Squares (S.S.)	Degrees of Freedom	Mean of Squares (M.S.)	Mean Squares Ratio (M.S.R.)	
				Calculated	Tabular 99.0%
Between foods	103.7	2	51.85	8.49	7.56
Between runs	79.58	5	15.92	2.61	5.64
Residual	61.08	10	6.11		
Total	244.36	17			

Table 4.12 Analysis of variance results for data from Table 4.11

99.0% level of confidence but there are not significant differences between runs either at 99.0% and 95.0% confidence levels. A L.S.D. value of 4.479 was used to seek significant differences between the samples at 95.0% level of confidence. The f_h-value means show that carrot and potato heating rates are significantly different, the piece of potato being the slowest for heat transfer (larger f_h-value), followed very closely by the meat's mean f_h-value while carrot presented the smallest f_h-value (fastest heat transfer rate). There is no significant statistical difference between either potato and meat means or meat and carrot means.

The F_o-value for each individual temperature was calculated and the results are given in Table 4.13 together with the corresponding analysis of variance table. The analysis of variance shows that potato F_o-values are significantly different from those of carrot at 99.0% confidence level. Also meat and carrot values are significantly different at 99.0% confidence level, while potato and meat F_o-values are not significantly different at 99.0% and 95.0% levels of confidence. Nevertheless, the mean F_o-value obtained for potatoes is slightly lower than the mean F_o-value obtained for meat.

Typical temperature-time profiles and their corresponding F_o-values for the three ingredients tested are shown in Figure 4.10, where the differences in heating rates can be easily observed. In this particular case, the piece of carrot was at

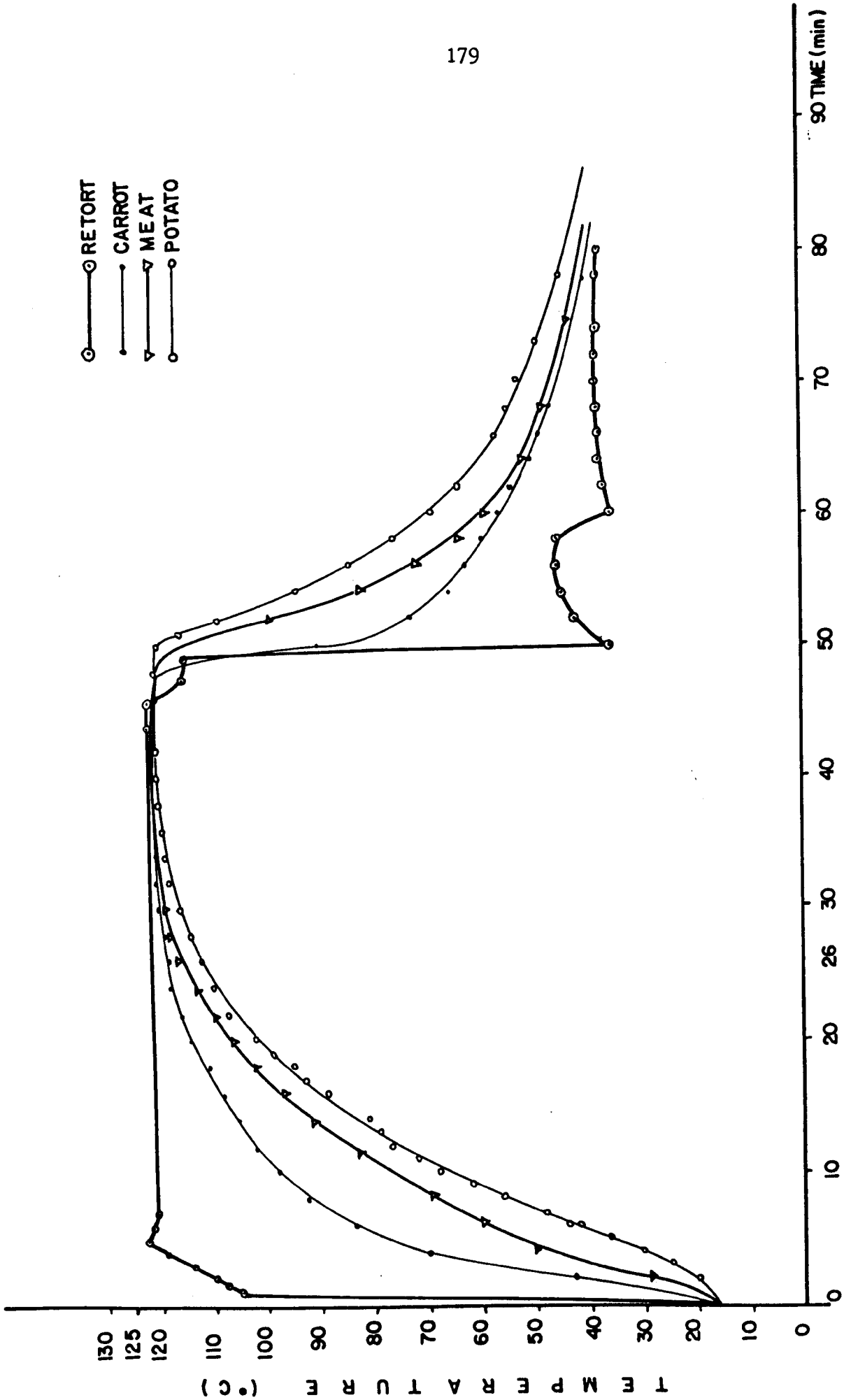


FIG. 4.10 TIME-TEMPERATUE PROFILE OF PORK CASSEROLE INGREDIENTS

Piece in Centre	F_o-values in Minutes		
	Potato	Carrot	Meat
Carrot	17.04	21.18	20.31
Carrot	16.93	20.13	17.82
Meat	19.53	20.94	16.23
Meat	18.23	21.33	18.13
Potato	17.53	22.65	18.73
Potato	18.96	21.94	19.63
$\bar{X}_i$	18.04	21.35	18.48
σ	1.058	0.863	1.438

ANALYSIS OF VARIANCE TABLE

Source of Variation	Degrees of Freedom (d.f.)	Sum of Squares (s.s.)	Mean of Squares (m.s.)	Mean Square Ratio (m.s.r.) Calculated	Tabular 99%
Between ingredients	2	39.194	19.597	14.96	6.36
Residual	15	19.649	1.31		
Total	17	58.842			

Table 4.13 Comparison of heating rates of pork casserole food ingredients

the geometric centre of the package with the pieces of meat and potato on either side on the long axis of the pouch. It can be seen in this Figure that the faster heat transfer of the carrot piece does not lead to a much larger F_o-value because of the more rapid cooling of the carrot pieces.

The semilogarithmic heating curves corresponding to the temperature-time profiles shown in Figure 4.10 are in Figure 4.11 where the broken type profile presented by the pork casserole ingredients can be seen.

According to the above results, the piece of potato was selected as the ingredient with the slowest heating rate. Therefore it was the piece of food used for process control and temperature monitoring in the pork casserole experiments. Potato rather than meat was preferred because its large size allowed easier handling when inserting the thermocouple.

4.6 Development and Evaluation of Rice Pudding Formulations

Information available from text books and food manufacturers (Campbell, 1950, Canning Trade, 1924, Co-operative Wholesale Society Limited, 1979, Cruess, 1958, Komarick et al, 1978, Fressler and Woodroof, 1978) was found to be adequate to produce acceptable vegetables and stew type products. However, products like rice pudding needed a full development procedure. This created the need for a considerable investment of effort and time, particularly when the production of acceptable products, in both cans and pouches, was regarded as being of major importance in setting up the comparison conditions in a

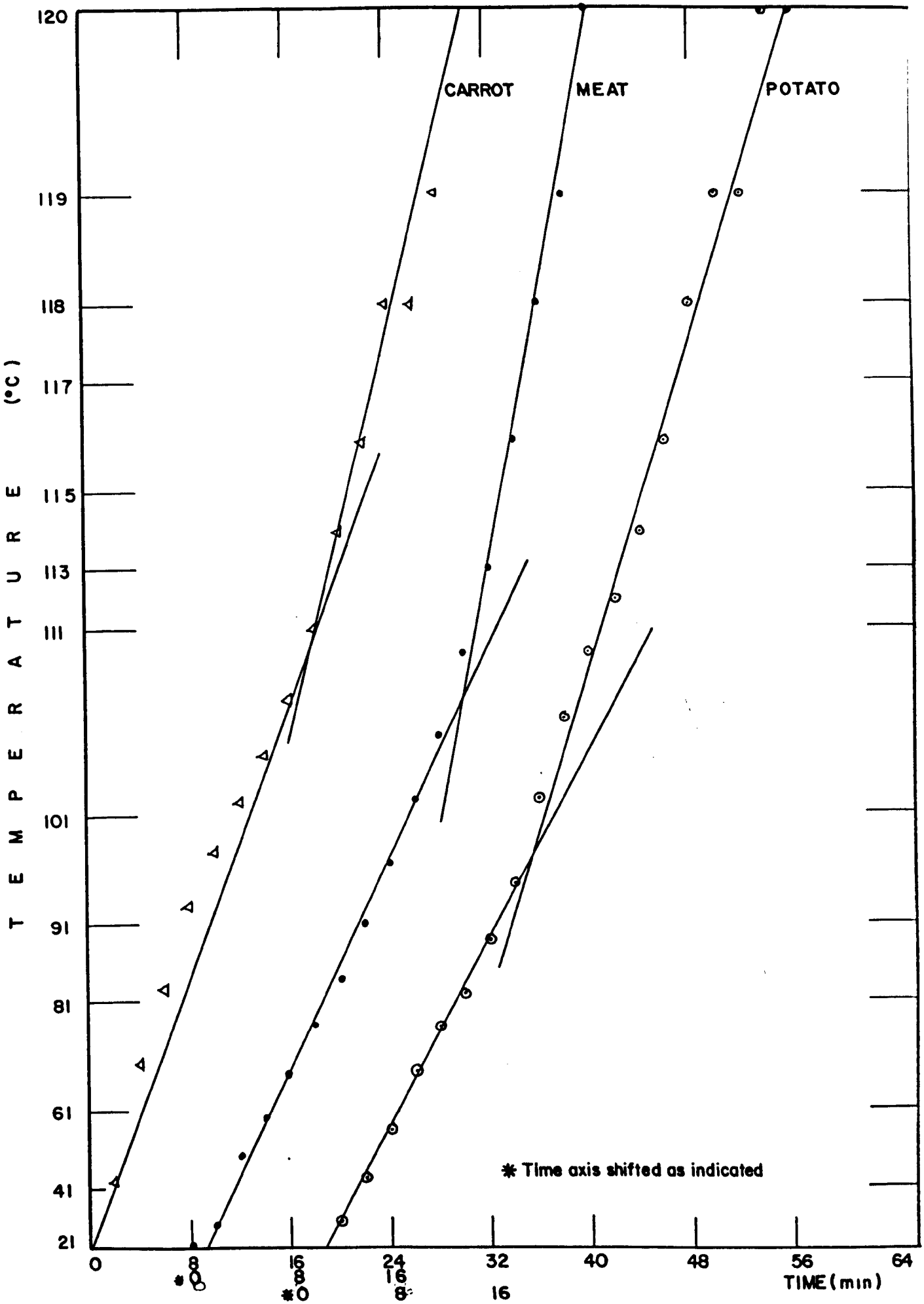


FIG. 4.11 HEAT PENETRATION CURVES OF PORK CASSEROLE INGREDIENTS CORRESPONDING TO FIG. 4.10

substantial part of this work.

4.6.1 Development of Rice Pudding Formulations

After several tests, four successful formulations were developed for the production of acceptable rice puddings. The main differences in these formulations were the ratios between each component in the package and the precooking process.

(i) Experimental Procedure

The products contained three basic ingredients, white granulated sugar, round short grain rice and fresh raw cow's milk. Large batches of sugar and rice were purchased, mixed and stored while the milk was collected fresh from the dairy and processed within four hours to avoid a "metallic flavour" development.

The milk was treated with 8% (w/v) Sodium bicarbonate solution to raise the pH by 0.1 units. This was done in order to stabilize the milk during processing, avoiding its separation. The four accepted formulations were as indicated in the following section.

Results and Discussion

Formulation 1: 30 grams of raw rice, 230 grams of treated milk and 15 grams of sugar were packaged. Precooking was carried out in a rotating retort at 100°C for 20 minutes before sterilization.

Formulation 2: 26 grams of raw rice, 230 grams of

treated milk and 13 grams of sugar were packaged and precooked in a rotating retort at 100°C for 30 minutes, before sterilization.

Formulation 3: Rice was precooked in a large catering pressure steam cooker (model 75, Hobart manufacturing company, Ohio). A pressure of 92.53 KP_a above atmospheric pressure was applied. The rice was steamed for 10 minutes in milk, and for every 100 grams of rice 200 cm^3 of treated milk were added. Precooked rice was then left to cool before filling. Finally, 120 grams of precooked rice, 170 grams of milk and 15 grams of sugar were used to fill the packages.

Formulation 4: 110 grams of processed rice (same as above), 170 grams of treated milk and 15 grams of sugar were used to fill the packages.

4.6.2 Rice Pudding Evaluation

Foods are normally submitted to sensory evaluation to obtain information about overall quality and possible changes due to processing. This procedure is of importance since acceptance or rejection of a commercially processed product is largely based on its sensory characteristics.

Therefore, two taste panel techniques were used to assess the overall acceptability of the formulations developed and to select the best products for processing and storage. The tests used were hedonic scaling and ranking tests. The ranking test was used to select, from four commercial rice pudding products,

the canned sample with the highest acceptability for the taste panel group. Hedonic scaling was used to determine the difference between the acceptability of the selected commercial product and the four experimental rice pudding formulations, and also to select the best two formulations for processing and storage.

(i) Experimental Procedure

All taste panels were conducted in standard taste panel booths with an adjacent food preparation area. Access to the preparation area from each one of the six booths was by a sliding hatch. Each booth was provided with cold running water and variable lighting conditions. The light controls were located in the preparation area and any mixture or intensity could be selected. White, maximum intensity light was selected. Each panelist was provided with a record sheet, pen and glass with cold water for mouth wash purposes. Examples of the taste panel sheets provided are shown in Figures 4.12 and 4.13.

Before tasting, all samples were heated in a water bath at 80°C in their packages and served at this temperature. Heating time for each package was controlled to a minimum of 20 minutes and a maximum of 30 minutes to avoid changes due to heating. The samples were presented on glass dishes which were kept warm in an oven. All glass dishes were of the same size and appearance. Similar amounts of foods were used for all samples.

TASTE PANEL RECORD SHEET

Ranking Test

Booth No. _____

Date _____

Name _____

Rank Order	Sample
1 (best)	
2	
3	
4	

Please taste all samples and place them in preferred order.

You may taste the samples as many times as you like to make a judgement.

Comments

_____

_____

Thank you

Figure 4.12 Taste panel sheet used for ranking test

TASTE PANEL RECORD SHEET

Hedonic Test

Booth No. _____

Date _____

Name _____

Sample

Like extremely

Like very much

Like moderately

Like slightly

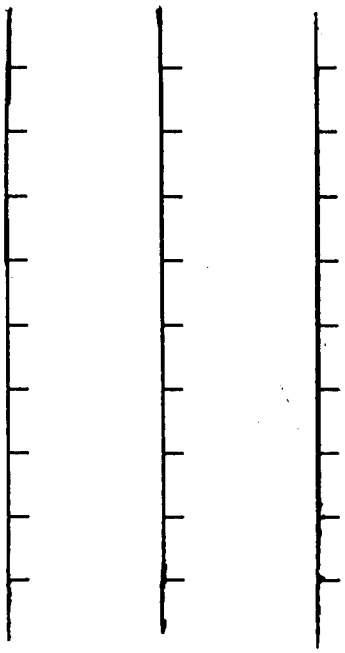
Neither like or dislike

Dislike slightly

Dislike moderately

Dislike very much

Dislike extremely



Please mark the scales anywhere on the vertical line.

The scale is continuous.

Comments

_____

_____

_____

Figure 4.13 Taste panel record sheet used for hedonic scaling

(a) Ranking Test

This test was used to select the best commercial samples, of those available in the market, rather than to test all samples thoroughly. Four samples were ranked by twenty-four judges. The samples were:

(A) Ambrosia Creamed Rice Pudding

(B) Heinz Creamed Rice Pudding

(C) Libby's Creamed Rice Pudding

(D) Co-op Creamed Rice Pudding

The four warm samples were tasted at the same time and the panelists were asked to rank them by preference on the record sheet provided (see Figure 4.12). The samples were labelled with symbols to avoid mental bias of the tasters with the use of numbers or letters. The values given to score the samples were 1 for the best, 2, 3 and 4 in descending order of preference.

The results were statistically analysed in two ways, namely, the Kendall(1948) coefficient of concordance method and the Kramer (1963) method for determining significance of differences from rank sums. The first method was employed to test the judges' degree of agreement in their rankings and the second was used to test significant differences in a group of samples to select one of the best quality samples.

(b) Hedonic Scaling

For this test there were eight experimental rice pudding

samples (four formulations in both pouches and cans) and a commercial product chosen from the ranking test results. Tasting nine rice pudding samples at one time was considered to be inadvisable since a possible reduction in accuracy and sensitivity by fatigue in the judge response could be induced. Consequently, the judges were presented with groups of three warm samples per session, two experimental samples and the commercial rice pudding chosen as standard. Each sample was identified by means of symbols. Record sheets with three nine point hedonic scales (see Figure 4.13) were given to the tasters and they were asked to qualify each sample by recording the score at any point of the continuous hedonic scale. A score of 5 indicated "neither like or dislike", whereas scores above 5 indicated degree of liking and scores below 5, degrees of dislike.

Each booth had a different set of samples to be tasted and each panelist tasted all samples within four different settings. The tasting sessions were carried out during mid-morning, between 10.00 and 11.30 a.m. or mid-afternoon between 2.30 and 4.30 p.m.

(ii) Results and Discussion

The results obtained and a resumé of their statistical analysis are given in Table 4.14. To test significant agreement between the rankings asigned to the products by the judges, the data were arranged in an analysis of variance pattern, and the coefficient of concordance, W,

was calculated. This statistical parameter was tested for significance using the F distribution. The F calculated value was 12.48, while from tables the value of F at 99.9% significance level is estimated as 6.12. From these results it can be seen that the calculated value of F is highly significant at 0.1% confidence level. Therefore, the judges exhibited a noticeable degree of agreement in their ranking. These judges were considered to be semi-trained panelists and were members of the Food Science department. To test if one of the commercial products was significantly different from the others on the basis of the rankings assigned by the panelists, Kramer (1963) tables were used. The values found in the tables for 1% significance level were 42-73 and 44-71. The first Kramer pair shows the limits outside of which the rank totals must fall to indicate significant differences. Following this criterion sample A, with a rank total of 80, shows significant difference against the other samples. The second Kramer pair indicates the significance between samples. The high rank total (80) of sample A indicates that this sample was ranked as significantly inferior to the other samples, while sample D was ranked as significantly superior.

According to these results, the Co-op Creamed Rice Pudding was selected as the best commercial product available in the market by the group of semi-trained panelists employed. Therefore, this product was used as the commercial standard against which the rice pudding formulations developed were tested.

	SAMPLES			
	A	B	C	D
T	80	71	47	42
$\bar{X}_i$	3.33	2.96	1.96	1.75

for $n = 24$
correction term $= 600$
Products sum of squares $= 42.25$
Total sum of squares $= 120$
then $W = 0.35$
and $F = 12.48$
degrees of freedom for numerator $= 3$
degrees of freedom for denominator $= 67$

Table 4.14 Ranking test. Taste panel data means and their summarized results from statistical analysis

(b) Hedonic Scaling

The mean score value for the control sample was calculated for each individual judge and the values for the sample scores were adjusted using the control mean value by a normalization procedure. Therefore the differences between sample and control were maintained. The mean values given in Table 4.15 are the means of the adjusted values. The analysis of variance of the results is presented in Table 4.16 which shows that there is no significant difference between the samples and the standard commercial product used as control. Therefore, the experimental samples have the same overall acceptability as the

Sample Formulation	Container	Adjusted means ($\bar{X}_i$)	Standard Deviation (σ)
1	pouch	5.97	±0.93
2	pouch	6.62	±0.69
3	pouch	5.88	±0.84
4	pouch	6.46	±0.72
1	can	5.97	±0.86
2	can	5.88	±0.99
3	can	6.80	±0.59
4	can	6.84	±0.63
Control	can	6.587	±0.73

Table 4.15 **Hedonic scaling adjusted means of experimental samples and control rice pudding**

commercial product. The mean scores for the experimental samples and the control were between 5.5 and 7.0, hedonic values that correspond to "like slightly" and up to "like moderately".

Source of Variation	Sum of Squares (S.S.)	Degrees of Freedom (D.F.)	Mean of Squares (M.S.)	Mean Square Ratio (M.S.R.) Calculated	Tabular 99.0%	Tabular 95.0%
Between rice puddings	21.83	8	2.73	1.4	1.94	2.51
Between judges	251.98	23	10.96	5.63	1.52	1.79
Residual	358.23	184	1.95			
Total	632.04	215				

Table 4.16 **Analysis of variance of adjusted scores of Table 4.15**

Several other statistical models, including various interaction effects, were tried on the results by the University statistical advisory service. The analysis of variance table for a model separating scores of the control and scores of the samples and seeking for significant differences for control scores, control and experimental smaples, judges and judges and control scores is shown in Table 4.17 where it can be seen that control and experimental samples are not significantly different. Control scores, judges and interaction of judges with control samples are significantly different. These results suggest that the difference between control and experimental sample is not the same for all judges probably due to different "mental rice pudding standards". As the analysis of these problems is out of the scope of this work, no further taste panels were designed to obtain a better set of results. Therefore in order to select the formulations to be processed and produced, the mean scores for each formulation were added and the two recipes with the higher combined scores were selected. These were formulation 2 and 4.

Source of Variation	Sum of Squares (S.S.)	Degrees of Freedom (D.F.)	Mean of Squares (M.S.)	Mean Square Ratio (M.S.R.)	Significance at
Between control scores	4.55	1	4.551	3.73	95%
Between control and experimental samples	14.57	7	2.085	1.71	not --
Between judges	213.531	23	9.298	7.62	99%-
Between judges on control scores	372.705	23	16.143	13.22	99%-
Residual	284.522	233	1.221		
Total		287			

Table 4.17 Analysis of variance for results presented in Table 4.15

4.7 Evaluation and Comparison of Vitamin C Assay Methods

For the present work two analytical methods for vitamin C assay were selected, the 2,6 dichlorophenol indophenol (DCP) visual titration and the o-phenylenediamene (OPDA) fluorimetric procedure. These are the A.O.A.C., (1975), recommended assay procedures. Following the recommendation given by Freed, (1966), Joslyn, (1970), Osborne and Voogt, (1978), Pearson, (1976), Deutsh, (1967), Hall and Deutsh (1965) and Deutsh and Weeks (1965) slightly modified procedures were used, which are fully described in Appendices 2.1 and 2.2.

It was considered important to evaluate the precision and accuracy of both analytical methods. Both procedures were followed and tested simultaneously and the results were carefully studied, permitting better understanding of the significance of the results and comparison of the performance of the two assay procedures.

4.7.1 Evaluation of the Visual Titration Method

The two stages of the evaluation of this method were the determination of the relationship between ascorbic acid concentration and dye consumption and the recovery of ascorbic acid in the assay procedure.

(i) Experimental Procedure

(a) Standard Curve

To evaluate the relationship between ascorbic acid concentration and the dye consumption for the titration,

ten dilutions of the ascorbic acid standard solution were prepared with concentrations ranging from 0.20 mg of AA/cm^3 to 0.005 mg of AA/cm^3. Duplicate 5 cm^3 aliquots were titrated with standardized 0.04% (w/v) indophenol dye solution as described in Appendix 2.1.

(b) Percentage of Ascorbic Acid Recovery during the Assay Procedure.

Known volumes of standard ascorbic acid solution were added to brussels sprouts and pork casserole extracts after maceration. The level of ascorbic acid added was similar to the ascorbic acid sample content.

(ii) Results and Discussion

The relationship between ascorbic acid concentration and dye consumption is given in Figure 4.14, where a clear linear response can be seen. When small volumes of dye are used for titration the results are erratic, especially below 0.2 cm^3 of dye. The same effect happens when large volumes of dye are required, particularly above 8 cm^3 of dye. It was decided to adjust sample size so that all sample titrations consumed less than 5 cm^3 and more thatn 0.2 cm^3, both to reduce variation in the experimental procedure due to refilling of the burette and also to avoid the erratic results obtained when small volumes of dye were consumed.

The ascorbic acid percentage recovery during the assay procedure was calculated from data shown in Table 4.18. The mean percentage recovery found was of 97.73%.

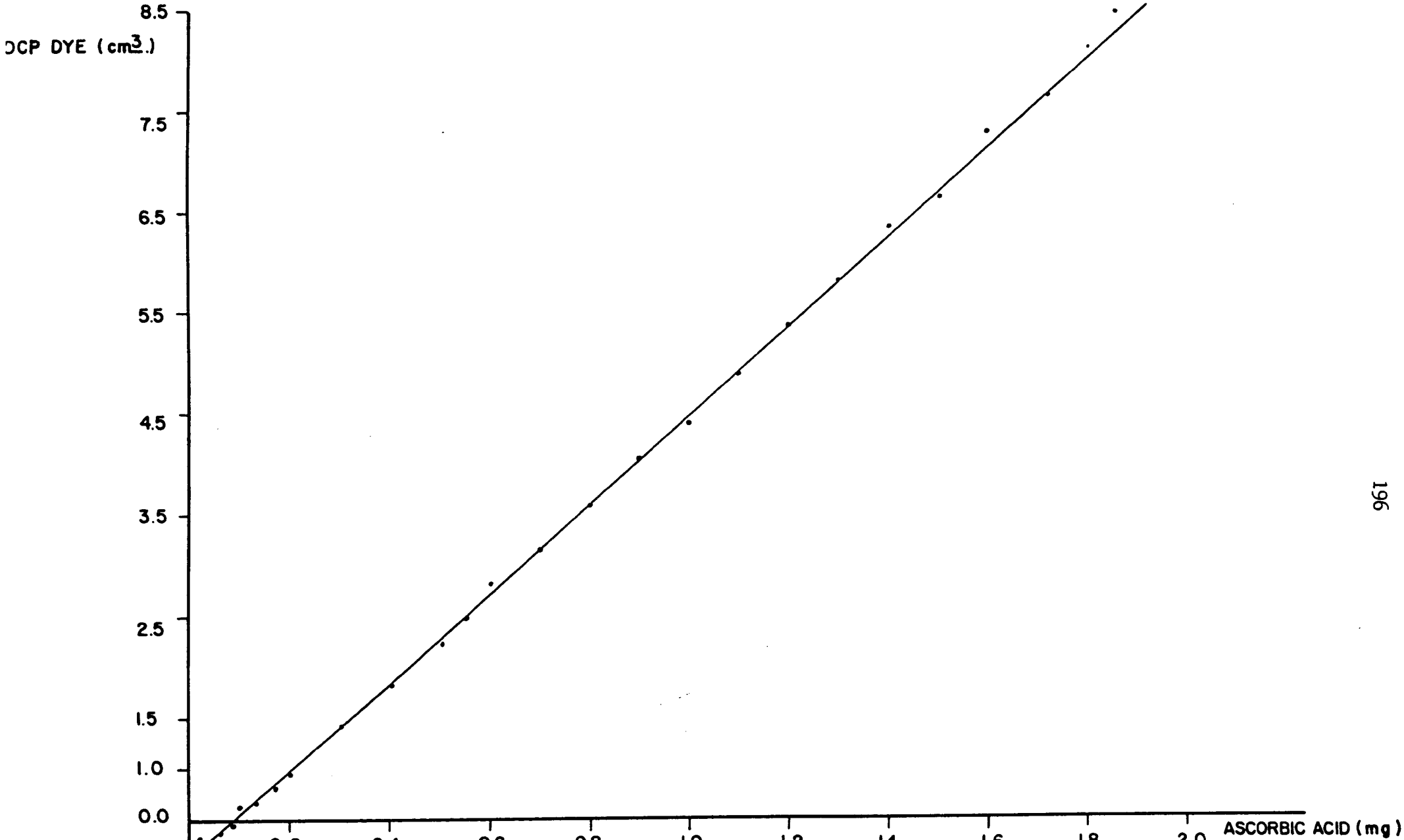


FIG.4.14 STANDARD CURVE FOR THE 2,6-DICHLOROPHENOL-INDOPHENOL VISUAL TITRATION

Sample	Milligrams of Ascorbic Acid			% Recovery
	Standard	Sample	Sample plus Standard	
BS_1	0.150	0.163	0.302	96.76
BS_2	0.100	0.149	0.243	97.80
PC 1	0.050	0.045	0.093	97.47
PC 2	0.050	0.0315	0.084	98.98
$\bar{X}$				97.73
σ				±1.35
S.E.				±0.39

Each individual value is the mean of three determinations

B.S. Brussels sprouts
P.C. Pork casserole

Table 4.18 Ascorbic acid recovery during DCP visual titration procedure

4.7.2 Evaluation of the Fluorimetric Procedure

The evaluation of this method was performed in two stages; the determination of the relationship between fluorescence developed and ascorbic acid concentration and the recovery of ascorbic acid during the assay procedure.

(i) Experimental Procedure

(a) Standard Curve

To check the relationship of fluorescence develop-

ment and ascorbic acid concentration in the range of interest thirteen dilutions were prepared from the ascorbic acid standard solution, ranging between 5 ng of AA/cm^3 to 120 ng of AA/cm^3 and treated as described in Appendix 2.2.

(b) Recovery of Ascorbic Acid during Assay Procedure

This assay procedure was only used for brussels sprouts samples. Therefore, for the recovery studies, known volumes of ascorbic acid were added to brussels sprouts extract solutions after maceration. The ascorbic acid in the solutions was oxidized with norit to dehydro ascorbic acid, which was measured fluorimetrically after reaction with OPDA as described in Appendix 2.2.

(ii) Results and Discussion

The standard curve obtained for OPDA fluorimetric procedure is shown in Figure 4.15. There it can be seen that a linear relation was found between 5 ng/cm^3 and 90 ng/cm^3 of ascorbic acid. Concentration above 90 ng/cm^3 of ascorbic acid depart from the linear relationship; therefore it was decided that the working concentration range should be under 80 ng/cm^3 of ascorbic acid. This also was the internal standard concentration used to calibrate the fluorimeter.

The percentage recovery of ascorbic acid obtained during the assay procedure was 97.2%, which figure was calculated from the data shown in Table 4.19.

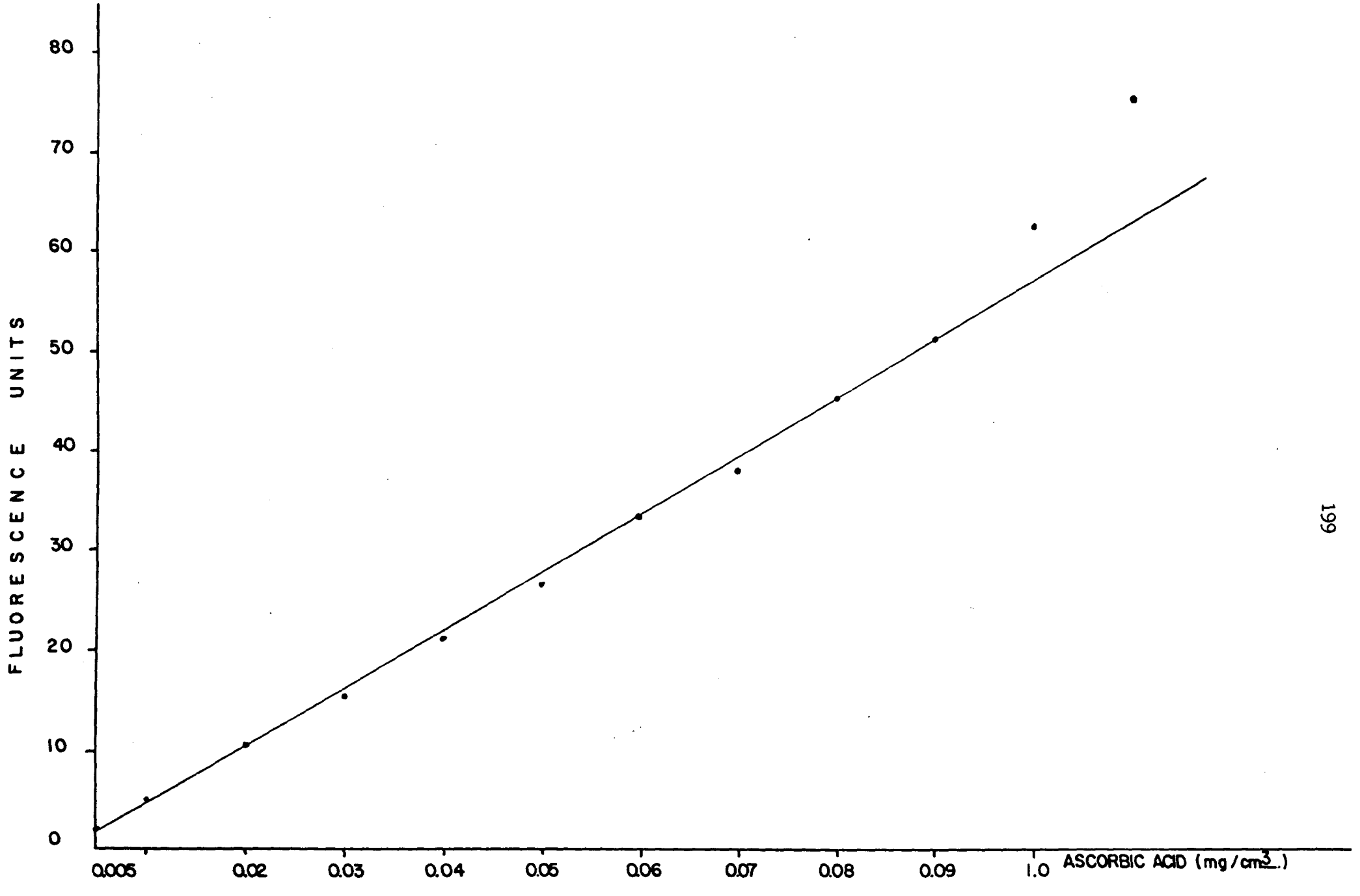


FIG. 4.15 STANDARD CURVE FOR THE O-PHENYLENEDIAMINE FLUORIMETRIC PROCEDURE.

Sample	Milligrams of Ascorbic Acid			% Recovery
	Standard Added	Sample	Sample plus Standard	
BS-4	0.150	0.143	0.2828	96.31
BS-7	0.150	0.162	0.302	96.98
BS-10	0.150	0.151	0.298	98.77
BS-12	0.150	0.163	0.300	95.81
BS-13	0.150	0.159	0.303	98.15
$\bar{X}_i$				97.204
σ				±1.509
S.E.				±0.302

Table 4.19 Ascorbic acid percentage recovery during the OPDA fluorimetric procedure

4.7.3 Comparison of Vitamin C Assay Methods

The performance of both assay methods was compared using brussels sprouts as the test material.

(i) Experimental Procedure

Miscellaneous samples were stabilized in a 5% (w/v)HPO_3 extraction solution and homogenized for 3 minutes. The resultant extract solution was used for the determination of ascorbic acid content using both the DCP visual titration and the OPDA fluorimetric procedure. In the latter, ascorbic acid content was obtained by difference after obtaining total vitamin C (ascorbic acid plus dehydroascrobic acid) and dehydroascorbic acid content.

(ii) Results and Discussion

The results obtained are given in Table 4.20, where it can be seen that the percentage agreement between the procedures was 99.82%. The coefficient of correlation obtained between both procedures was 0.9991 which also shows the good agreement between both methods.

4.8 Evaluation of the Vitamin B_1 Assay Method

The procedure chosen for the determination of vitamin B_1 was the fluorimetric procedure, proposed by Jansen, (1936). This method is one of the recommended methods of the A.O.A.C., (1975). Following the recommendations of A.O.A.C., (1975), Douglas and Hennessy, (1941), Edwin et al, (1975), Freed, (1966), Hennessy, (1942) and Voight and Eitenmiller, (1978), the procedure was slightly modified. The full description of the method used is given in Appendix 2.3.

The evaluation and setting up of this method was performed in three stages; checking fluorescence linearity against thiamine concentration, determination of whether the base exchange purification procedure was needed and the percentage recovery of added thiamine during the assay method.

(i) Experimental procedure

(a) Standard curve

A standard curve was prepared to check fluorescence linearity in the working range of concentrations. Several

Sample No.	Milligrams of Ascorbic Acid per 100 grams of Sample (Brussels Sprouts)				% Agreement
	DCP Visual Titration Method	OPDA Fluorimetric Procedure			
	AA	Total	DAA	AA	
1	35.76	39.52	4.19	35.33	98.82
2	33.82	34.95	2.07	32.88	97.22
3	43.39	45.24	3.98	41.26	95.09
4	42.98	43.12	0.56	42.56	99.03
5	52.38	56.83	3.15	53.68	102.48
6	56.57	57.05	0	57.05	100.84
7	5.94	7.16	0.85	6.31	106.05
8	5.66	6.70	0.70	6.00	102.47
9	1.90	1.91	0	1.91	100.52
10	1.86	1.78	0	1.78	95.69
$\bar{X}$					99.82
σ					±3.37
S.E.					±0.76
C.C.					0.9991

Table 4.20 Comparison of performance of DCP visual titration and OPDA fluorimetric procedure

dilutions were prepared from the standard thiamine solution obtained as described in Appendix 2.3. The dilutions were prepared using 38 cm^3 of 0.2 N HCl and distilled water to make up to 100 cm^3.

The range of concentrations covered was from 3 $\mu g/cm^3$ to 0.5 $\mu g/cm^3$ of thiamine.

(b) Base exchange purification procedure

To evaluate whether there was a need for the base

exchange purification procedure as suggested by Freed, 1966, Glick, 1944, Hennessy and Cerecedo, 1939, Ministry of Food, 1942 and Rice and Beuk, 1945, duplicates of two standard solutions and three samples (pork casserole) were prepared, i.e. two sets of five identical solutions. One group of solutions was treated by the base exchange purification procedure (described in Appendix 2.3 section B) and was omitted for the second set. Thiochrome was developed and measured as described in Appendix 2.3.

(c) Thiamine percentage recovery during the assay method

Known volumes of a standard thiamine solution were added to homogeneous samples in duplicate before the extraction stage. the level of thiamine added to the sample extract solutions was similar to the expected thiamine content in the sample. Four different samples were weighed in duplicate. To one set of duplicates, 10 cm^3 of thiamine standard solution were added, while for the second set 10 cm^3 of distilled water were added instead. Thiamine was extracted and thiochrome was developed and measured as described in Appendix 2.3, omitting the base exchange purification procedure.

(ii) Results and Discussion

The standard curve obtained is shown in Figure 4.16, which indicates that there is a clear linear relationship for the range of concentrations used.

The results of thiamine recovery from the ion exchange purification procedure are presented in Table 4.21. The mean percentage recovery found was 94.62%, although the results obtained for the various samples showed a significant scatter,

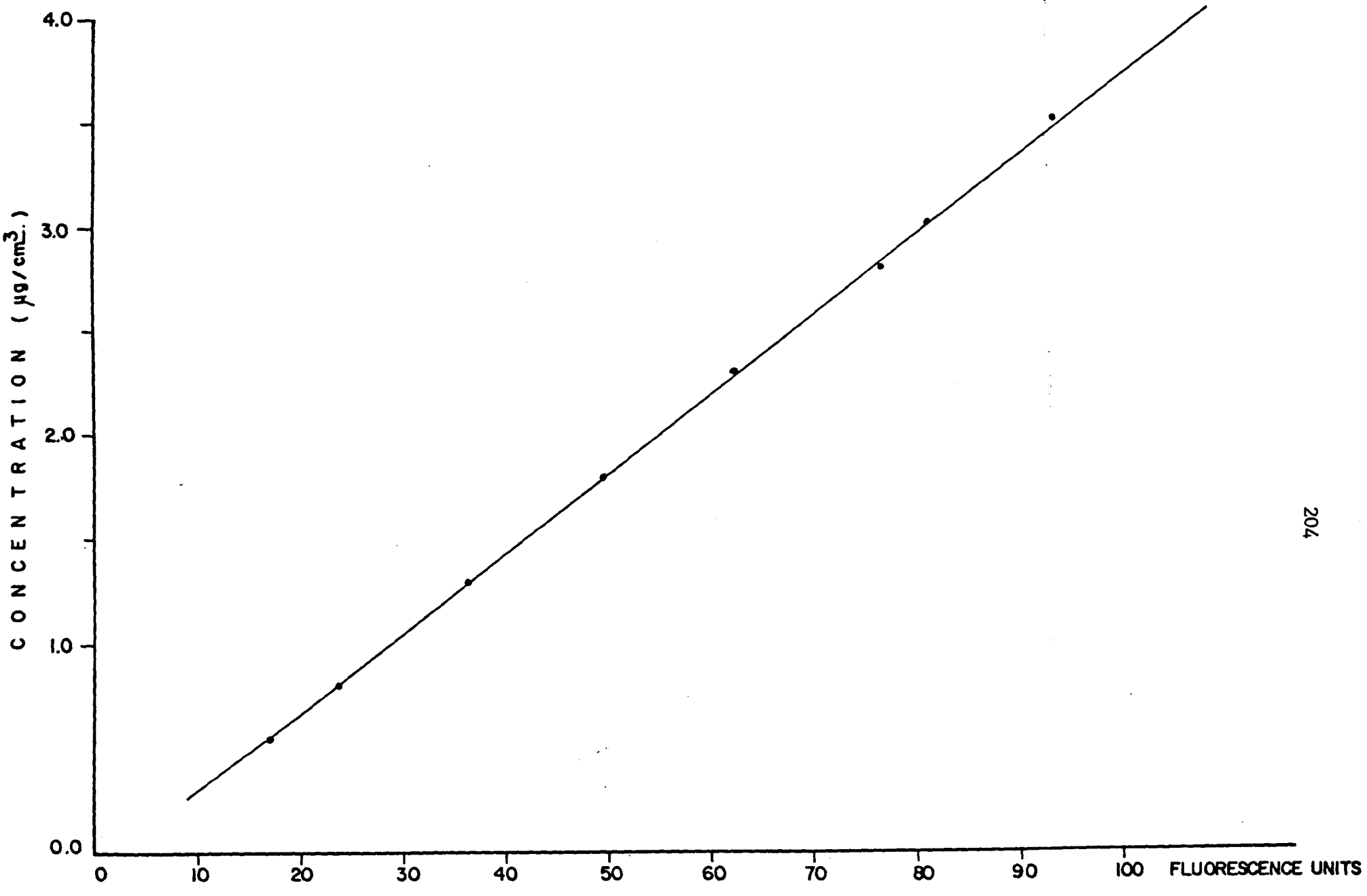


FIG. 4.16 STANDARD CURVE FOR THIAMINE FLUORIMETRIC DETERMINATION.

Sample No.	Micrograms of Thiamine in Sample, Measured		% Recovery
	Without Ion-Exchange Procedure	With ion-Exchange Procedure	
Standard Solutons			
1	16.00	14.66	91.64
2	13.00	12.26	94.31
Food Samples			
3	6.72	6.32	97.10
4	3.78	3.41	94.65
5	7.50	7.15	95.39
$\bar{X}$			94.62
σ			±3.04
S.E.			±0.78

All values reported are the mean of three determinations

Table 4.21 Recovery of thiamine with the ion-exchange purification procedure

as shown by the calculated standard deviation (σ = ±3.04). Also it was found that the recovery of the standard was lower than the recovery of the sample extracts. These findings, together with the very low blank values obtained for the sample extracts, suggested that there was no evidence of the presence of interfering materials which could be removed by the ion exchange purification procedure (Andrews and Nordgren, 1941, Glick, 1944, Hennessy and Cerecedo, 1939, Ministry of Food, 1942 and Rice, 1945). Therefore, there was no benefit in using this procedure when recovery of thiamine from the ion-exchange column was so erratic. Consequently, in all further studies, the ion-exchange procedure was omitted. According to Freed, (1966), this may eliminate the possibility of any loss of thiamine due to incomplete absorption and elution and decrease errors of manipulation.

The recovery of thiamine through the assay procedure, omitting ion-exchange, was also investigated. The results are presented in Table 4.22. There the mean percentage recovery found was 97.41%.

Sample No.	Micrograms of Thiamine			% Recovery
	Standard	Sample	Sample plus Standard	
1	3.60	4.38	7.88	98.76
2	3.60	5.06	8.80	96.07
3	8.00	9.50	16.87	96.37
4	10.00	10.18	19.87	98.46
$\bar{X}$				97.41
σ				±1.67
S.E.				±0.48

Each individual value is the result of triplicate determinations on a pork casserole extract.

Table 4.22 Recovery of thiamine in the assay procedure

4.9 Evaluation and Comparison of Available Lysine Assay Procedures

Two different methods were used for available lysine assay determination. One was Carpenter, (1960), 2,4 fluorodinitro-benzene , (FDNB), direct determination as modified by Booth, (1971) and the second was the acid orange -12, (AO-12), dye binding capacity method as improved by Hurrell and Carpenter, (1979). Both methods are fully described in Appendices 2.4 and 2.5 respectively. Both determinations were set up and their performances evaluated using standard proteins, casein and sodium caseinate. The results obtained were used later to compare both methods.

4.9.1 Evaluation of FDNB Direct Method

Preliminary tests were carried out with this procedure to determine overall performance and conditions of the analytical assay method. Nitrogen content in all samples was determined by the Macro Kjeldahl method as described in Appendix 2.7, since the results were expressed in terms of millimoles or milligrams of available lysine per gram of nitrogen.

(i) Experimental Procedure

(a) Standard curve

To assess the relationship between colour measurement and ε-DNP-lysine added, several dilutions of ε-DNP-lysine standard solutions were prepared contianing from 0.5×10^{-3} mg/cm^3 to 23×10^{-3} mg/cm^3 of ε-DNP-lysine HCl. The absorbance of these solutions was read against distilled water at 435 nm.

(b) Available lysine in both casein and sodium caseinate

Samples of approximately 0.2 grams were used to determine the available lysine of casein and sodium caseinate. All determinations were carried out in duplicate.

(c) Recovery of ε-DNP-lysine after ether extraction

Known volumes of ε-DNP-lysine concentrated standard solution were added to sodium caseinate hydrolyzates before ether extraction. The volumes added to the sample contained similar levels of lysine to the calculated available lysine value for the sample.

(d) Recovery of ε-DNP-lysine after hydrolysis and ether extraction

Several 0.2 grams sodium caseinate samples were treated with FDNB as described in Appendix 2.4. Before acid hydrolysis, instead of 30 cm^3 of 8.1 N HCl, 20 cm^3 of 8.1 N HCl were added followed by 10 cm^3 of ε-DNP-lysine standard concentrated solution dissolved in 8.1 N HCl.

(e) Recovery of ε-DNP-lysine in presence of starch and glucose

Several samples were prepared using 0.2 grams of sodium caseinate and 1.2 grams of starch or glucose. The samples were treated with FDNB and 20 cm^3 of 8.1 N HCl followed by 10 cm^3 of concentrated ε-DNP-lysine standard solution, were added before hydrolysis. After hydrolysis the resulting ε-DNP-lysine was measured following the normal procedure.

(f) Effect of thiodiglycodic acid on the recovery of ε-DNP-lysine in the presence of starch or glucose

Samples were prepared containing 0.20 grams of sodium caseinate and 1.20 grams of glucose or starch, and then treated with FDNB. Before hydrolysis and after the addition of 20 cm^3 of 8.1 N HCl and 10 cm^3 of ε-DNP-lysine standard soltuion, 1.0 gram of thiodiglycolic acid was added. The solution was mixed by hand and the analysis completed as described in Appendix 2.4.

(g) Recovery of ε-DNP-lysine in the presence of rice pudding

Several samples of rice pudding ranging from 1.8 grams to 2.3 grams were treated with FDNB. Before hydrolysis, 25.0 cm^3 of 8.1 N HCl followed by 5.0 cm^3 of ε-DNP-lysine concentrated solution were added. Analysis was completed as described in Appendix 2.4.

(h) Recovery of ε-DNP-lysine from added sodium caseinate in the presence of rice pudding

Several duplicate samples of homgenised rice pudding, between 1.5 grams and 2.0 grams, were weighed and then 0.10 grams of sodium caseinate were added. Those operations were followed by treatment with FDNB and hydrolysis with 6 N HCl. The procedure was completed following the standard operations.

(ii) Results and Discussion

The standard curve obtained for ε-DNP-lysine is shown in Figure 4.17. The straight line obtained indicates that Beer's law is obeyed over the range of concentrations investigated.

Table 4.22 contains the results obtained for casein and sodium caseinate samples when measuring available lysine using Carpenter's method. Each determination was carried out in

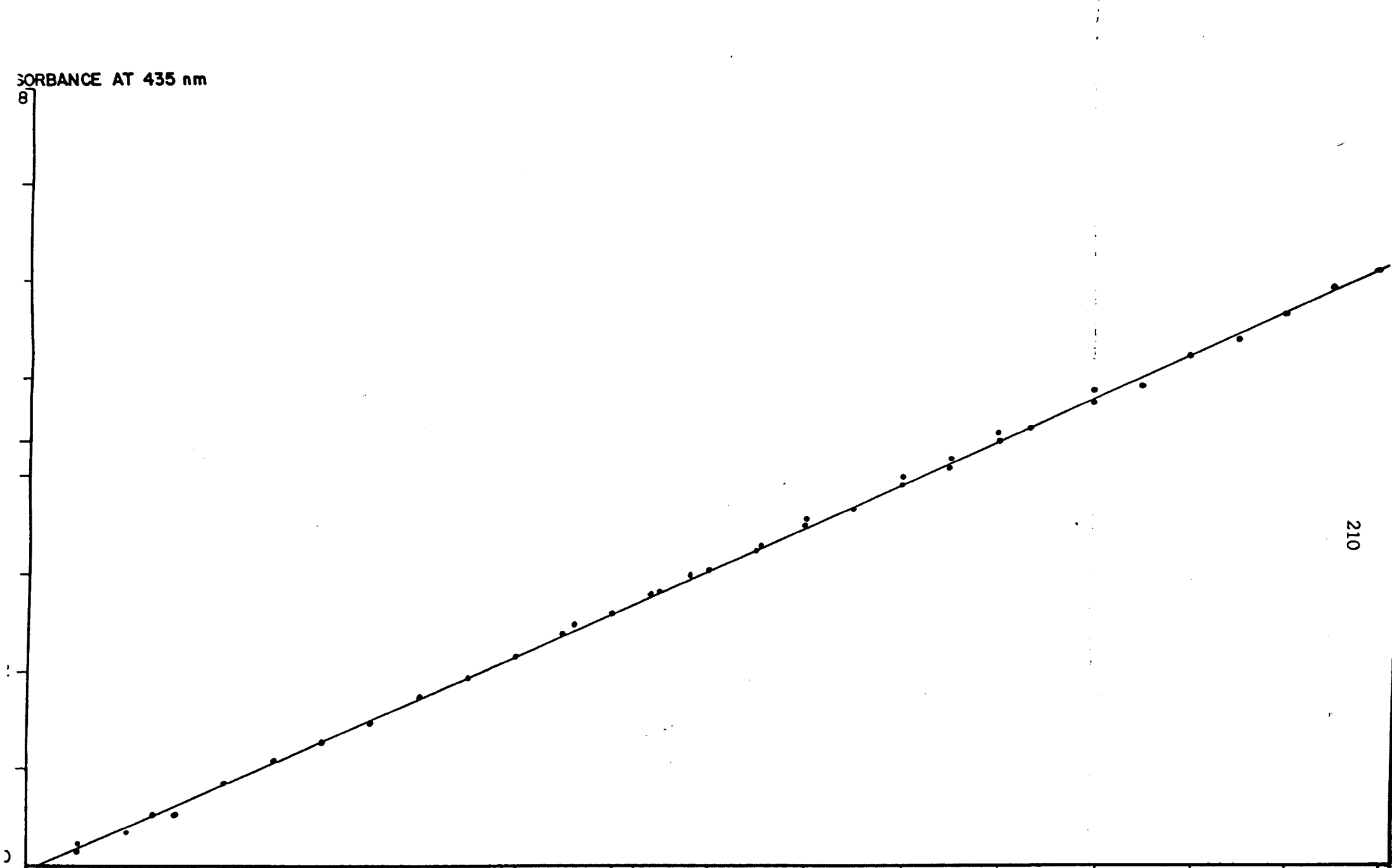


FIG. 4.17 STANDARD CURVE FOR ε-DPN-LYSINE.

duplicate. The values given are not corrected with the recovery factor and were used as such for the evaluation of the assay procedure.

	milli moles of available lysine/ gram of nitrogen	
	Casein	Sodium Caseinate
$\bar{X}$	2.8807	2.9908
n	23	24
σ	±0.1625	±0.0984
S.E.	±0.0332	±0.0205
C.V.	±5.43%	±3.41%

Table 4.23 **Available lysine in Casein and Sodium Caseinate using Carpenter's FDNB direct procedure**

The standard deviations, standard errors and coefficients of variation are also given in Table 4.23. These values show good reproducibility of the assay procedure with slightly more variability for the Casein samples.

The results obtained for the assessment of losses of ε-DNP-lysine due to the ether extraction are given, with their corresponding % recovery, in Table 4.24 These results show that losses of ε-DNP-lysine during the ether extraction are less than 0.5%.

The losses of ε-DNP-lysine obtained during acid hydrolysis and ether extraction are given in Table 4.25, from which it can be seen that the loss is of the order of 6.6%. These results show that the hydrolysis procedure alone produced approximately a 6.0% loss.

Sample No.	Absorbance at 435 nm Standard	Sample	Sample Plus Standard	% Recovery
1	0.464	0.34	0.888	98.77
2	0.464	0.17	0.881	99.99
3	0.464	0.443	0.908	100.05
4	0.464	0.443	0.894	98.67
5	0.464	0.428	0.889	99.69
6	0.464	0.443	0.904	99.67
7	0.464	0.436	0.897	99.52
8	0.464	0.412	0.884	101.27
9	0.464	0.407	0.870	99.77
10	0.464	0.427	0.880	98.76
$\bar{X}$	0.464	0.429	0.889	99.621
σ	0.0	±0.013	±0.012	±0.776
S.E.		±0.004	±0.004	±0.246

All samples were done by duplicate

Table 4.24 Recovery of ε-DNP-lysine after ether extraction of the hydrolyzate

The recovery of ε-DNP-lysine in the presence of starch and glucose was also investigated. The results are presented in Table 4.26. The mean recovery of ε-DNP-lysine in the presence of glucose, in a ratio similar to the expected ratio of starch and sugar to protein in the rice pudding (six to one), was 90.88%. The losses seem to be greater when starch was added; the mean recovery value obtained in this case being 85.06%. The effect of thiodiglycolic acid (TDG) on these recoveries was also investigated. The results are presented in Table 4.27, where it

Sample No.	milligrams of available lysine 100 g of sample: Standard	Sample	Sample Plus Standard	% Recovery
1	5977.0	6125.84*	11 296.25	93.34
2	5977.0	6125.84	11 140.66	92.05
3	5977.0	6125.84	11 281.25	93.27
4	5977.0	6125.84	11 257.81	93.02
5	5977.0	6125.84	11 343.75	93.73
6	5977.0	6125.84	11 500.00	95.02
7	5977.0	6125.84	11 562.00	95.54
8	5977.0	6125.84	11 125.00	91.92
$\bar{X}$			11 434.51	93.486
σ			±219.025	±1.804
S.E.			±77.668	±0.639

* Value calculated from mean available lysine obtained for sodium caseinate in Table 4.23.

All samples were done by duplicate

Table 4.25 Recovery of ε-DNP-lysine after hydrolysis and ether extraction

can be seen that the mean percentage recoveries are slightly higher than when no thiodiglycolic acid was added. The mean percentage recovery values obtained when glucose and TDG were present is 92.02%, while 87.28% was obtained when starch and TDG were present.

To search for significant differences between the effects of thiodiglycolic acid, glucose and starch on the recovery of ε-DNP-lysine, statistical analysis was used. The results were arranged

Sample No.	Sodium Caseinate mg available lysine / g N_2	In presence of glucose sample mg available lysine / g N_2	% Recovery	In presence of starch sample mg available lysine / g N_2	% Recovery
1	434.20*	394.19	90.78	361.25	83.99
2	434.20	387.16	89.16	372.34	85.76
3	434.20	396.15	91.23	366.79	84.47
4	434.20	395.16	91.23	366.79	84.47
5	434.20	392.54	90.41	387.67	89.28
6	434.20	401.67	92.50	355.45	85.06
$\bar{X}$		394.478	90.885	368.85	85.06
σ		±4.741	±1.101	±10.166	±2.261
S.E.		±1.935	±0.449	±4.149	±0.923

* Calculated from mean available lysine in sodium caseinate obtained in Table 4.23

Table 4.26 Recovery of ε-DNP-lysine in the presence of glucose and starch

as shown in Table 4.28 for analysis of variance, for which summarized results are given. The M.S.R. value calculated, 14.0, exceeds the estimated tabular value, 4.82, at 99.0% confidence level. This shows that significant differences are present.

No.	Sodium Caseinate mg available lysine / g N_2	In the presence of glucose mg available lysine / g N_2	% Recovery	In the presence of starch mg available lysine / g N_2	% Recovery
1	434.20	398.86	91.86	361.25	83.19
2	434.20	407.61	93.87	379.19	87.73
3	434.20	382.03	87.98	381.67	87.90
4	434.20	409.51	94.31	385.24	88.72
5	434.20	406.22	93.56	368.79	84.94
6	434.20	396.48	91.31	396.12	91.23
7	434.20	396.41	91.29	377.87	87.56
$\bar{X}_1$		399.588	92.026	378.71	87.285
σ		9.446	2.177	12.313	2.845

* Calculated from the mean available lysine value obtained for sodium caseinate in Table 4.23

Table 4. 27 Effect of thiodiglycolic acid on the recovery of ε-DNP-lysine in the presence of starch or glucose

The student's (t) test was used to seek these differences. The results shown in Table 4.28, when underscored by the same line, indicate that no significant difference exist. Therefore, recovery values in presence of both glucose and glucose with thiodiglycolic acid are not significantly different. The same happens between samples with both starch and starch with

Sample No.	mg of Available Lysine/g of N_2 in sodium caseinate assayed in presence of:-			
	Glucose	Starch	Glucose Thiodiglycolic Acid	Starch and Thiodiglycolic Acid
1	394.19	361.25	398.86	361.25
2	387.16	372.34	407.61	379.19
3	396.15	366.79	382.03	381.67
4	395.16	366.79	409.51	385.24
5	392.54	387.67	406.22	368.79
6	401.67	355.45	396.48	396.12
7	-	-	396.41	377.87
$\bar{X}$	394.48	368.85	399.59	378.71
σ	±4.74	±10.16	±9.44	±12.31

ANALYSIS OF VARIANCE TABLE

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	Mean Square Ratio		Confidence Level
				Calculated	Tabular	
Between glucose, starch and thiodiglycolic acid	3	4158.635	1386.212	14	4.82	99%
Residual	22	2041.841	92.811			
Total	25	6200.476				

Least significant differences.

Comparison of sample means

Starch	Starch + TDG	Glucose	Glucose + TDG
368.85	378.71	394.486	399.59

Table 4.28 Analysis of variance for data from Tables 4.26 and 4.27

thiodiglycolic acid. Significant differences were found between samples with glucose and samples with starch. From these results it can be seen that no significant beneficial effect was found on ε-DNP-lysine recovery in presence of glucose or starch when thiodiglycolic acid was used. These findings agree with Carpenter's (1973) who was unable to confirm the claim of Lyman and Thomas (1965) that the addition of thiodiglycolic acid reduced the destruction of DNP-lysine due to carbohydrates presence during the assay procedure.

The results obtained to evaluate the losses of standard ε-DNP-lysine and ε-DNP-lysine from added sodium caseinate in the presence of rice pudding are shown in Table 4.29. These results show that the losses of standard ε-DNP-lysine.HCl are greater than the losses of sodium caseinate ε-DNP-lysine. Also the standard deviation (σ) values show that the results obtained for ε-DNP-lysine from added sodium caseinate are more reproducible than the results obtained with standard ε-DNP-lysine solution.

These findings agree with Ruderus and Kehlherg, (1970) but other authors (Carpenter, 1973 and Booth, 1971) claimed that soluble and easily digested proteins such as casein are unsuitable recovery agents for vegetable origin foods and meat and fish meals. Nevertheless, Booth, (1971) suggested that an ideal recovery marker should resemble the protein being analyzed which is the case of casein for rice pudding samples. Therefore, in any further studies sodium caseinate was used as the recovery agent.

Rice Pudding m moles/g of N_2	Rice Pudding and ξ-DNP-lysine.HCl			Rice Pudding and Sodium Caseinate		
	m moles of Lysine/ g of N_2		% Recovery	m moles of Lysine/ g of N_2		% Recovery
	Expected	Experimental		Expected	Experimental	
2.4572	6.94	5.95	85.73	8.29	7.38	89.02
2.5752	6.29	5.48	87.12	7.92	7.07	89.26
2.3609	6.90	5.87	84.78	7.91	6.99	88.37
2.3934	6.43	5.59	80.02	7.89	6.87	87.07
2.4237	6.86	5.45	82.53	8.20	7.22	88.04
2.5402	7.05	6.12	86.80	8.65	7.54	87.16
2.5682						
2.3124						
2.4808						
$\bar{X}_1$ 2.4568			84.33			88.153
σ 0.0929			3.748			± 0.9161

Table 4.29 Recovery of standard ξ-DNP-lysine and ξ-DNP-lysine from sodium caseinate in the presence of rice pudding

4.9.2 Evaluation of Acid Orange-12 Dye Binding Capacity Method

Several preliminary tests were carried out with this assay procedure to determine the overall performance and conditions for analysis. The tests were carried out with sodium caseinate and casein as standard protein samples.

(i) Experimental Procedure

(a) Standard curve

A standard curve was prepared by diluting the dye with buffer solution to several concentrations

and the absorbance of these dilutions was measured at 482 nm. The standard curve was used to check the linearity of the readings in the range of concentrations used.

(b) Determination of the relationship between dye bound and reaction time.

Several duplicate samples of sodium caseinate were treated with AO-12, varying the time of shaking necessary to bind the dye with the protein. All samples had approximately the same weight.

(c) Determination of the relationship between protein weight and amount of dye bound

Several pairs of sodium caseinate samples were prepared covering a wide range of weights, to investigate the effect of varying the amount of protein used for the assay procedure. One sample of each pair was treated with AO-12 to obtain the HARL-values, (Histidine, Arginine and Lysine), while the second set of samples was treated with propionic anhydride, prior to the AO-12 treatment, to determine the HAR-values, (Histidine and Arginine). The analysis was carried out, as described in Appendix 2.5, maintaining all conditions constant but the sample weights.

(d) Available lysine in sodium caseinate and casein

Several samples of casein and sodium caseinate were treated to obtain both HARL- and HAR-values. These values were subtracted to obtain the available lysine present in the samples. The weights of samples

used were as determined above (see results on this section). The results obtained were used later for the comparison of performance between the dye binding capacity method and the FDNB direct procedure.

(ii) Results and Discussion

The standard curve obtained for AO-12 dye absorbance at 482 nm is given in Figure 4.18. There it can be seen that Beer's law is followed in the range of concentrations investigated.

The relationship between dye bound and reaction time is shown in Figure 4.19. These results show that, for sodium caseinate samples, a minimum of 5 hours is necessary to complete the dye binding. Also, the dye binding reaches a maximum after 8 hours of reaction time by shaking. In accordance with these findings, the time of shaking selected in all subsequent studies was 5 hours.

Figure 4.20 shows the relationship obtained between dye bound and sample weight used. The results show an "S" shape relationship. The limits within which a linear relationship is followed are from 2 to 3 millimoles of dye bound per dm^3, which corresponds to 0.075 grams to 0.125 grams of sodium caseinate per 40 cm^3 of standard dye solution (see Appendix 2.5).

Figure 4.21 shows the same relationship but expressed as millimoles of basic residues bound in sodium caseinate (HARL-value) and excess of AO-12 dye

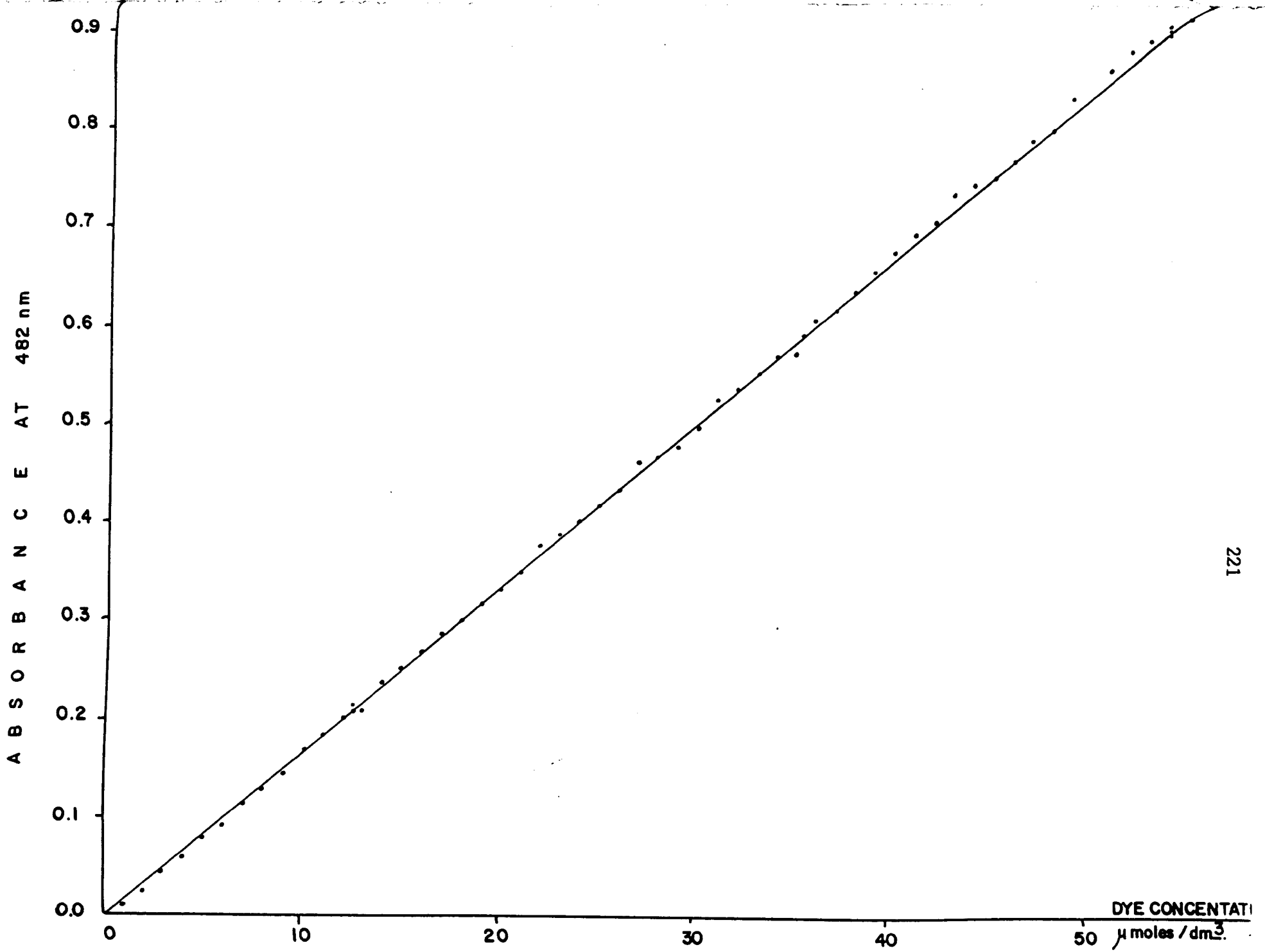


FIG.4.18 STANDARD CURVE FOR ACID ORANGE-12 DYE BINDING CAPACITY

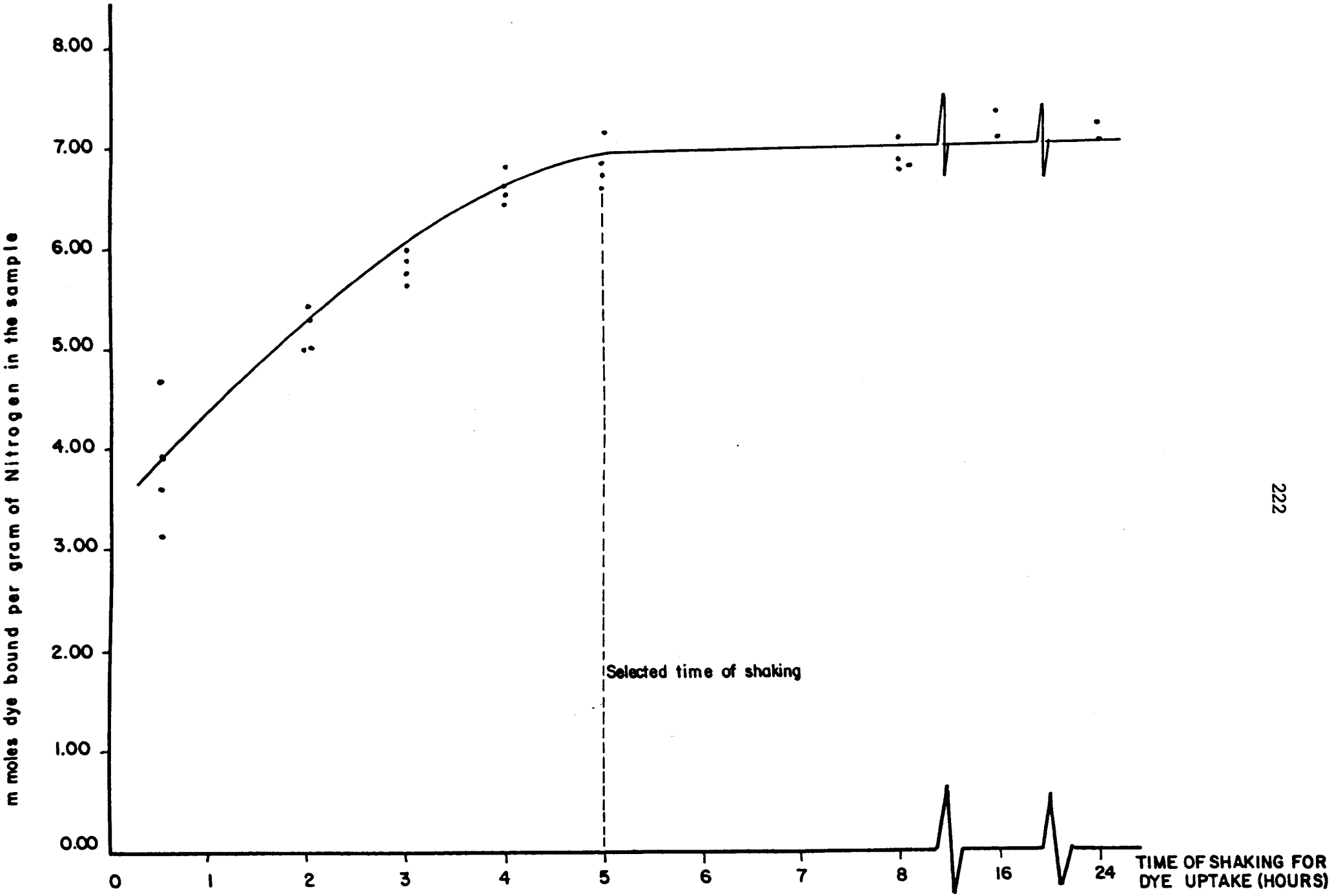


FIG.4.19 RELATIONSHIP BETWEEN ACID ORANGE-12 BOUND AND REACTION TIME.

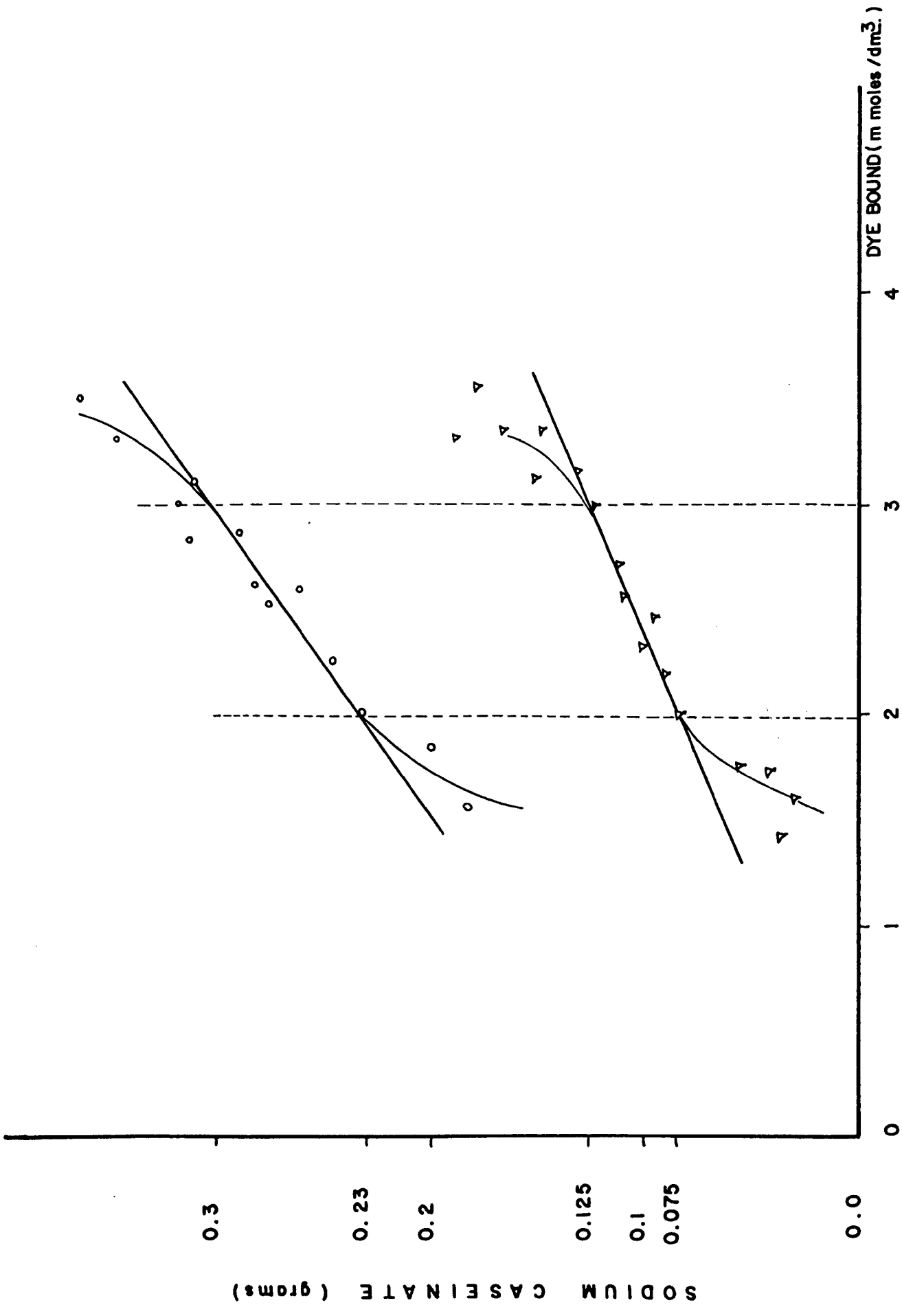


FIG. 4.20 RELATIONSHIP BETWEEN ACID ORANGE-12 BOUND AND THE NUMBER OF BASIC RESIDUES IN cm^3. OF DYE (3.89 m moles/dm^3.)

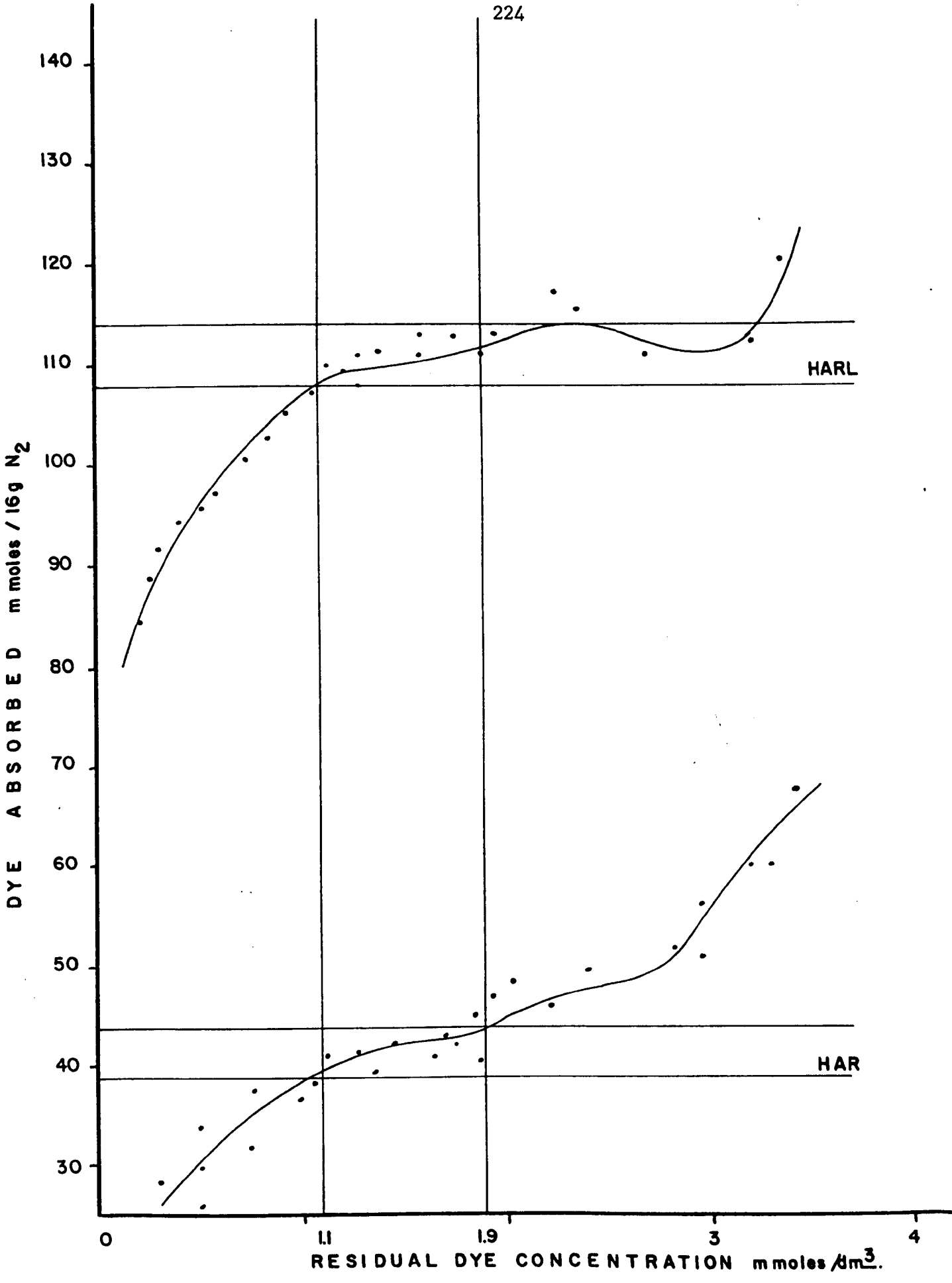


FIG. 4.21 EFFECT OF RESIDUAL ACID ORANGE-12 CONCENTRATION IN THE AMOUNT OF DYE BOUND.

present. These results show that, when a greater amount of sample is used, a lower quantity of dye is bound per gram of sodium caseinate or if a small weight of sample is used, a high HARL-value is obtained. Hurrell and Carpenter (1979) recommended 1.2 to 1.8 m moles of residual dye/dm^3 as working limits. Figure 4.21 shows good agreement with this recommendation, indicating a slow and steady increase of dye bound as the level of excess dye increases between 1.35-2.0 m moles/dm^3. Outside these limits the response of millimoles of basic residues bound per 16 g of nitrogen, with increasing excess dye concentration is greatly raised.

The values obtained for available lysine in casein and sodium caseinate samples are presented in Table 4. 30 together with the HARL- and HAR-values obtained for each sample. From this table it can be seen that the casein results are very erratic as shown by the coefficients of variation (C.V.) obtained. Casein presented severe problems during the dye uptake shaking period, producing an incomplete AO-12 dye binding with the protein, because it did not disperse in the dye solution. These findings lead to the elimination of casein as standard protein sample, leaving only sodium caseinate for the evaluation and comparison studies of the available lysine assay procedures.

Sample No.	Casein values in m moles/g N_2			Sodium caseinate values in m moles/g N_2		
	HARL-value	HAR-value	Available Lysine	HARL-value	HAR-value	Available Lysine
1	6.23	2.98	3.26	6.71	3.12	3.59
2	6.09	2.85	3.23	6.65	2.95	3.70
3	7.35	2.82	4.53	6.80	3.28	3.52
4	5.23	1.80	3.43	6.65	3.98	3.67
5	4.14	2.44	1.70	7.02	3.16	3.86
6	2.13	1.93	0.19	6.66	3.01	3.65
7	1.34	2.04	0.70	6.38	2.89	3.49
8	2.22	1.46	1.45	6.51	3.26	3.25
9	4.42	1.97	2.45	6.48	3.19	3.29
10	6.25	1.89	4.35	6.52	2.90	3.62
11	5.26	1.72	3.54	6.75	3.16	3.59
12	4.72	1.43	3.29	6.66	3.23	3.43
13	5.70	1.87	3.83	6.43	3.06	3.37
14	5.36	1.86	3.50	6.80	3.35	3.46
15	5.06	2.07	2.98	6.92	3.34	3.59
16	6.98	2.72	4.26	6.70	3.04	3.66
$\bar{X}$	6.98	2.12	2.98	6.67	3.12	3.54
s	1.77	0.49	1.39	0.17	0.15	0.16
c.v.	25.36%	23.11%	46.64%	2.55%	4.8%	4.51%

Table 4.30 HARL, HAR and available lysine values for casein and sodium caseinate using the dye binding capacity procedure

4.9.3 Determination of Available Lysine by Indirect FDNB Method

This method was used to determine the total and the unavailable lysine content in the sodium caseinate and rice

pudding samples. The results obtained with this assay procedure were used to compare with the performances of the AO-12 dye binding capacity and the FDNB direct method.

(i) Experimental Procedure

The samples were hydrolyzed in 6 N HCl solutions during 24 hours. The total lysine and the available lysine content of the hydrolyzates were determined by a technical service department using an automatic amino acid analyzer. This amino acid analyzer was a Beckman model 120C linked to an integrator for the for the analysis of the results. This technique was used only as a reference and not as a routine assay.

(ii) Results and Discussion

The results obtained for sodium caseinate using the FDNB indirect assay for available lysine were 3.955 m moles of total lysine per gram of nitrogen and 0.510 m moles of unavailable lysine per gram of nitrogen. These values are the mean of three determinations and were used to compare the performances of the AO-12 dye binding capacity, FDNB direct determination and the FDNB indirect assay procedure, as indicated below.

The available lysine content on sodium caseinate using both FDNB direct procedure and AO-12 dye binding capacity were given in Tables 4.22 and 4.29, respectively. The mean value for available lysine obtained by the FDNB direct method, 2.99 m moles per

gram of nitrogen, needed to be corrected with a recovery factor. Booth, V. H., 1971, suggested a recovery factor of 1.05 while Carpenter, K. J., 1960, suggested 1.09. For the present work, the recovery factor was calculated from the mean value obtained for standard ξ -DNP-lysine when added to sodium caseinate. This value was reported in Table 4.23 as 93.5%. Therefore, the recovery factor used was 1.07, giving a mean of 3.2 m moles per gram of nitrogen for available lysine.

The values obtained with the AO-12 dye binding capacity assay and the FDNB indirect and direct procedure are shown in Table 4.31 There it can be seen that the three methods give reasonable agreement taking into account the following points: the FDNB direct method is expected to give a lower value than the FDNB indirect method which is known (Carpenter, 1973 and Mauron, 1979) to measure as lysine some of the early maillard products and the terminal lysine. Likewise, according to Hurrell (1981), in the dye binding method, there is some evidence that the deoxy ketosyl lysine residues bind the dye and some may also react with the propionic anhydride. This effect would increase the DBC-lysine value relative to FDNB direct procedure. It is interesting to note that the lysine in the sodium caseinate used was partly unavailable to FDNB. This is probably due to residual lactose present in the sample.

Method employed	Available lysine in millimoles/gram of N_2
FDNB direct determination	3.21
AO-12 dye binding capaicty	3.54
FDNB indirect procedure	3.44

Table 4.31 **Sodium caseinate available lysine mean values obtained with three assay procedures**

The results obtained with the AO-12 dye binding procedure were very encouraging as shown by the comparison of dye binding capacity lysine (DBC-lysine) and FDNB-reactive lysine obtained by both difference and direct methods with sodium caseinate samples. Unfortunately when this procedure was applied to the rice pudding samples (Table 4.32) large differences were found between DBC-lysine and FDNB-reactive lysine. The FDNB-reactive lysine presented always smaller values than the DBC-lysine values which were even larger than the total lysine content values (2.99 m moles/g of N_2). Consequently the Dye Binding Capacity Method was abandoned for any further studies with the experimental samples due to lack of time for further investigation of the method.

4.10 Evaluation of Colour Measurement of Rice Pudding

A colour measurement technique was used to measure the colour development of the rice pudding samples during processing and storage.

Determinations	Available lysine in milli moles of lysine/gram of N_2			
	DBC Capacity			FDNB direct detn FDNB-lysine Corrected Values
	HARL	HAR	DBC-Lysine	
1	6.28	2.71	3.57	2.36
2	6.05	3.04	3.01	2.32
3	6.18	2.31	3.87	2.34
4	6.33	2.82	3.51	2.29
5	6.45	3.65	2.80	2.29
6	6.39	3.26	3.03	2.32
$\bar{X}$				
σ				

Table 4.32 **Available lysine values for a rice pudding sample using both Dye Binding Capacity Method and Fluorodinitrobenzene direct method**

(i) Experimental Procedure

Colour was measured with a Colormaster, model V, differential colorimeter. This instrument can reproducibly measure C.I.E. (Commission Internationale de l'Eclairage) tristimulus values x, y and z directly.

The homogenous rice pudding sample was placed in a specially designed glass bottomed brass cup which fitted on to the Colormaster. Before measurement it was checked that no air bubbles were trapped between the sample and the bottom glass to obtain a homogeneous flat surface for colour measurement. The instrument

was zeroed using two standard white tiles. One of the tiles was replaced by the sample and the values for percentage reflectance of green (G), red (R) and blue (B) were obtained. A full description of the method used for colour measurement can be found elsewhere (Glasser and Troy, 1952).

To evaluate the reproducibility of the colour measurements, the colour of a sample was measured several times. The zero calibration of the instrument was altered and reset again before each measurement.

SAMPLE	G Value	R Value	B Value
1a	49.82	54.56	35.44
1b	49.92	54.66	34.02
1c	50.32	54.98	36.12
1d	49.82	54.23	35.04
1e	50.16	54.75	35.66
$\bar{X}_i$	50.008	54.636	35.256
σ	0.3097	0.2750	0.7934

Table 4.33 Reproducibility of colour measurements

(ii) Results

The results obtained for the colour measurement of a particular sample are shown in Table 4.33, which shows a high level of reproducibility.

CHAPTER 5

PRODUCTION AND EVALUATION OF FOOD SAMPLES

This chapter contains the central work of this dissertation which is intended to evaluate the differences in nutritional quality of thermally sterilized foods packaged in both pouches and cans. For this purpose it was considered that useful results could be obtained from a study carried out on real food systems with commercial potential as menu items.

5.1 Food Sample Production

For the selection and production of food samples, several factors were taken into account. One of the most important factors was the selection of the nutrients to be studied, since they had to be heat labile under processing and storage conditions, readily analysable and found in adequate quantities in the foods finally chosen.

The heat sensitivity and water solubility characteristics of vitamins C and B_1 make them the most vulnerable vitamins during sterilization conditions and long term storage. They were also chosen because their chemical analysis is well established. The third nutrient selected for analysis was lysine, as "available" lysine, because it is one of the essential and often limiting aminoacids in many foods. Lysine is labile during heat treatment and long term storage, particularly in the presence of reducing sugars. This degradation is due mainly to Maillard reactions, as discussed in chapter two.

Once the nutrients were chosen, the selection of appropriate food samples was carried out. This was done taking into consid-

eration nutrient content, cost and availability of the raw materials. Two vegetables, a main dish and a pudding were selected for development and production. These were:

(a) whole brussels sprouts
(b) whole Jersey new potatoes
(c) pork casserole
(d) rice pudding

Additional important factors which influenced product selection were:

(i) Brussels sprouts and Jersey new potatoes could be packaged with brine in cans or without it in pouches. This packaging characteristic is one of the main differences between the two types of package. Consequently, a significant difference in the extent of leaching of water soluble nutrients was expected.

(ii) Pork casserole is a conductive type of food. Therefore it was expected that significant differences would be obtained in process time due to differences in the heating rates, caused by the differences in package critical dimensions.

(iii) Rice pudding presents potential conditions for development of Maillard reactions, with significant browning effect which leads to lysine damage.

5.1.1 Preparation and Blanching

It was considered essential to maintain identical compositions for both canned and pouched products. Consequently, all food preparation, blanching and filling conditions were maintained constant in both pouches and cans, as described in Appendix 4.

Blanching prior to processing was applied to all vegetables, except for pork casserole potatoes, which presented serious overcooking problems. Blanching procedures are described in Appendix 4

and a summary of the blanching conditions used is given in Table 5.1.

Sample	Time (in minutes)	Temperature	Solution Used
Brussels sprouts	3	Boiling point	0.2% (w/v) calcium chloride and2.5% (w/v) sodium chloride solution in distilled water
New potatoes	5	Steam	Steam
Pork casserole:			
Carrots	1	Boiling point	0.25% (w/v) calcium chloride solution in distilled water
Celery	1	Boiling point	1.0% citric acid solution in distilled water
Onions	2	Boiling point	1% (w/v) citric acid solution in distilled water

Table 5.1

Blanching treatments

5.1.2 Packaging and Sealing

The amount of each component in the packages was carefully controlled during the packaging procedure which is fully described in Appendix 5. Sealing for A-1 cans, A-1 pouches and A-10 pouches was carried out under vacuum, while A-10 cans were exhausted at 90 °C before seaming, as described in section 3.1.2.

5.1.3 Processing

The basis for the comparison between the food samples

prepared in both cans and pouches was that the only variable should be the package itself. All processing factors were standardized and equivalent microbiological heat treatments were applied for both canned and pouched food samples.

(a) Process selection

It was considered essential to retain the highest possible level of nutrients. Consequently, the process time was chosen to be as mild as possible without diminishing the bacteriological safety of the food.

It has been widely accepted, (Charm, 1978, Joslyn and Heid, 1963, Jackson and Lamb, 1981, Leniger and Baverloo, 1975, Stumbo, 1965), that the minimum thermal treatment for low acid foods, (pH > 4.5), should be adequate to reduce any population of heat resistant *Clostridium botulinum* spores by 10^{12} , which is equivalent to a minimum F_o -value of 2.52 min. Most of the low acid foods are processed beyond that limit, mainly because of the demand for an adequate safety margin and the occurrence of spoilage bacteria of greater heat resistance. Food manufacturers, (Campbell, 1950, Canning Trade, 1924, Leniger and Baverloo, 1975, Stumbo, 1965, Tressler et al, 1978,), estimate that a 10^5 fall in the population of these bacteria is a sufficiently good preventive measure. Considering average D and Z values for these bacteria that fall corresponds to a F_o-value of 5 min, which was the value selected for new potatoes and rice pudding. In the case of brussels sprouts, a F_o -value of 3 min was chosen to avoid overcooking problems, particularly in the canned product..

Pork casserole was considered likely to be contaminated with thermophilic organisms (Brennan et al, 1976, Herson and

Hulland, 1980, Leniger and Baverloo, 1975, and Stumbo, 1965) therefore a more severe heat treatment was needed. This is in agreement with the information available from food manufacturers (Campbell, 1950, Canning Trade, 1924, Lyall, 1965, and Komarick et al, 1978), which indicates that for meat products the minimum F_o-value should be of 10 min. This was the F_o-value finally chosen for pork casserole.

A F_o -value of 10 min was also chosen for rice pudding since a greater heat treatment would increase the chance of finding differences in available lysine retention between cans and pouches.

To summarize, the target thermal sterilization treatments selected for the food samples were: brussels sprouts, 3 min; Jersey new potatoes, 5 min; pork casserole, 10 min. and rice pudding, 5 min and 10 min.

(b) Temperature Monitoring

The pieces of food selected for thermocouple insertion for temperature monitoring and process control were always in the upper limit of size. However, in the case of rice pudding the thermocouple was simply centrally located in the package by means of the supporting rig. For pork casserole potato was the slowest heating component under study conditions, (section 4.4), and was used for insertion of the thermocouple. The pieces of food selected to insert the thermocouple for both pouches and cans were of similar size and characteristics.

All thermocouple assemblies, monitoring devices and process control equipment used were described in Chapter 3.

During processing in the static canning retort and the

pouch water retort, three A-1 packages or two A-10 packages containing thermocouples were used in each run, while at least one thermocouple was placed in the heating medium. One thermocouple was used for process control (see section 3.6) and the other two were used as a back-up monitoring system in case of failure in the process control system. These two therm - couples were connected to a chart recorder for record keeping.

For the rotating retort, as only one thermocouple could be used in each run, this was employed for process control. The retort temperature was checked during processing using a mercury in-glass thermometer fitted to the retort body.

(c) Processing Conditions

Temperature and Pressure. The processing temperature selected for the three retorts was 121.1 ^{o}C. The pressure used for processing in the static and rotating canning retorts was approximately 104. ⁻. KPa, above atmospheric pressure, which is the corresponding saturated steam pressure at 121.1 ^{o}C. In pouch processing counter pressure above the process equilibrium is necessary to avoid seal damage or bursting (section 2.1.4). Therefore superimposed air pressure during pouch processing was used. The working pressure selected was 172 KPa above atmospheric pressure.

Procedure. All the packages were numbered and placed inside the retorts. The process was monitored from the opening of the heating medium value by feeding the first temperature reading into the computer. The process and the computer monitoring were then carried out until the target F_o -value was reached and cooling was immediately started. Thirty A-1 pouches or A-1 cans were always processed in each run.

Cooling. Cold chlorinated tap water at approximately 18^{o}C

was used as cooling medium. The packages were cooled until the temperature monitored in the centre of the package was reduced to approximatley 40°C. During the cooling process also, an adequate over-riding air pressure was maintained to prevent bursting or seam damage to the packages, 172.37 KPa for pouch processing and 82.74 KPa for can processing.

(d) Post-processing handling

It has been mentioned that several codes of practice for post-processing handling of retortable pouches have been reported in the literature (section 2.1. 6). Therefore, following these recommendations, the retortable pouches were dried immediately after processing, and handling was reduced to a minimum. The pouches were dried using a forced air cabinet, in which the pouches were placed in horizontal wire trays, while air at 15 °C was circulated until all the water was removed. The same criteria were also applied to the cans. Here, drying was carried out manually using clean cloths. Once the packages were dried, they were ready for storage and analysis.

5.1.4 Storage

(a) Selection

Three storage temperatures were selected to cover the range of temperatures that could be found under normal storage conditions of heat sterilized products. These temperatures were:

(i) 37°C ± 1°C or incubation temperature

(ii) 20°C ± 1°C or room temperature

(iii) 5°C ± 2°C or refrigeration temperature

(b) Procedure

Storage at these temperatures was achieved using two automatically controlled Hearson incubation units, for 37°C and 20°C and an auto freeze refrigerator unit for 5°C for all A-1 size pouches and cans. Due to the lack of space, A-10 containers were stored under ambient conditions which fluctuated between 10°C and 25°C during the storage period. Table 5.2 shows the storage conditions for the different food samples produced.

Table 5.2 Storage conditions for food samples produced

Samples in A-1 Containers	F_o-values aimed minutes	Temperatures of storage °C	No. of samples stored
Canned Brussels sprouts	3	5	8
		20	8
		37	9
Pouched Brussels sprouts	3	5	8
		20	8
		37	9
Canned new potatoes	5	20	23
Pouched new potatoes	5	20	24
Canned pork casserole	10	20	20
		37	24
Pouched pork casserole	10	20	23
		37	24
Canned Rice Pudding Formulation 2	5	5	9
		20	9
		37	9
Canned Rice Pudding Formulation 2	10	5	9
		20	9
		37	9
Canned Rice Puding Formulation 4	5	20	10
Pouched Rice Pudding Formulation 4	5	5	9
		20	9
		37	9
Pouched Rice Pudding Formulation 4	10	5	9
		20	8
		37	9
Pouched Rice Pudding Formulation 2	5	20	10

Table 5.2 contd.

Samples in A-10 Containers			
Canned Pork Casserole	10	A.T.*	1
Pouched Pork Casserole	10	A.T.*	1
Canned Rice Pudding	5	A.T.*	2
Formulation 2	10	A.T.*	2
Pouched Rice Pudding	5	A.T.*	2
Formulation 4	10	A.T.*	2
Pouched New Potatoes	5	A.T.*	1 (damaged)
Canned New Potatoes	5	A.T.*	1

* Ambient Temperature ($10^{\circ}C$ - $25^{\circ}C$)

5.2 Results and Discussion

The mathematical method for calculating temperature changes in unsteady state heat transfer developed by Ball, (1923), and Olson and Jackson, (1957), has been widely used in food engineering and it is based on the assumption that the can cold point will approach retort temperature at a logarithmic rate. According to this method, several heating factors are necessary to define the heat treatment received by a particular type of food under certain processing conditions. These factors include: the negative reciprocal slope of the asymptote to the logarithm of temperature gradient versus time, (f), lag factor, (j), pseudo-initial temperature, (JI), difference between retort temperture and the can cold point maximum temperature at the end of the heating cycle, (g), difference between cooling water and the temperature of the can cold point and the end of heating cycle, (m), thermal death time at retort temperature, (U). For these parameters the subscript "h" stands for heating cycle, and the subscript "c" for the cooling cycle.

To obtain these heating parameters semilogarithmic temperature - time plots were prepared for each one of the food samples produced. These plots are shown in Figures 5.1 to 5.10. The heating parameters obtained from these semilogarithmic plots are given in Tables 5.3 to 5.6.

5.2.1 f_h-values

The parameter f_h depends on container dimensions and food solid particle size and nature of the product. The food particles for each type of product in both pouches and cans are of similar size and dimensions and with similar thermal properties. Therefore the differences obtainedin f_h-values for the same food product in the various containers should be attributable to the geometry and dimensions of the containers.

Brussels sprouts and New Potatoes

In the case of brussels sprouts (Figure 5.1), and new potatoes (Figure 5.2), which are solid particles in brine, conduction was expected to be the main mechanism of heat transfer in the solid particles while heat transfer mainly by convection was expected in the brine. The low f_h-values obtained for brussels sprouts and new potatoes (see Table 5.3) , in A-1 containers could be considered typical of convection packs which are characterized by low f_h-values, about 5 to 20 minutes. But for the brussels sprouts and new potatoes, the low f_h-value obtained may be the effect of the small size food particles used.

To explain the differences between f_h-values obtained for the same product packaged in both pouches and cans it was considered that the heating rates from the heating medium to package wall and from package wall to food surface are very

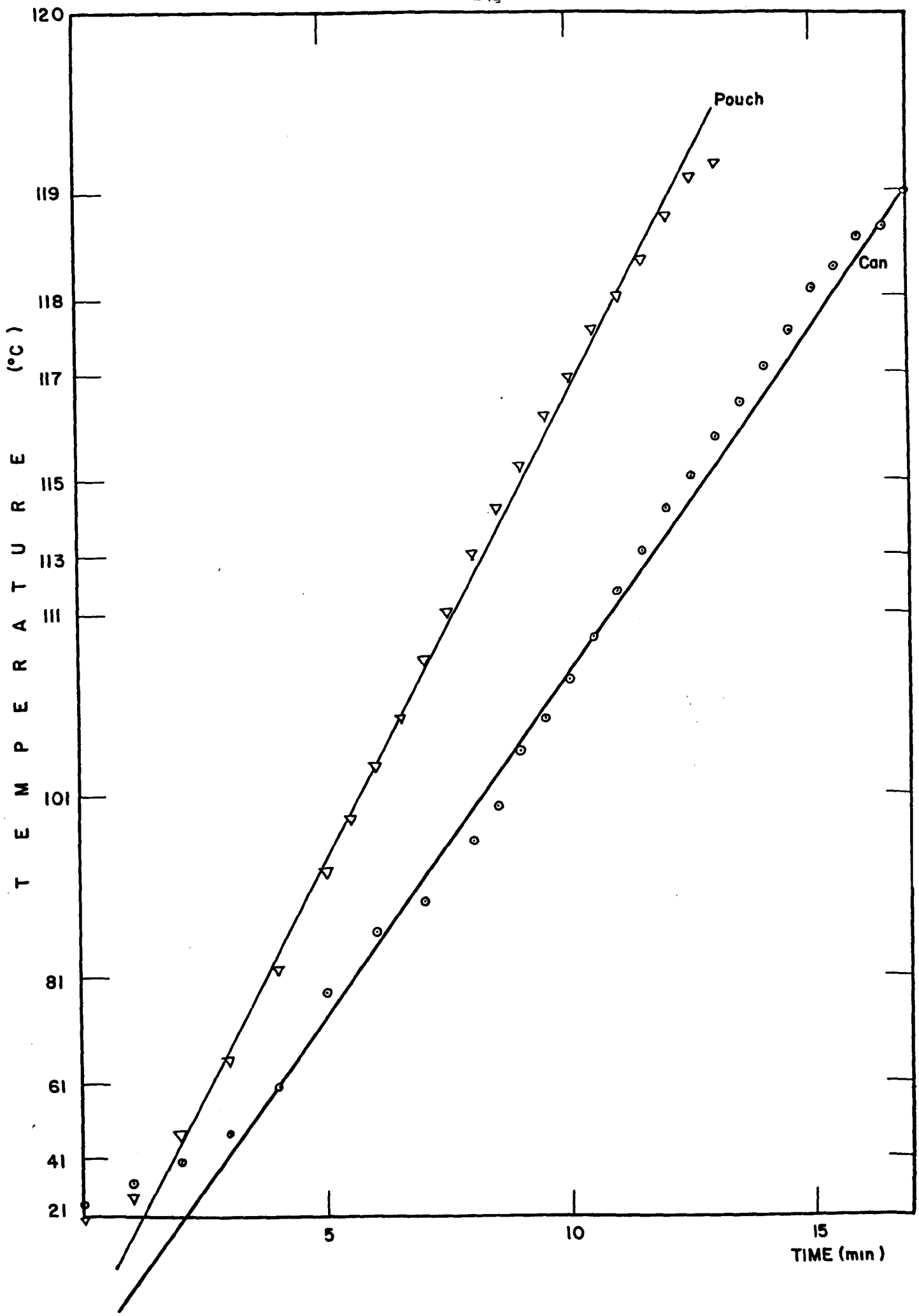


FIG. 5.1 HEAT PENETRATION CURVES - BRUSSELS SPROUTS IN A-1 CONTAINERS.

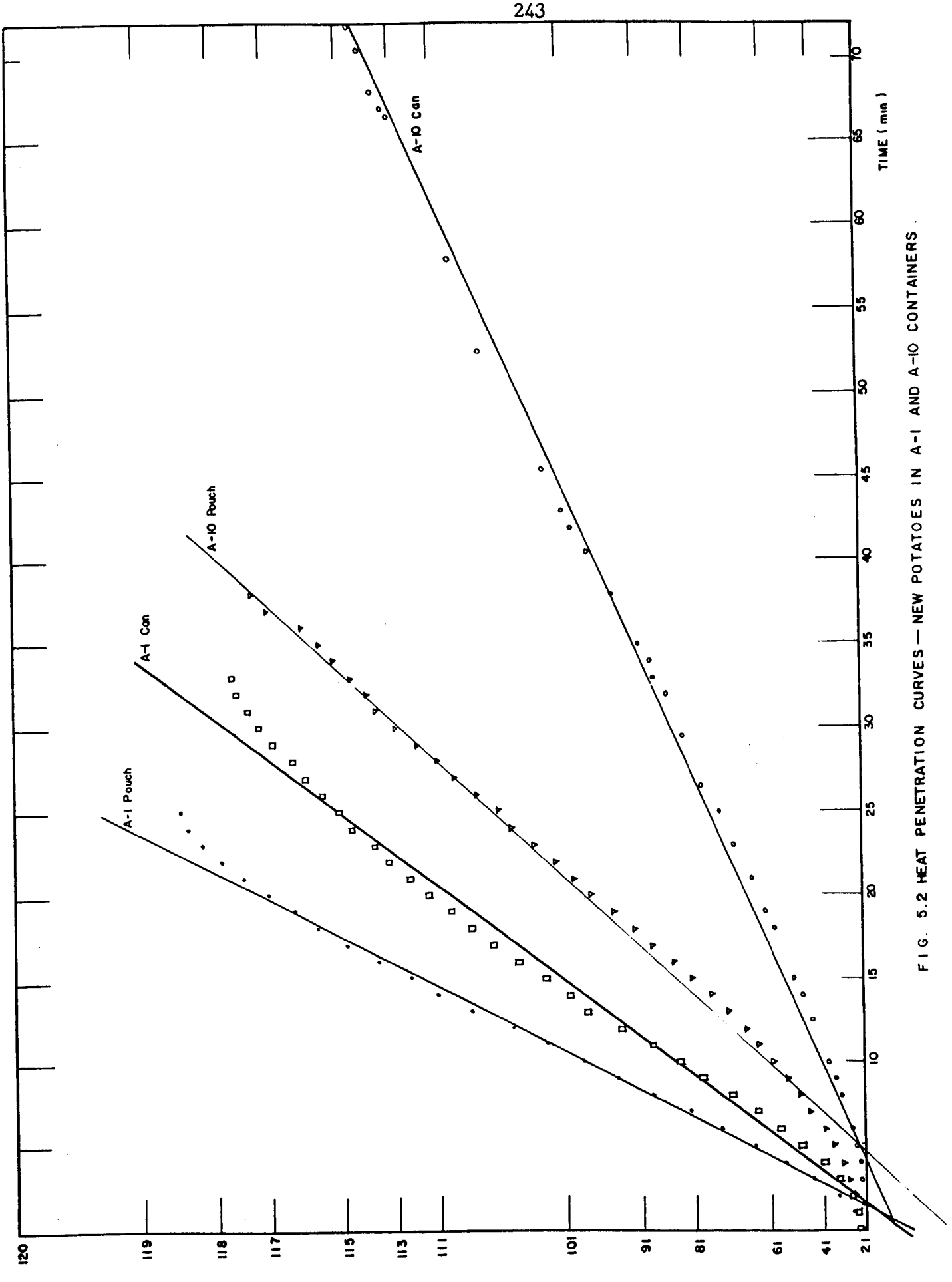


FIG. 5.2 HEAT PENETRATION CURVES — NEW POTATOES IN A-1 AND A-10 CONTAINERS.

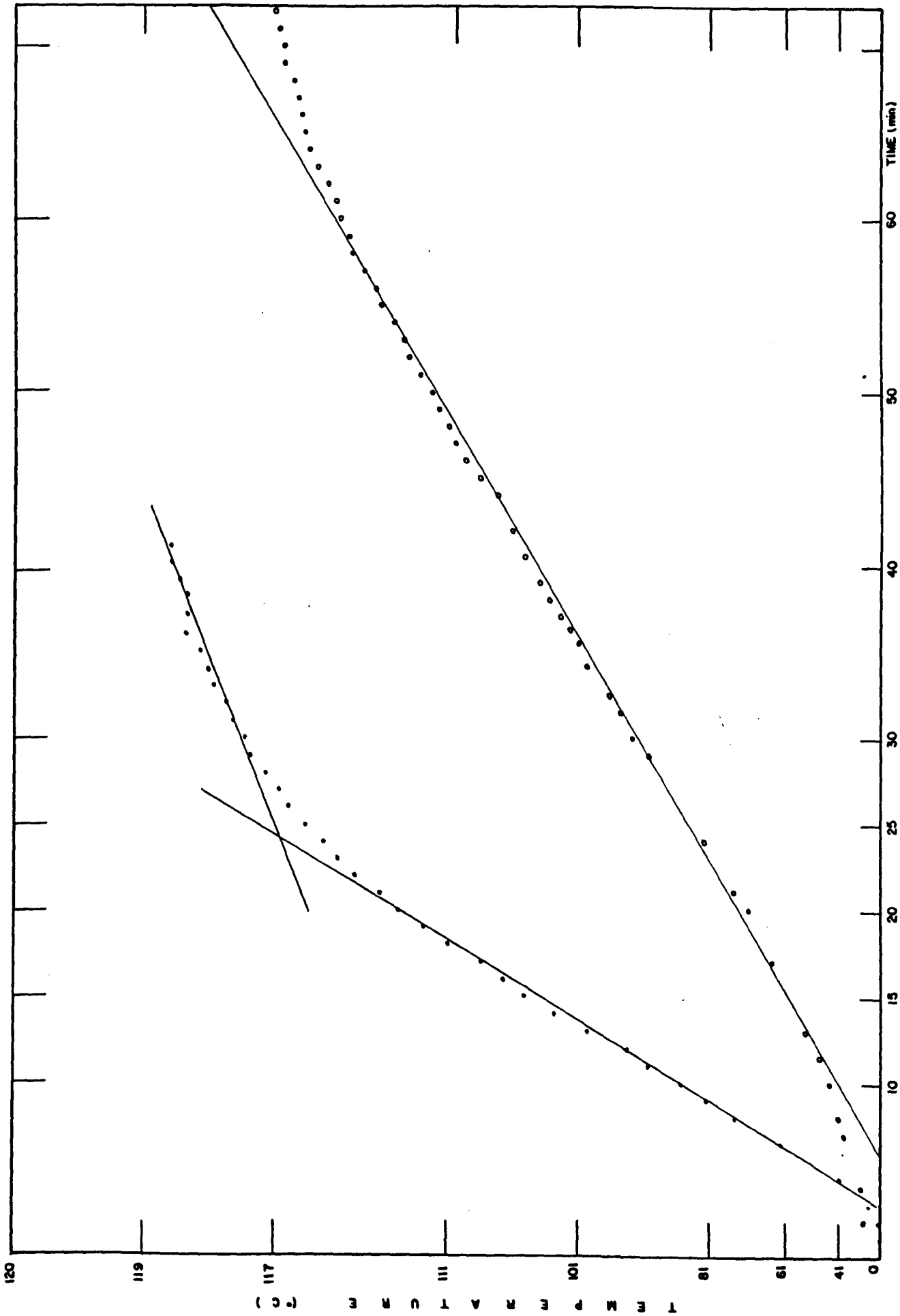


FIG. 5.3 HEAT PENETRATION CURVES - PORK CASSEROLE IN A-1 CONTAINERS.

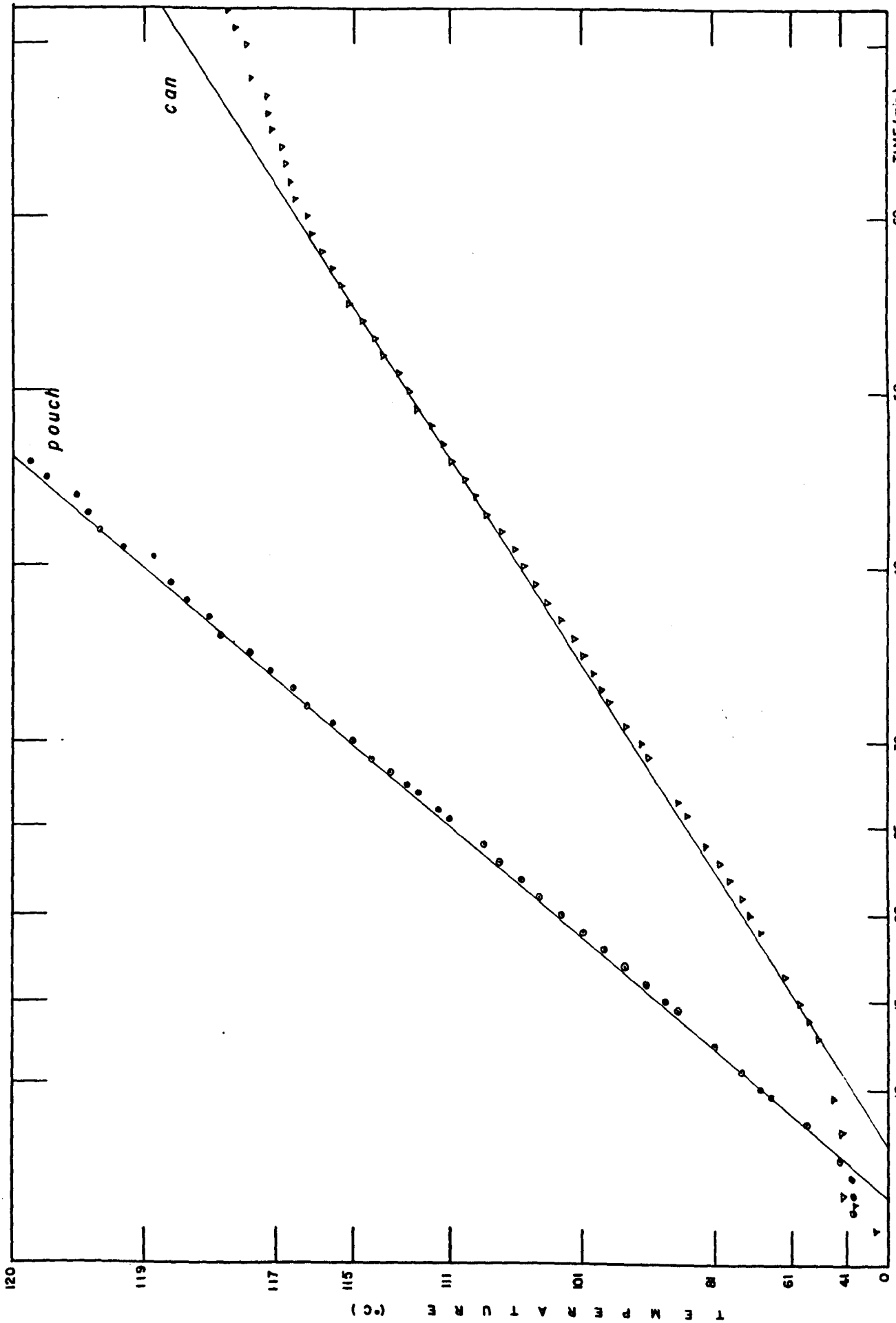


FIG. 5.4 HEAT PENETRATION CURVES – PORK CASSEROLE IN A-1 CONTAINERS.

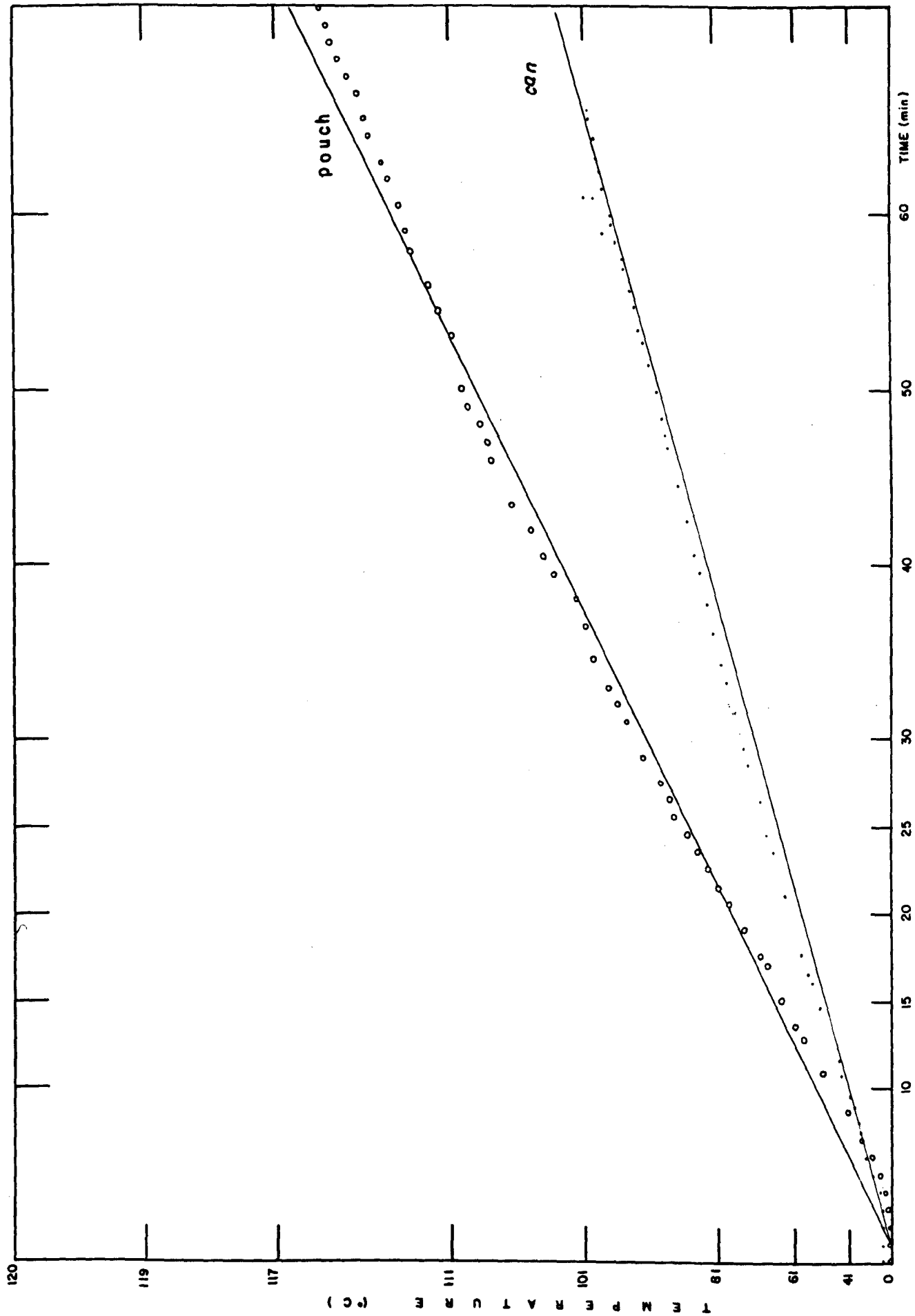


FIG. 5.5 HEAT PENETRATION CURVES - PORK CASSEROLE IN A-10 CONTAINERS

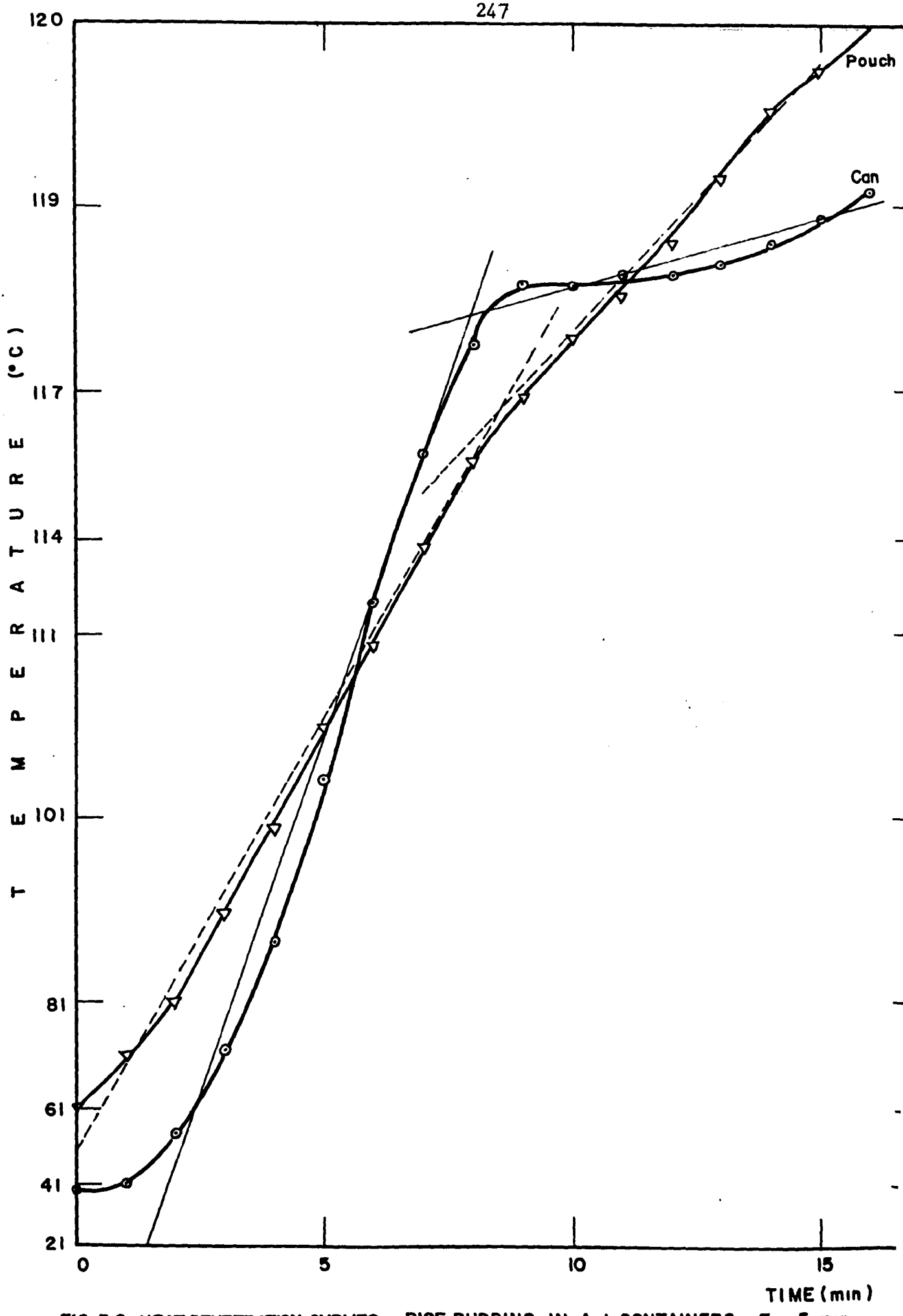


FIG. 5.6 HEAT PENETRATION CURVES – RICE PUDDING IN A-1 CONTAINERS. $F_o \approx 5$ min. FORMULATION 4

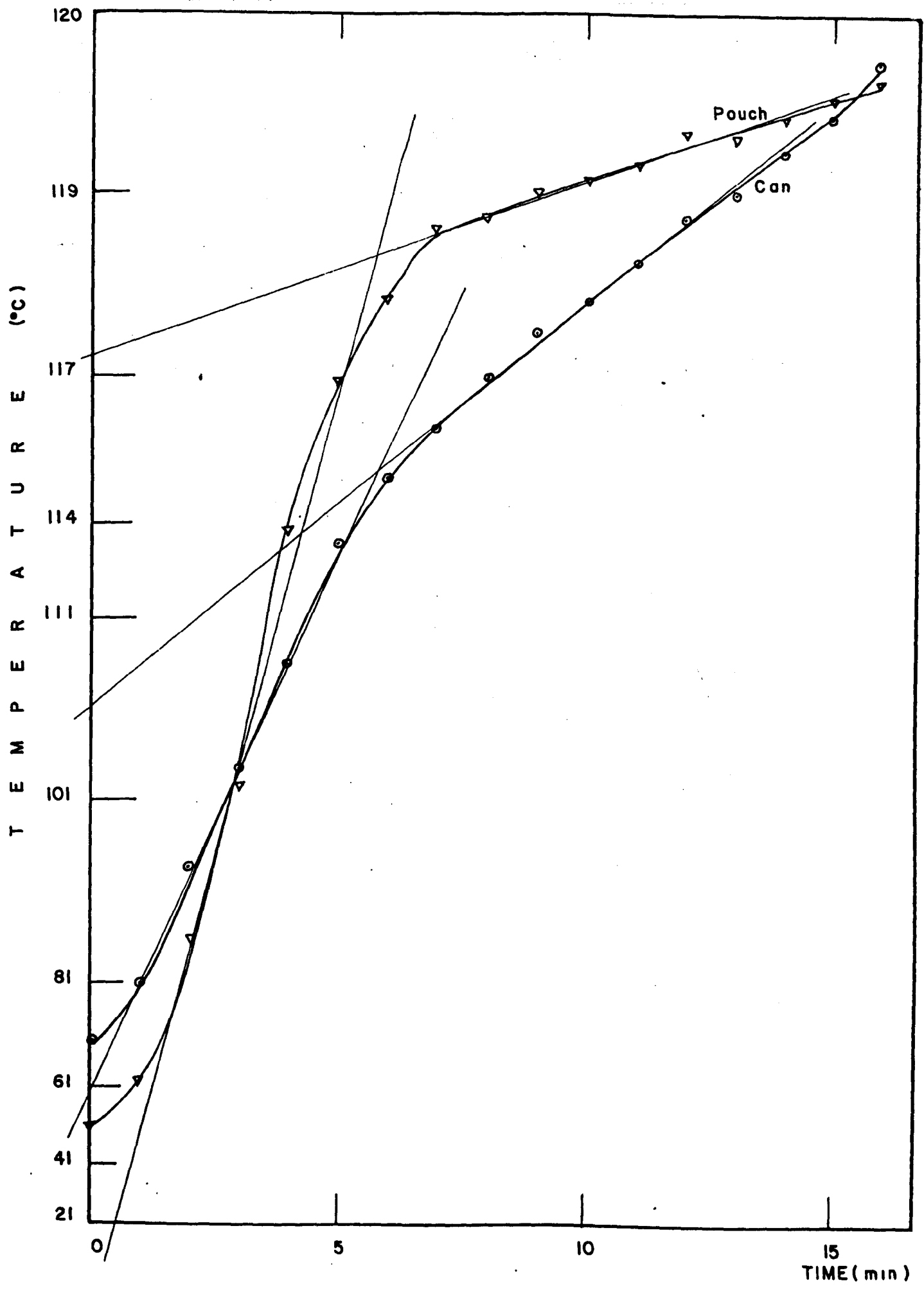


FIG. 5.7 HEATING RATE CURVES—RICE PUDDING IN A-1 CONTAINERS.

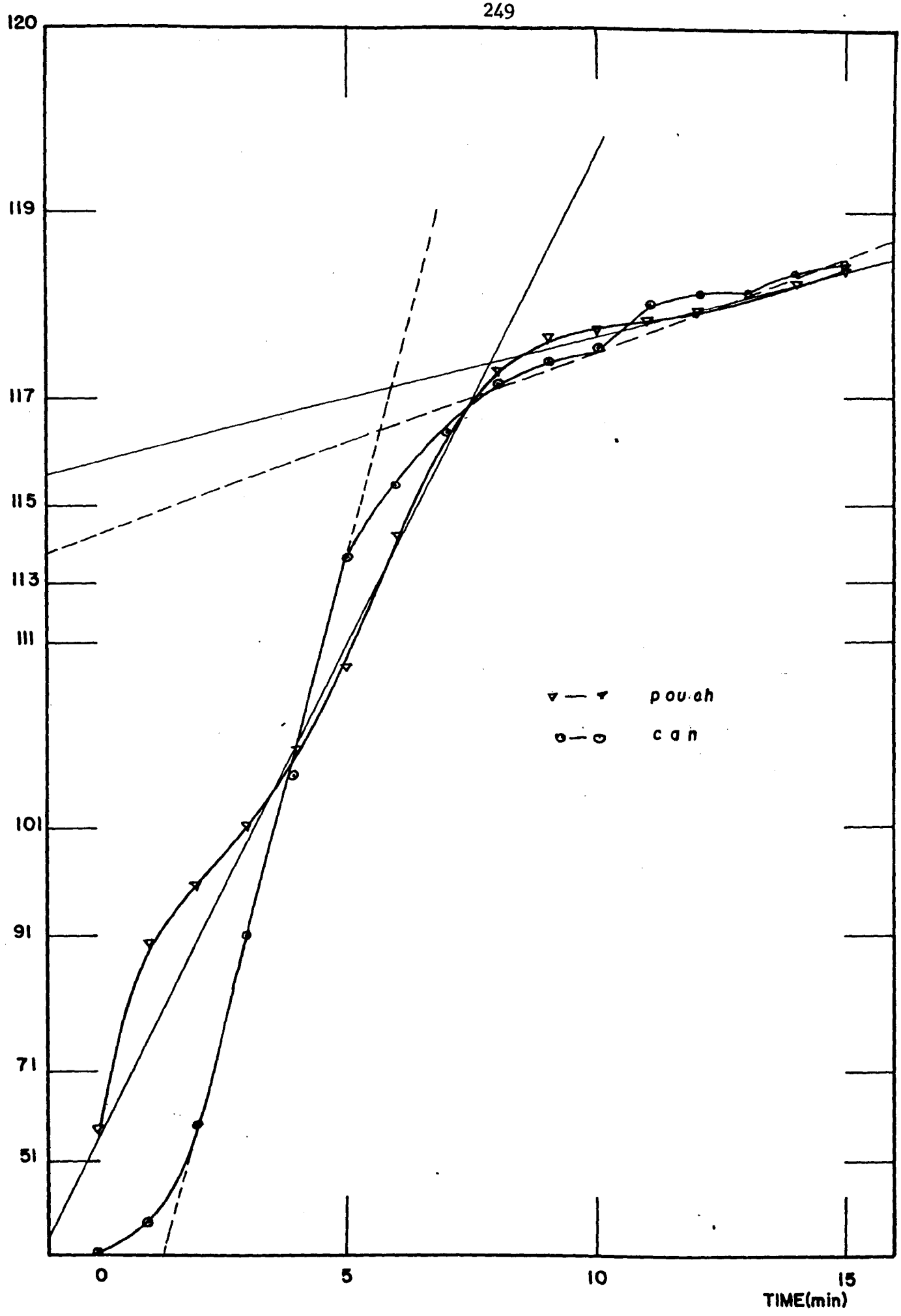


FIG. 5.8 HEAT PENETRATION CURVES - RICE PUDDING IN A-1 CONTAINERS. F_0=5 min. FORMULATION 2.

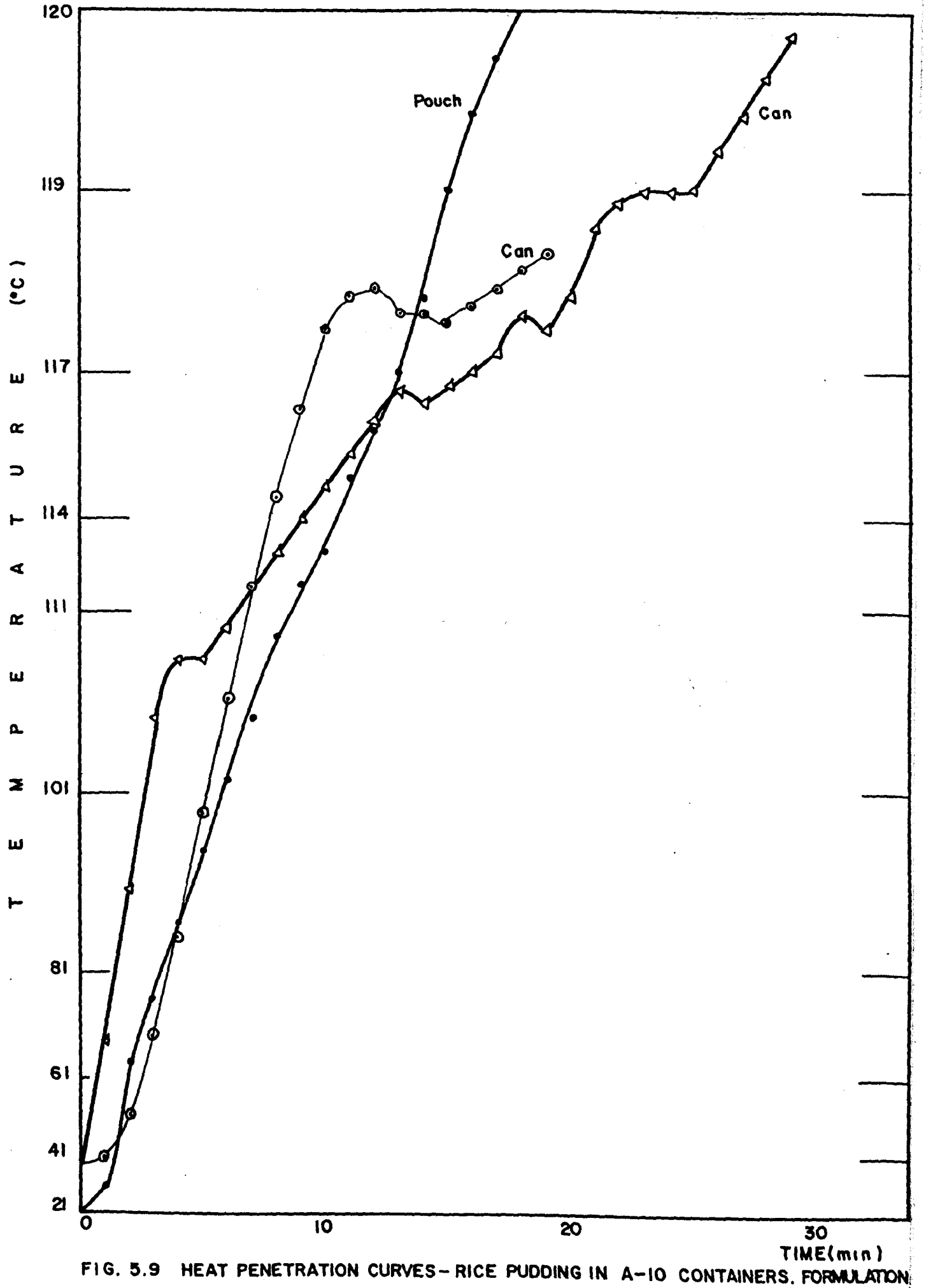


FIG. 5.9 HEAT PENETRATION CURVES - RICE PUDDING IN A-10 CONTAINERS. FORMULATION

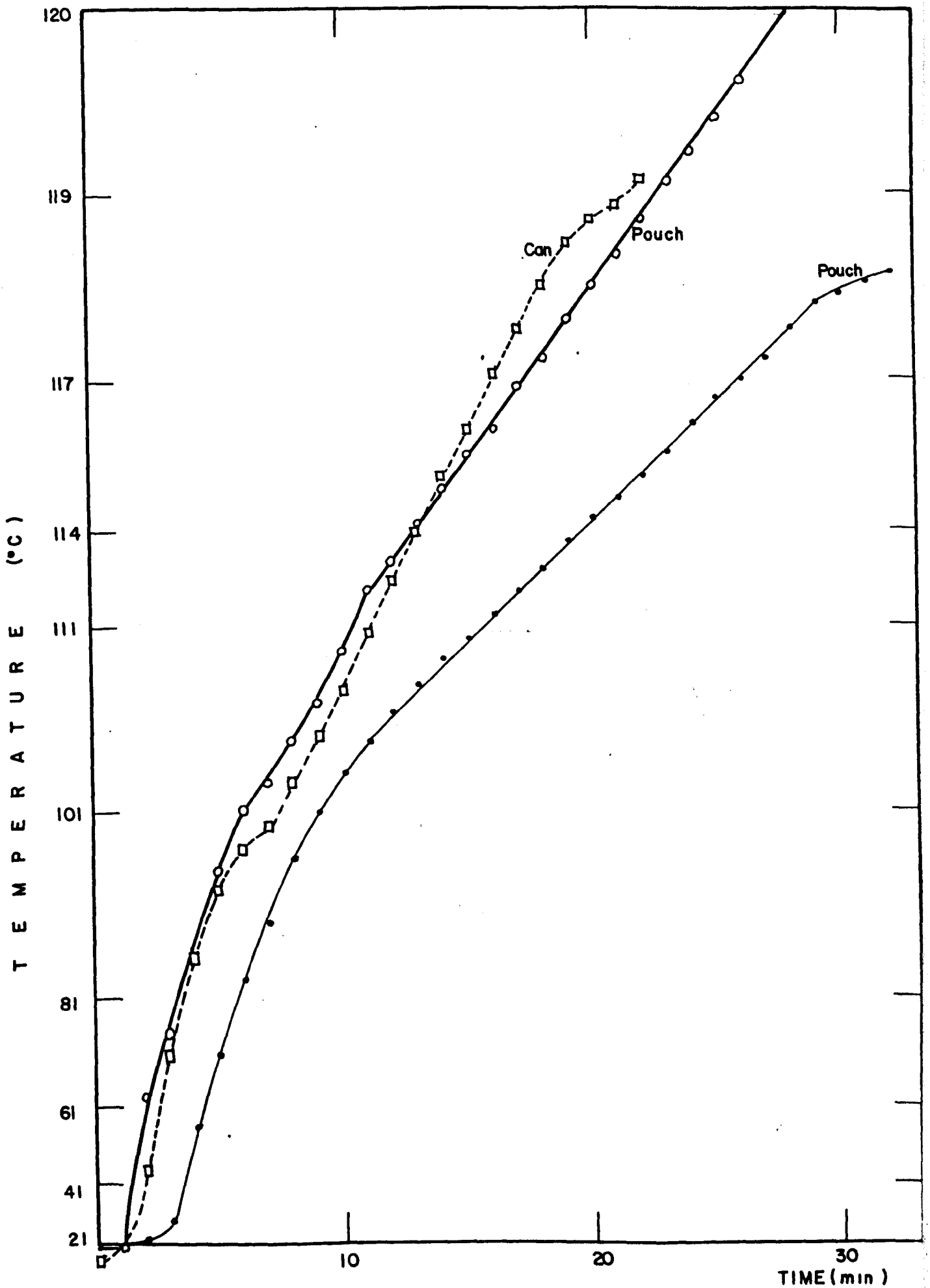


FIG. 5.10 HEAT PENETRATION CURVES – RICE PUDDING IN A-10 CONTAINERS. FORMULATION 4

high when compared with food heating rates. Therefore their effect on the food heating rate is negligible and it can be ignored. Consequently food surface can be considered to be at retort temperature.

The A-1 pouches had only one layer of potatoes or brussels sprouts so the food particle diameter was the critical dimension. In the canned products the brine was the food component in contact with the package wall. Therefore any difference between pouched and canned brussels sprouts or new potatoes f_h-values could be attributed mainly to a brine delaying heating effect. This effect can be simplified by saying that extra time was required for the brine to reach retort temperature and consequently extra time was needed for the solid particle surface to reach retort temperature. Although it is known that water heats by convection, (which is very fast), it is possible that the high density of solid particles in the cans could restrict the convection currents, decreasing the heat transfer rate of the brine as suggested by Sarmad, (1977).

The A-10 size containers showed bigger differences in f_h-values for new potatoes as expected from the package dimensions. The A-10 pouches had two staggered layers of new potatoes so the A-10 pouch critical dimension was one and a half that of the A-1 pouches, (3.75 cm). This difference between A-1 and A-10 thickess explains the difference in f_h-values encountered.

Pork Casserole

The high f_h-values reported for pork casserole products in Table 5.4 suggest that conduction is the main mechanism of heat transfer in these products, as expected from the high viscosity

of the gravy. In this case the differences are caused mainly by the package dimensions, particularly package thickness. The A-1 pouches had an average thickness of 1.7 cm while the A-10 pouches had an average thickness of 2.9 cm, when filled with pork casserole.

Olson and Jackson, (1942), equations were used to estimate the f_h-values for the A-10 packages. The estimated mean f_h-value for the A-10 can, (201.60 min), is very similar to the obtained value, (205.0 min). But for the A-10 pouches, the esttimated f_h-value of 61.08 min is larger than the actual f_h-value obtained of 49.4 min, which means a faster heating rate in A-10 pouches than in A-1 pouches. This may be explained by the non-homogeneous nature of the product as follows:
The temperature was measured in the centre of a potato piece contained in a homogeneous gravy. The potato piece was of the same size, (2.54 cm x 1.5 cm), in all containers but due to the differences in packages thickness, for the A-1 pouch the heating rate of the potato was the critical contributor while for the other three containers a combination of the heating rates of the potato and gravy were the determining factor. Potato being a solid material was expected to have a slower heat transfer rate than the gravy, (Mohsenin, 1980).

The differences in f_h-values between A-1 pouches and A-1 cans are considerable as shown in Figure 5.3 and 5.4. As expected the difference between A-10 containers is extremely large (see Figure 5.5). These results show that big reductions in process time can be obtained in conduction heating products by packaging and processing in pouches.

Table 5.3 Brussels sprouts and new potatoes heating parameters

PARAMETERS	Brussels sprouts		New Potatoes			
	A-1 pouch	A-1 can	A-1 pouch	A-1 can	A-10 pouch	A10 can
Retort temp (RT)	12.1°C	121.1°C	121.1°C	121.1°C	121.1°C	121.1°C
Initial temp (IT)	17.8°C	26.6°C	22.7°C	22.4°C	21.3°C	24.0°C
Come-up time CUT	5 min	3 min	13 min	6.5 min	13 min	6.5 min
Maximum food temp at end of heating (T_{MAX})	119.2°C	119.1°C	118.6°C	117.7°C	117.5°C	115.2°C
Heating rate (f_{h1})	6.4 min	9.2 min	13.0 min	18.8 min	24.0 min	57.6 min
Pseudo initial temp (JI)	56.1°C	110.1°C	35.1°C	78.1°C	71.1°C	100.0°C
I = RT - IT	103.3°C	94.5°C	98.4°C	98.7°C	99.8°C	97.1°C
Lag factor (J) J = JI/I	0.54	1.17	0.36	0.79	0.71	1.03
g = RT - T_{MAX}	1.9°C	2.0°C	2.5°C	3.4°C	3.6°C	4.9°C
Cooling water temp(CW)	28°C	18°C	23°C	16.0°C	28°C	16°C
m = T_{MAX} - CW	91.2°C	101.1°C	95.6°C	101.7°C	89.5°C	100.2°C

Table 5.4 Pork Casserole heating parameters

Parameters	Pork Casserole					
	A-1 pouch	A-1 pouch	A-1 can	A-1 can	A-10 pouch	A-10 can
Retort temp (RT)	121.1°C	121.1°C	121.1°C	121.1°C	121.1°C	121.1°C
Initial temp (IT)	14.3°C	18.0°C	16.5°C	23.5°C	22.6°C	26.4°C
Come up time (CUT)	6 min	6 min	3 min	3 min	6 min	3 min
Maximum food temp at end of heating time (T_{MAX})	119.9°C	118.8°C	118.2°C	117.7°C	117.8°C	115.8°C
heating rate (f_{h1})	21 min	15.4 min 66.0 min	38.8 min	42.4 min	49.4 min	205 min
Pseudo initial temp(JI)	108.1°C	94.1°C	142°C	142°C	100.1°C	96.1°C
I = RT - IT	106.8°C	103.1°C	104.6°C	97.6°C	98.5°C	94.7°C
Lag factor (J) J = JI/I	1.01	0.91	1.36	1.46	1.02	1.02
g = RT - T_{MAX}	1.2°C	2.3°C	2.9°C	3.4°C	3.3°C	5.3°C
break point (g_{bh})		4.2°C				
(X)		24.2 min				
Cooling water temp(CW)	24°C	24°C	18°C	18°C	26°C	18°C
m = T_{MAX} - CW	95.9°C	94.8°C	100.2°C	99.7°C	91.8°C	97.8°C

Contrary to expectations one of the pouched pork casserole products presented a broken type curve (Figure 5.5). This effect could be caused by either a change in the product heating rate and/or because there was a thermocouple movement in the piece of food. A sudden change in the heat transfer rate was considered improbable as the gravy was already thickened when filled into the packages. Movement of the thermocouple was more likely to be the cause of the change in the heating rate, although no visible breakage of the potato occurred and the experimental procedure for thermocouple insertion (section 3.4) was designed to avoid this happening.

Rice Pudding

Most of the rice pudding samples (Figures 5.6 to 5.10) show broken type curves (excluding A-1 cans curves) with an initial high heat penetration rate which suggests convection heating and a subsequent reduction in the heating rate which may be taken as a change from convection to conduction type heat transfer. This can be explained by starch from the rice leaching into the milk during processing and its subsequent gelatinisation after a heating period. This would reduce the heating rate by increasing the product viscosity.

The A-1 cans heating curves present a slowing down in the heating rate at a high temperature. Because of this it is not clear if this change in heating rate is a true break due to the thickening of the sample or simply a reduction in the heating rate due to the small temperature difference between the rice pudding and the retort or a combination of both effects. Nevertheless two f_h-values are reported for these products in Tables

5.5 and 5.6.

There was no real f_h-value difference between pouched and canned rice puddings in A-1 and A-10 containers with either of the two formulations employed. This effect can be explained since canned rice puddings were processed in a rotating retort and it is well known that movement greatly increases the heat transfer rate. It is worth mentioning that it was found very difficult (almost impossible) to produce a reasonable canned rice pudding in the static retort. Movement was found to be necessary to avoid the rice from cohering to the bottom of the can, separating from the milk and burning and also to produce a "creamy" consistency in the final product.

5.2.2 Lag Factor (J_h value)

It has been suggested, (Hayakawa, 1972, Olson and Jackson 1942, and Stumbo, 1965), that the lag factor is a function of the position of the measured point, the initial temperature distribution and the shape and dimensions of the object being heated. Also it had been mentioned by Stumbo C.R., (1965) that a large change, (about 10°C), h, in the initial temperature difference, (I = RT - IT), results in only a small variation in the lag factor (of about 0.1)

In all samples produced, the package's geometrical centre was the measured point. The initial temperature distribution was assumed to be uniform. But for different batches of brussels sprouts, new potatoes and pork casserole the initial temperature difference varied by 10° C. Therefore the differences in J-values obtained might be attributed mainly to the shape (pouch or can) and dimensions of the container.

Table 5.5 Rice Pudding heating parameters (Formulation 2 - no precooking)

Parameters	Rice Pudding Formulation 2					
	A-1 pouch	A-1 can	A-1 can	A-10 pouch	A-10 can	A-10 can
Retort temp (RT)	121.1°C	121.1°C	121.1°C	121.1°C	121.1°C	121.1°C
Initial temp (IT)	59.5°C	39.2°C	51.8°C	20.0°C	38.9°C	37.0°C
Come-up time (CUT	6 min	2 min	2 min	6 min	2 min	2 min
Maximum food temp at end of heating (T_{MAX})	118.5°C	119.1°C	119.8°C	120.3°C	119.9°C	118.5°C
heating rate f_{h1}	3.3 min	4.3 min	3.3 min	15.8 min	4.6 min	5.6 min
f_{h2}	33.75 min	43.5 min	37.5 min	-	24.0 min	77.5 min
Pseudo initial temp (JI)	22.6°C	96.1°C	66.0°C	77.1°C	114.1°C	101.1°C
I = RT - IT	61.6°C	81.9°C	69.3°C	101.0°C	82.2°C	84.1°C
Lag factor (J) J = JI/I	0.37	1.17	0.95	0.76	1.39	1.20
$g = RT - T_{MAX}$	2.6°C	2.0°C	1.3°C	0.8°C	1.2°C	2.5°C
break point g_{bh}	4.5°C	3.1°C	2.6°C	-	13.1°C	4.7°C
X	2.2 min	6.9 min	4.6 min	-	2.4 min	8.2 min
Cooling water temp (CW)	26°C	18°C	18°C	26°C	18°C	18°C
$m = T_{MAX} - CW$						

Table 5.6 Rice Pudding heating parameters (Formulation 4 - precooked rice)

Parameters	Rice Pudding Formulation 4					
	A-1 pouch	A-1 pouch	A-1 can	A-10 pouch	A-10 pouch	A-10 can
Retort temp (RT)	121.1°C	121.1°C	121.1°C	121.1°C	121.1°C	121.1°C
Initial temp (IT)	61.3°C	70.0°C	23.3°C	21.4°C	19.5°C	12.2°C
Come-up time (CUT)	6 min	6 min	2 min	6 min	6 min	2 min
Maximum food temp at end of heating time (T_{MAX})	119.9°C	120.6°C	118.87°C	118.4°C	120.1°C	118.6°C
heating rate f_{h1}	7.0 min 11.4 min	5.7 min 15.2 min	6.1 min 52.5 min	9 min 26 min	9 min 17.5 min	6.6 min 12.8 min
Pseudo inital temp (JI)	24.5°C	14.6°C	43.6°C	46.6°C	52.6°C	114.0°C
I = RT - IT	59.8°C	51.1°C	97.8°C	99.7°C	101.6°C	98.9°C
Lag factor (J) J = JI/I	0.41	0.29	0.45	0.47	0.52	1.15
break point g_{bh}	4.6°C	5.9°C	3.6°C	18.1°C	21.1°C	24.6°C
X	5.2 min	2.3 min	6.6 min	3.7 min	3.5 min	4.0 min
Cooling water temp (CW)	26°C	26°C	18°C	26°C	26°C	18°C
$m = T_{MAX} - CW$ $g = RT - T_{MAX}$	1.2°C	0.7°C	2.4°C	2.7°C	1.0°C	0.3°C

Brussels sprouts and new potatoes

The lag factors of brussels sprouts and new potatoes samples are smaller in pouches than their equivalent in cans (see Table 5.3). It has been suggested that J_h-values close to and less than one are characteristic of convection heated packs while J-values close to two are characteristic of conduction heated packs, (Charm, S.E., 1978, Jackson, A. T. and Lamb, J, 1981, and Stumbo, C.R., 1965). This does not mean that convection was the mechanism of heat transfer for pouched products but probably that the differences in package geometry produce smaller lag factors.

The J_h-values reported here for new potatoes in A-10 containers are different from the J_h-values in the smaller containers.

It has been normally assumed in heat sterilization process calculations, (Leniger and Beverloo, 1975, National Canners Association, 1968, and Stumbo, 1965) that J-value is constant for the same food packaged in different can sizes. However Olson and Jackson, 1942, showed that the lag factor (J) varied with the ratio of can length to can diameter. They suggested that a can with a ratio of 1.0 presented the maximum J-value obtainable while cans with either smaller or bigger ratios showed a decrease in the lag factor. Therefore the lag factors of the A-10 cans with a ratio of 1.12 are understandably bigger than the lag factor for A-1 cans with a ratio of 1.45. The A-10 pouches also presented a larger J-value than the A-1 pouch but there is no information available in the literature on lag factors for this type of package.

Pork Casserole

The same general approach as for brussels sprouts and new

potatoes can be taken for pork casserole. The J_h-values of the pouched product were also smaller than their equivalent in cans as were those for sprouts and potatoes. However the J_h-values of A-10 and A-1 pouches were very similar in agreement with the literature.

A-10 cans have a smaller lag factor than A-1 cans, contrary to expectations from consideration of the length/diameter ratio (Olson and Jackson, 1942). No explanation can be given for these results due to the complexity of the lag factor relationship with the other heating parameters which results in lack of information in the literature.

Rice Pudding

Variation in the initial temperature difference (I) and the use of two formulations complicate the interpretation of J_h-values. It seems likely (Stumbo, 1965) that the J_h-value differences between different batches of A-1 pouches and different batches of A-10 cans for both formulations are small enough to be attribuated to differences in I. It is therefore difficult to rationalise the J_h-value for A-10 pouches and A-1 cans which appear to show a formulation effect. However pouch J_h-values were significantly lower than those of cans.

5.2.3 g-values

g-value is an indirect measure of the process time and being affected by most of the heating variables it is difficult to relate the variation of g-values to specific changes in the processing conditions or product properties. Nevertheless the g-values are included in the tables as important information parameters.

5.2.4 Come-up time

The come-up time (CUT) of the canning retorts (2 - 3 min), are smaller than CUT of the pouch water retort, (5 - 6 min) for all processes. During the processing of potatoes the longer come-up times of the canning retort, (5 - 6 min) and the pouch water retort, (13 min) were caused by a reduction in the process steam supply.

As mentioned by Ball, (1924), longer come-up times can influence the f_h-values of fast heat transfer products by slowing down the heat transfer rate thus increasing the f_h-value. And as mentioned by Stumbo, (1965), the heating curve lag factor (J) can also be influenced by long CUT by changing the pseudo initial temperature, (JI), during the correction for zero time of the process, therefore reducing the J_h-value.

These two effects may be present in the pouched product J_h and f_h parameters when compared with the canned J_h and f_h -values, partly explaining the fact that pouch J_h-values were always lower than comparable can J_h-values.

The f_h-values obtained for the pouched food samples were of the same value or smaller than the f_h-values for the equivalent canned samples. The increase in the f_h-values due to the longer come-up times suggests a way of increasing the advantage of shorter process time for foods packaged in pouches by reducing pouch retort come-up times.

The come-up time is an indirect measure of the efficiency of the sterilization process equipment used. This indicates that the canning process equipment used was more efficient than the pouch processing equipment used.

5.2.5 Cooling curve parameters

Cooling water temperature

The cooling water temperatures, (CWT), reported in tables 5.5 to 5.8 are the first temperatures measured by the thermo-couples inside the retort, not the tap water temperature. For the canning retorts these CWT can be considered constant, for practical purposes, during the cooling cycle. But, for the pouch water retort CWT increased with time until a second batch of cool water was left in the retort. Due to this variability, cooling curve parameters, such as J_c and f_c, were not evaluated for the food samples produced.

5.2.6 F_o-values

The lethal effect of heat on micro-organisms is usually determined by the time and temperature of the heat applied. In order to evaluate this lethal effect in the containers used - cans and pouches- the temperature-time relationship at the geometrical centre of the containers was established and the thermal death time curve for *Clostridium botulinum* was used as a basis for the thermal evaluation of the sterilization process.

The actual F_o-values obtained for each product, after the heating cycle and the total time of the process are given in Table 5.7. The total process time reported in this Table was defined as the time elapsing from the corrected zero time (0.58 of CUT) until the temperature of the centre of the control pouch fell to 87.7°C.

The F_o-values obtained, (Table 5.7), were very similar for equivalent pouched and canned products. These results show the high degree of precision achieved in the operation of the manual retorts used with the process control system employed.

Table 5.7 Actual F_o-values at the centre of the container for the food samples produced

Product and container	Heating cycle (minutes) time	F_o-value	Total process (minutes) time	F_o-value
Brussels sprouts				
A-1 pouch	13.2	3.03	15.5	4.08
A-1 can	16.3	3.04	20.0	3.78
New Potatoes				
A-1 pouch	19.5	5.01	25.4	6.23
A-1 can	33.2	5.02	38.9	5.91
A-10 pouch	37.5	5.02	45.5	6.26
A-10 can	78.2	5.15	91.1	6.20
Pork Casserole				
A-1 pouch 1	45.5	10.03	55.0	13.20
A-1 pouch 2	38.5	10.02	44.5	11.48
A-1 can 1	76.2	10.55	91.9	13.43
A-1 can 2	78.2	10.01	93.6	11.96
A-10 pouch	81.5	10.00	95.0	11.96
A-10 can	244.2	9.38	271.2	11.84
Rice Pudding Formulation 2				
A-1 pouch	14.0	5.07	17.0	6.09
A-1 can	15.3	5.06	17.0	5.66
A-1 can	19.6	10.04	33.0	10.50
A-10 pouch	33.5	10.10	54.5	11.90
A-10 can	28.1	10.02	44.8	10.85
A-10 can	19.4	5.04	33.2	6.62
Rice Pudding Formulation 4				
A-1 pouch	13.0	5.01	15.7	6.32
A-1 pouch	19.5	10.08	22.5	10.49
A-1 can	17.8	5.05	18.8	5.44
A-10 pouch	29.0	5.05	34.0	6.52
A-10 pouch	28.5	10.04	33.5	10.94
A-10 can	30.8	5.00	36.2	5.81

It can be assumed that the true variable in pouch and can processing was the processing time if the following assumptions are made:

- The heating characteristics of the food are considered identical for the same food in different packages.
- The retort temperature was the same in all cases.
- The effect of retort operational variables such as the come-up time had a minimal effect on the heating rate of the food.

Therefore, the process time would be mainly affected by the pouch, (thickness), and the can, (diameter), critical dimensions for heat transfer. The actual processing times are also given in Table 5.7.

Brussels sprouts and new potatoes

The difference in processing time for brussels sprouts packaged in both A-1 cans and A-1 pouches is small, (5.5 min), while for new potatoes the difference is larger (13.5) min. The processing time difference for new potatoes in A-10 containers is increased to 45.6 min. As already mentioned in section 5.2.1 for brussels sprouts and new potatoes in pouches the heat transfer is affected mainly by the food particle diameter while in the canned samples a combined effect of brine and solids is present.

Pork Casserole

Differences in processing time for pork casserole in A-1 containers is large, (30 - 40 min). As expected, no difference in processing time between different batches of A-1 cans was found but a big difference was obtained between different batches of A-1 pouches. This has been discussed in section 5.2.1 in terms of the unexplained broken type curve presented by one of the A-1

pouched pork casserole samples.

The process time difference between A-10 pouches and cans was extremely large (176.2 min), this suggests that higher nutrient retention should be obtained in conduction heated foods when packaged in large pouches instead of large cans.

Rice Pudding

For the rice pudding samples in A-1 containers, there were small differences in the process time resulting in slightly longer times for the canned products. But in A-10 containers slightly shorter times were obtained for the canned products, which can be explained by the use of a canning rotating retort. As mentioned earlier it is well known that movement greatly improves the heat transfer. It is important to mention that the speed of rotation used was high by commerical standards, (7 r.p.m.), and rotation was of the "end over end" type. The short processing times obtained for the canned samples are thus satisfactorily explained.

5.2.7 Thermal diffusivity

Thermal diffusivity can be calculated from experimental heating curves of conduction heated foods by Olson and Jackson, (1942), procedure. The only food sample produced showing a conduction curve was the pork casserole. Rice pudding presented broken curves. Brussels sprouts and new potatoes were heated by both conduction and convection in cans because of the brine and solid effects. Although brussels sprouts and potatoes in pouches were heated by conduction the shape of the pouches was irregular, since due to their flexibility they take the shape of the food. Consequently no heat transfer mathematical model is available for these conditions.

Thermal diffusivity was calculated by substituting the inner dimension values of the filled packages and the heating rates (f_h), experimentally obtained in the equations proposed by Olson and Jackson,(1942) for rectagular shape objects (for pouches) and for finite cylinders, (for cans) (see section 2.2.2). These values together with the thermal diffusivities obtained are summarized in Table 5.8.

The thermal diffusivity values obtained for the two batches of A-1 and one batch of A-10 canned pork casserole are very similar. These values are the average thermal diffusivity of the potato piece (where the thermocouple was inserted) and the gravy. The values obtained for pork casserole in pouches are mainly the potato thermal diffusivity due to the proximity of the pouch wall to the potato. Therefore it is not surprising that the thermal diffusivities obtained for pouched and canned pork casserole were different. Nevertheless these average thermal diffusivities are important to the food scientist as they are used for the theoretical calculations of heat transfer in foods despite their limitations in extrapolation and in prediction theory.

5.3 Brussels Sprouts Evaluation

5.3.1 Experimental Procedure

Sampling was carried out as described in Appendix 1.1. Duplicate weights were taken to determine both ascorbic acid content using the DCP visual titration method (see Appendix 2.1) and moisture content (see Appendix 2.8)

5.3.2 Results

The amount of ascorbic acid in both liquid and solids was determined in order to study the leaching effect together with the

Table 5.8 Thermal diffusivity of pork casserole in different containers

Pork casserole container	Container dimensions in metres(m)			f_h-value in inseconds (s)	thermal diffusivity in square metres per second (m^2/s)
	2a	2b	2c		
A-1 pouch	0.181	0.137	0.017	1260	5.223×10^{-8}
A-10 pouch	0.419	0.205	0.029	2964	6.458×10^{-8}
A-1 can 1	0.066	0.096		2544	14.175×10^{-8}
A-1 can 2	0.066	0.096		2328	15.093×10^{-8}
A-10 can	0.155	0.175		12300	14.558×10^{-8}

2a = length of pouch or diameter of can

2b = width of pouch or length of can

2c = thickness of pouch

sterilization process and storage temperature effects. The vitamin C retention results obtained for total contents, liquid and solids are shown in Table 5.9, for samples stored at 5°C in both cans and pouches, in Table 5.10 for brussels sprouts stored at 20°C and in Table 5.11 for samples stored at 37°C. In these tables the results are the mean of four determinations and are given in milligrams of ascorbic acid per 100 grams of wet sample. The mean moisture content obtained for brussels sprouts in pouches was 90.56% with 39 degrees of freedom and 0.42% standard deviation while the mean moisture content for canned samples was 91.66% with 39 degrees of freedom and 0.98% standard deviation.

Table 5.9 Ascorbic acid content in milligrams per 100 grams of sample in brussels sprouts stored at 5°C

Storage days	Pouched brussels sprouts			Canned brussels sprouts		
	Liquid*	Solid	Total	Liquid*	Solid	Total
B.P.	-	95.38 ± 0.73	95.38	-	94.43 ± 1.29	94.43
A.P.	13.58 ± 0.25	69.99 ± 0.66	83.58	26.55 ± 0.44	32.92 ± 0.22	59.48
9	11.08 ± 0.64	69.75 ± 1.12	80.82	33.23 ± 0.37	25.77 ± 0.40	58.99
18	7.65 ± 0.55	53.29 ± 0.85	60.94	30.10 ± 1.07	23.44 ± 0.21	53.54
31	6.11 ± 0.09	52.08 ± 0.50	58.19	30.52 ± 0.19	22.05 ± 0.21	52.57
99	5.51 ± 0.15	34.34 ± 0.21	39.85	21.77 ± 0.38	17.36 ± 0.19	39.13
202	4.01 ± 0.09	30.61 ± 0.31	34.62	23.44 ± 0.03	18.26 ± 0.31	41.70
346	5.74 ± 0.01	30.13 ± 0.09	35.87	18.08 ± 0.33	13.66 ± 0.27	31.74

B.P. Before Processing
A.P. After Processing

Each value reported is the mean of four determinations and it is followed by ± 2σ (two standard deviations).

* The amount of ascorbic acid in the liquid refers to ascorbic acid leached out from 100 grams of solids.

Table 5.10 Ascorbic acid content in milligrams per 100 grams of sample in brussels sprouts stored at 20°C

Storage days	Pouched brussels sprouts			Canned brussels sprouts		
	Liquid*	Solid	Total	Liquid*	Solid	Total
B.P.	-	95.38 ± 0.73	95.38	-	94.43 ± 1.29	94.43
A.P.	13.58 ± 0.25	69.99 ± 0.66	83.58	26.55 ± 0.44	32.92 ± 0.22	59.48
8	10.58 ± 0.13	66.85 ± 0.40	77.43	27.15 ± 0.49	23.19 ± 0.50	50.34
18	8.79 ± 0.66	45.45 ± 0.42	54.25	19.06 ± 0.08	15.09 ± 0.99	34.15
30	6.54 ± 0.29	32.48 ± 0.49	38.02	20.92 ± 0.99	16.20 ± 0.26	37.12
97	5.58 ± 0.51	32.79 ± 0.21	38.37	20.99 ± 0.20	15.72 ± 0.32	36.72
201	4.47 ± 0.07	31.63 ± 0.31	36.11	20.43 ± 0.15	15.24 ± 0.53	35.67
346	5.14 ± 0.02	28.00 ± 0.17	33.14	20.82 ± 0.65	15.87 ± 0.16	36.67

B.P. Before Processing
A.P. After Processing

Each value reported is the mean of four determinations and it is followed by $\pm 2\sigma$.

* The amount of ascorbic acid in the liquid is the ascorbic acid leached out by 100 grams of brussels sprouts.

Table 5.11 Ascorbic acid content in milligrams per 100 grams of sample in brussels sprouts stored at 37°C

Storage days	Pouched brussels sprouts			Canned brussels sprouts		
	Liquid*	Solid	Total	Liquid*	Solid	Total
B.P.	-	95.38 ± 0.73	95.38	-	94.43 ± 1.29	94.43
A.P.	13.58 ± 0.25	69.99 ± 0.66	83.58	26.55 ± 0.44	32.92 ± 0.22	59.48
9	9.15 ± 0.28	50.55 ± 0.52	69.70	32.35 ± 0.85	25.16 ± 0.37	57.51
18	6.35 ± 0.58	40.45 ± 0.22	46.80	22.94 ± 0.91	17.34 ± 0.21	40.38
30	6.31 ± 0.74	35.75 ± 0.50	42.06	21.39 ± 0.37	16.86 ± 0.82	38.04
63	4.24 ± 0.39	25.01 ± 0.19	29.25	19.19 ± 0.35	16.29 ± 0.37	35.48
200	2.45 ± 0	17.50 ± 0.21	19.94	20.17 ± 0.37	14.82 ± 0.51	34.99
347	0.99 0.01	8.83 0.19	9.87	19.70 0.78	17.46 0.67	37.16

B.P. Before Processing
A.P. After Processing

Each value reported is the mean of four determinations and it is followed by $\pm 2\sigma$.

* The amount of ascorbic acid in the liquid is the ascorbic acid leached out from 100 grams of brussels sprouts.

The results obtained were plotted as ascorbic acid % retention against the storage time. 100% was considered the ascorbic acid content immediately after blanching; while the starting point for the curves on y-axis represents the ascorbic acid retention immediately after processing. The ascorbic acid retention curves obtained for 5°C, 20° C and 37°C storage temperatures are shown in Figures 5.11, 5.12 and 5.13. It can be seen that the loss due to can processing (37.1%) is larger than the loss due to pouch processing, (12.4%). Although the pouched brussels sprouts were packaged without brine, some liquid was leached out from the solids, and a further 17.2% of the ascorbic acid was found in the liquid immediately after processing. However in the canned product 29.8% of the ascorbic acid was found in the brine immediately after processing.

(a) Storage at 5°C

In Figure 5.11 it can be seen that storage at 5 °C for 4 months produced a further ≃ 45.0% loss in the total ascorbic acid content of the pouched samples while for the canned product only an extra ≃ 25.0% was lost. Consequently, at the end of the storage period, pouched and canned samples were no different in ascorbic acid total content, although, for the edible part (brussels sprouts) the pouch solids were higher in ascorbic acid retention than the can solids due to the detrimental leaching effect. During the last five months of storage no change in ascorbic acid retention was detected in either products.

(b) Storage at 20°C

Storage at 20° C (Figure 5.12) produced basically the same results as for 5° C but most of the ascorbic acid destruction took

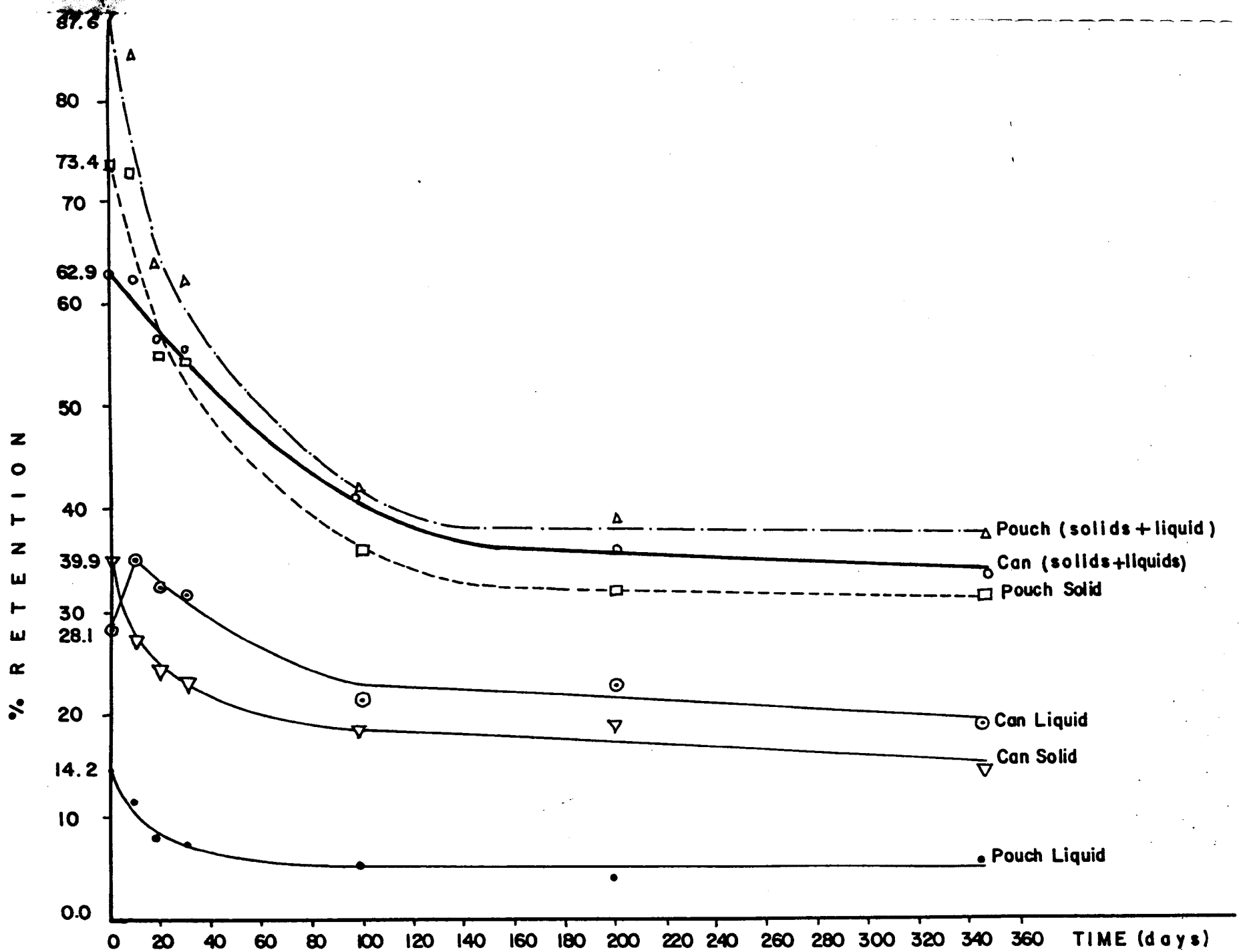


FIG. 5.11 ASCORBIC ACID RETENTION IN BRUSSELS SPROUTS AFTER STERILISATION AND DURING STORAGE AT 5° C

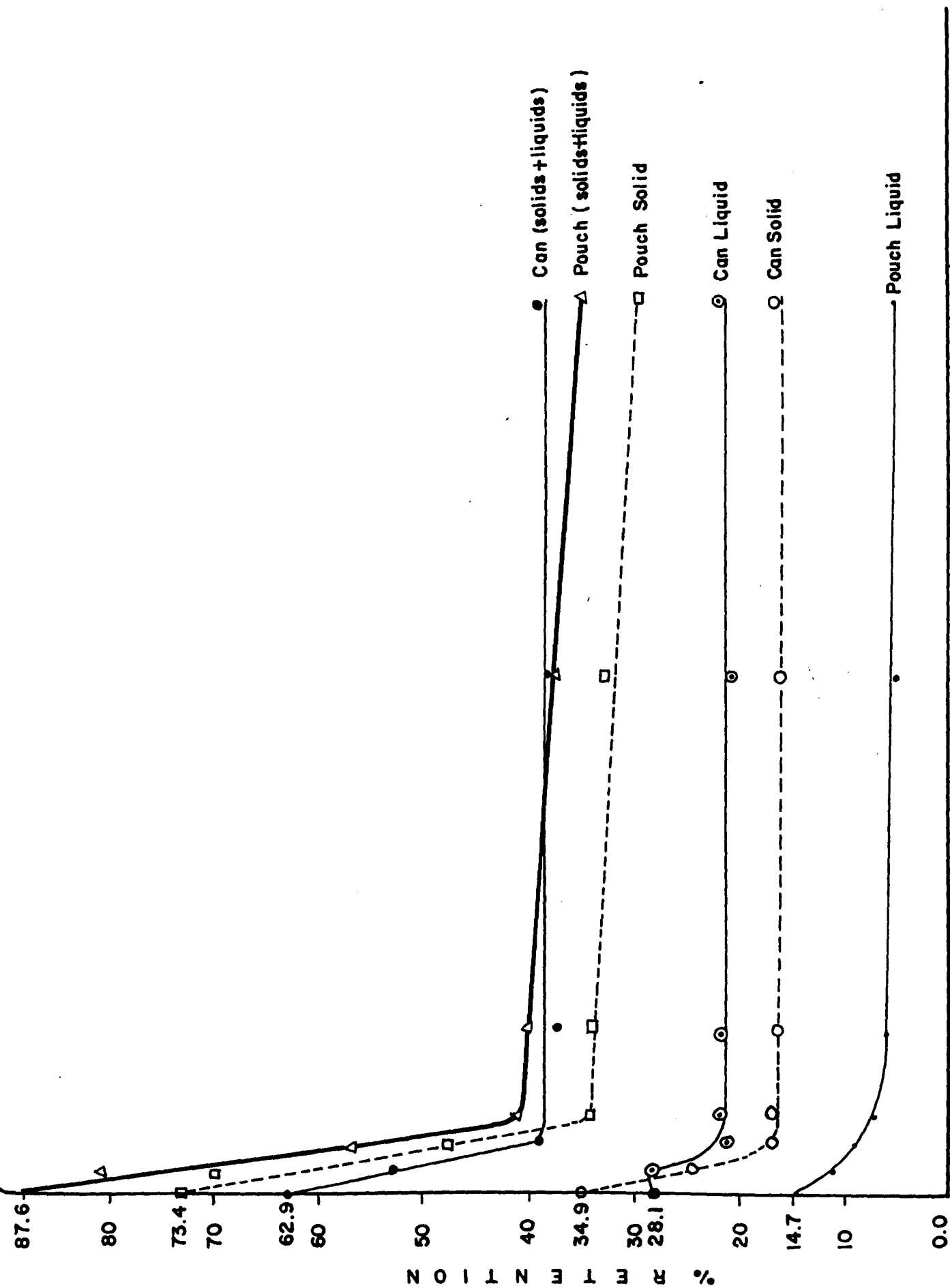


FIG. 5.12 ASCORBIC ACID RETENTION IN BRUSSELS SPROUTS AFTER STERILISATION AND DURING STORAGE AT 20°C

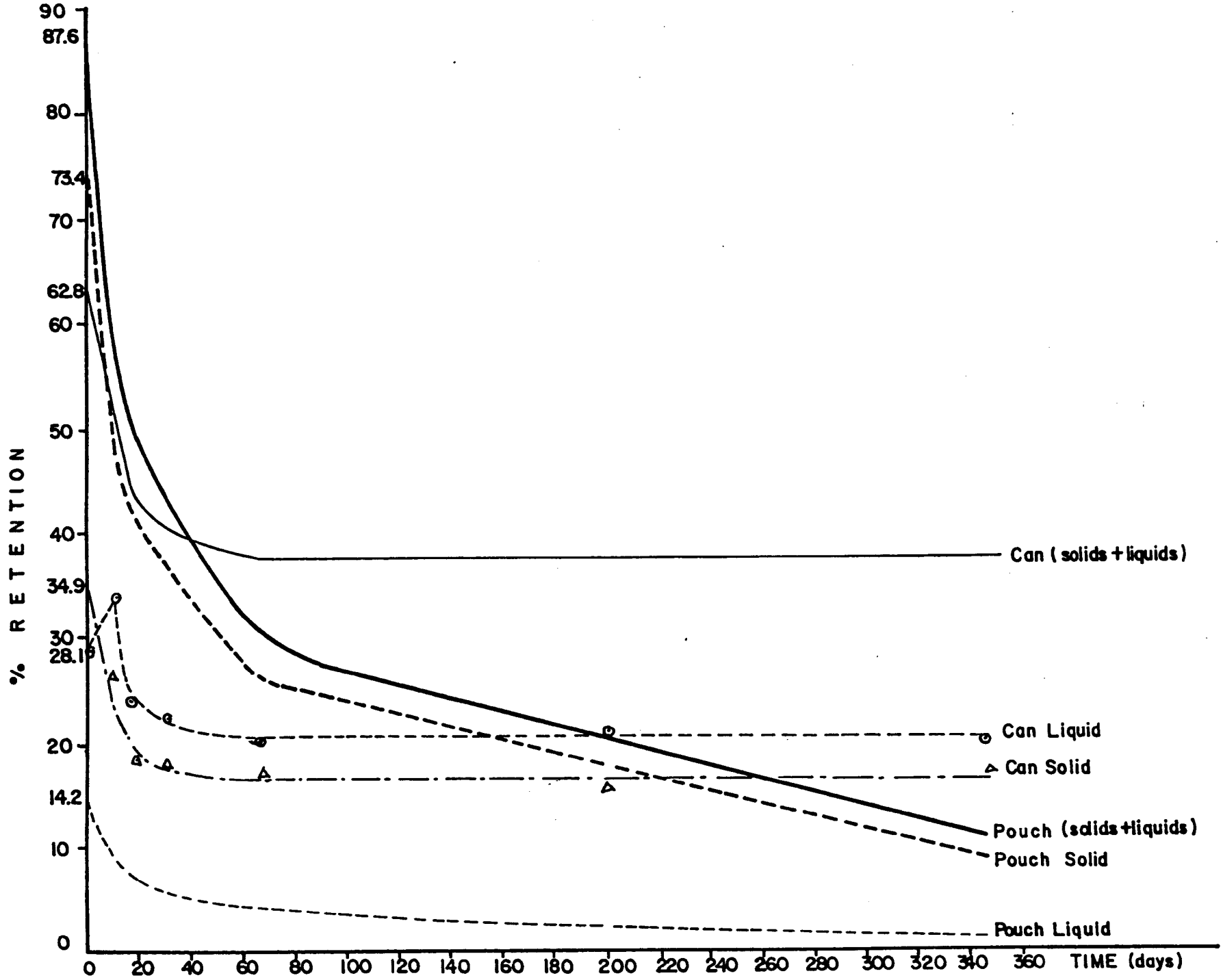


FIG. 5.13 ASCORBIC ACID RETENTION IN BRUSSELS SPROUTS AFTER STERILISATION AND DURING STORAGE AT 37 °C

place in the first two months of storage, instead of four months. Also in this case the overall ascorbic acid retention at the end of storage was slightly better in the canned product. While no change was detected in the total ascorbic acid in the canned product after two months of storage, in the pouched product a further slight decrease of ascorbic acid retention was noticed.

(c) Storage at 37°C

In Figure 5.13 it can be seen that for the canned product, storage at 37°C produced the same general behaviour as storage at both 20°C and 5°C with most of the ascorbic acid loss taking place during the first month and a half of storage and with no detectable ascorbic acid destruction during the following ten months of storage. For the pouched brussels sprouts totally different results were obtained. A surprisingly large decrease in the ascorbic acid content was obtained over the whole of the storage period. Therefore most of the vitamin C present in the sample was lost(≃ 90.0%) at the end of the storage period.

(d) Storage temperature effect

The effect of storage temperature on ascorbic acid retention on both pouched and canned brussels sprouts is shown in Figure 5.14, where it can be seen that pouches at 5°C and cans at both 5°C and 20°C show no difference in ascorbic acid retention while pouches at 20°C and cans at 37°C are slightly lower. But pouches at 37°C show an extremely large ascorbic acid destruction, which is further investigated in Chapter 6.

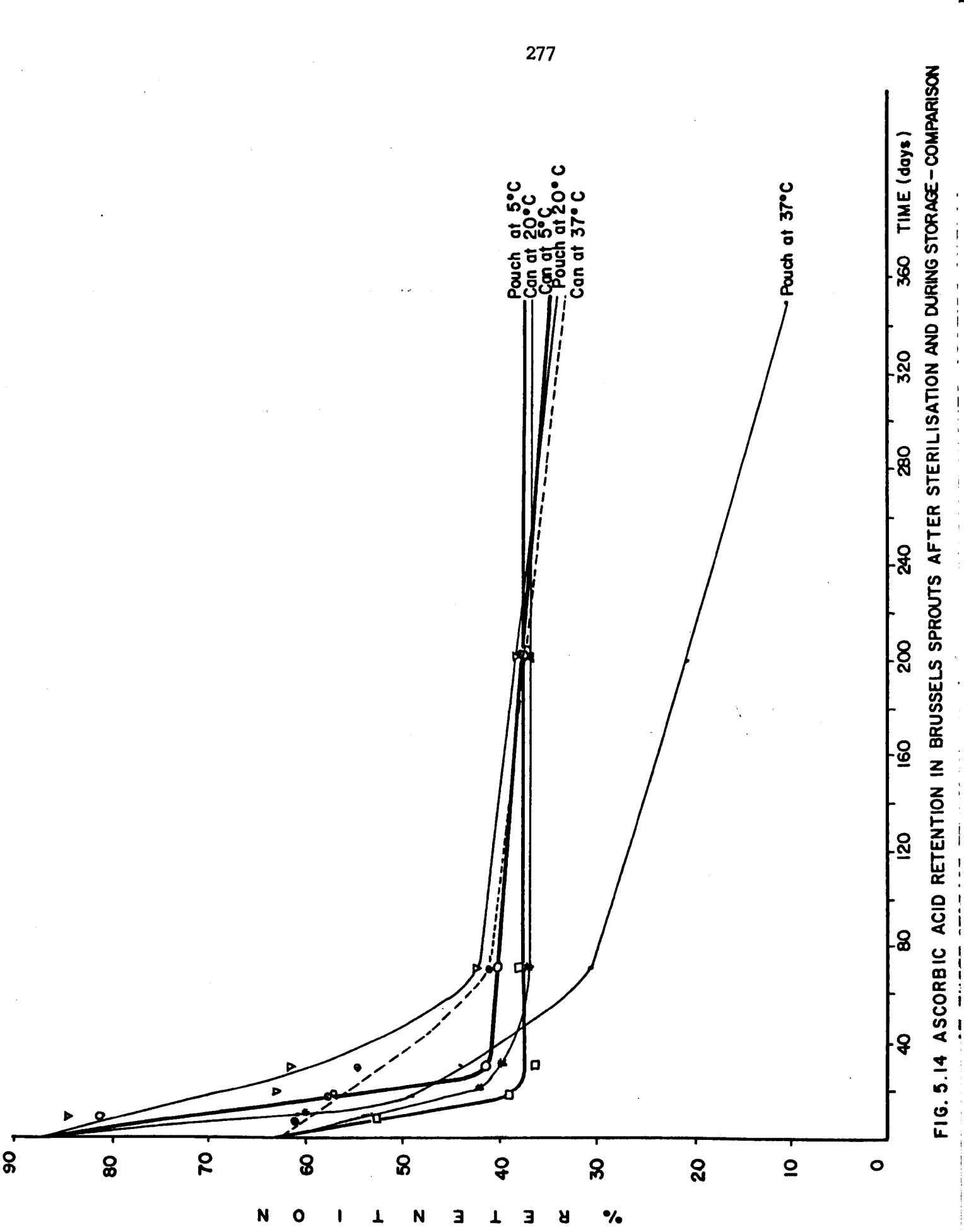


FIG. 5.14 ASCORBIC ACID RETENTION IN BRUSSELS SPROUTS AFTER STERILISATION AND DURING STORAGE – COMPARISON

(e) Leaching effect

For the three groups of canned brussels sprouts, (stored at 5 °C, 20 °C and 37 °C), there was an initial increase in the brine ascorbic acid content after nine days of storage. This effect suggests that the leaching rate of ascorbic acid into the brine was very high, consequently any ascorbic acid destruction taking place in the brine was covered by the leaching effect.

The effect of the ascorbic acid leaching into the brine, at three different storage temperatures, on the solids (edible portion) ascorbic acid retention is shown in Figure 5.15. Here it can be seen that for both types of packages storage at higher temperatures produced larger ascorbic acid losses than storage at lower temperatures. Also in Figure 5.15 the detrimental effect of leaching on the canned products can be easily observed. Although pouched brussels sprouts lost ascorbic acid heavily during storage, the combination of initial high post processing ascorbic acid content and the minimal leaching of vitamin C during storage resulted in a final product with a solids ascorbic acid content very similar to that of the canned product.

5.4 Jersey New Potatoes Evaluation

5.4.1 Experimental Procedure

All sampling was carried out as described in Appendix 1. Three packages were opened each time, taking two different samples from each package for both the ascorbic acid determination by the DCP visual titration method (Appendix 2.1) and moisture content (Appendix 2.8).

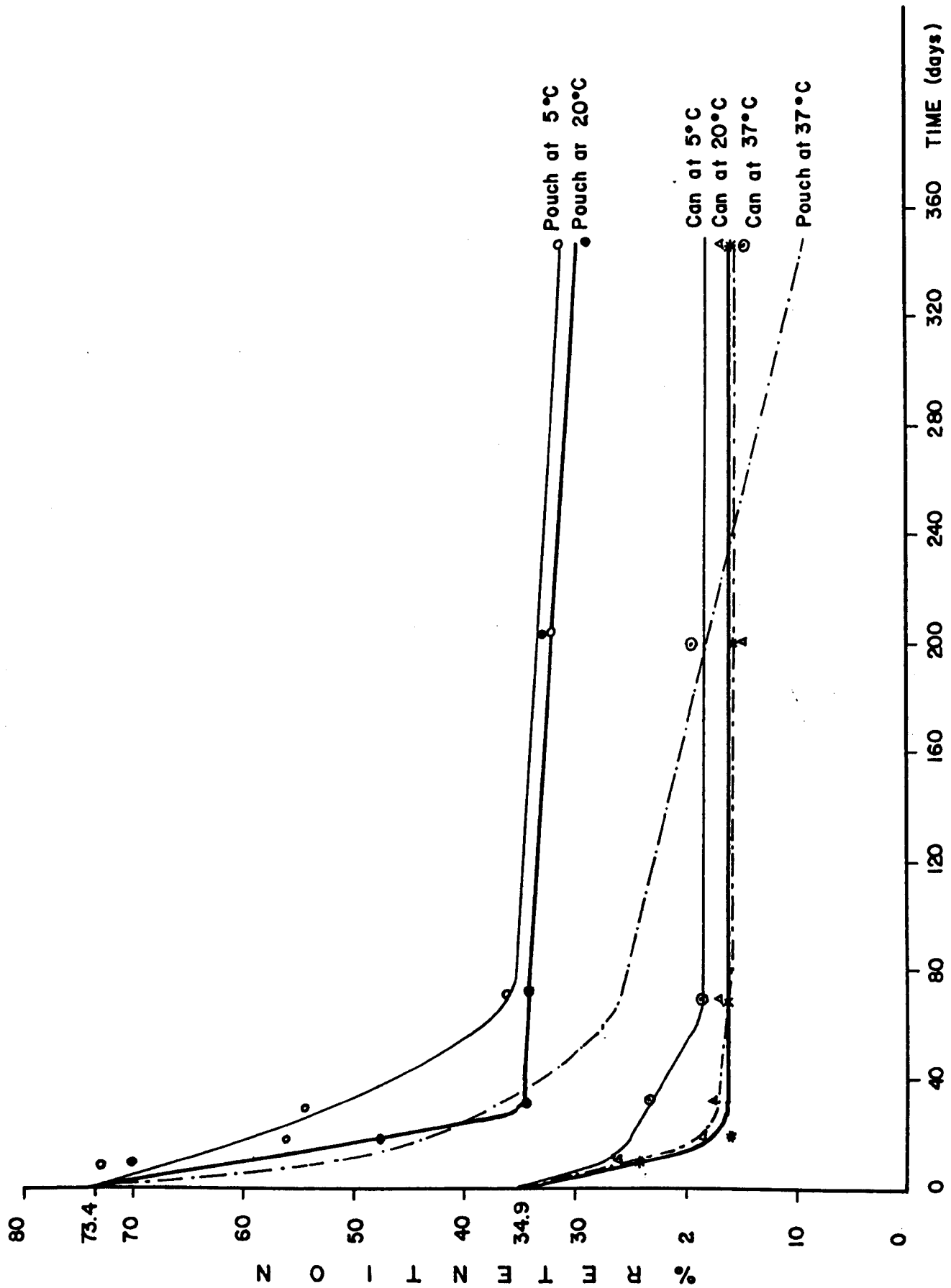


FIG. 5.15 ASCORBIC ACID RETENTION IN BRUSSELS SPROUTS AFTER STERILISATION AND DURING STORAGE – COMPARISON AT THREE STORAGE TEMPERATURES (SOLIDS ONLY).

5.4.2 Results and Discussion

Processing and storage condition effects on ascorbic acid retention in canned and pouched Jersey new potatoes are shown in Table 5.12. The values are given as milligrams of ascorbic acid per 100 grams of wet sample. The average moisture content of the pouched new potatoes is 81.75% with 21 degrees of freedom and 2.12 ± standard deviation and for the canned product the mean moisture content is 83.32% with 22 degrees of freedom and ±1.26 standard deviation.

(a) A-1 containers

The results obtained were plotted as percentage of ascorbic acid retention vs storage time at 20°C for both canned and pouched products. The curves obtained are shown in Figure 5.16 where 100% is the ascorbic acid content immediately after blanching and the starting points on the y-axis are the % of ascorbic acid retained after the sterilization process.

The loss due to can processing is larger, (26%), than the loss for pouch processing, (12%). In the canned new potatoes a further 22% is lost due to leaching of ascorbic acid into the brine immediately after processing.

In the pouched product most of the ascorbic acid loss took place during the first month of storage with no further destruction detected during the following 12 months. Also some liquid leached out from the pouched potatoes with a very small amount of ascorbic acid, (2.0%). The net effect being a very high ascorbic acid retention value, (76.0%), in the edible portion of the product. No ascorbic acid destruction was detected in the pouch liquid.

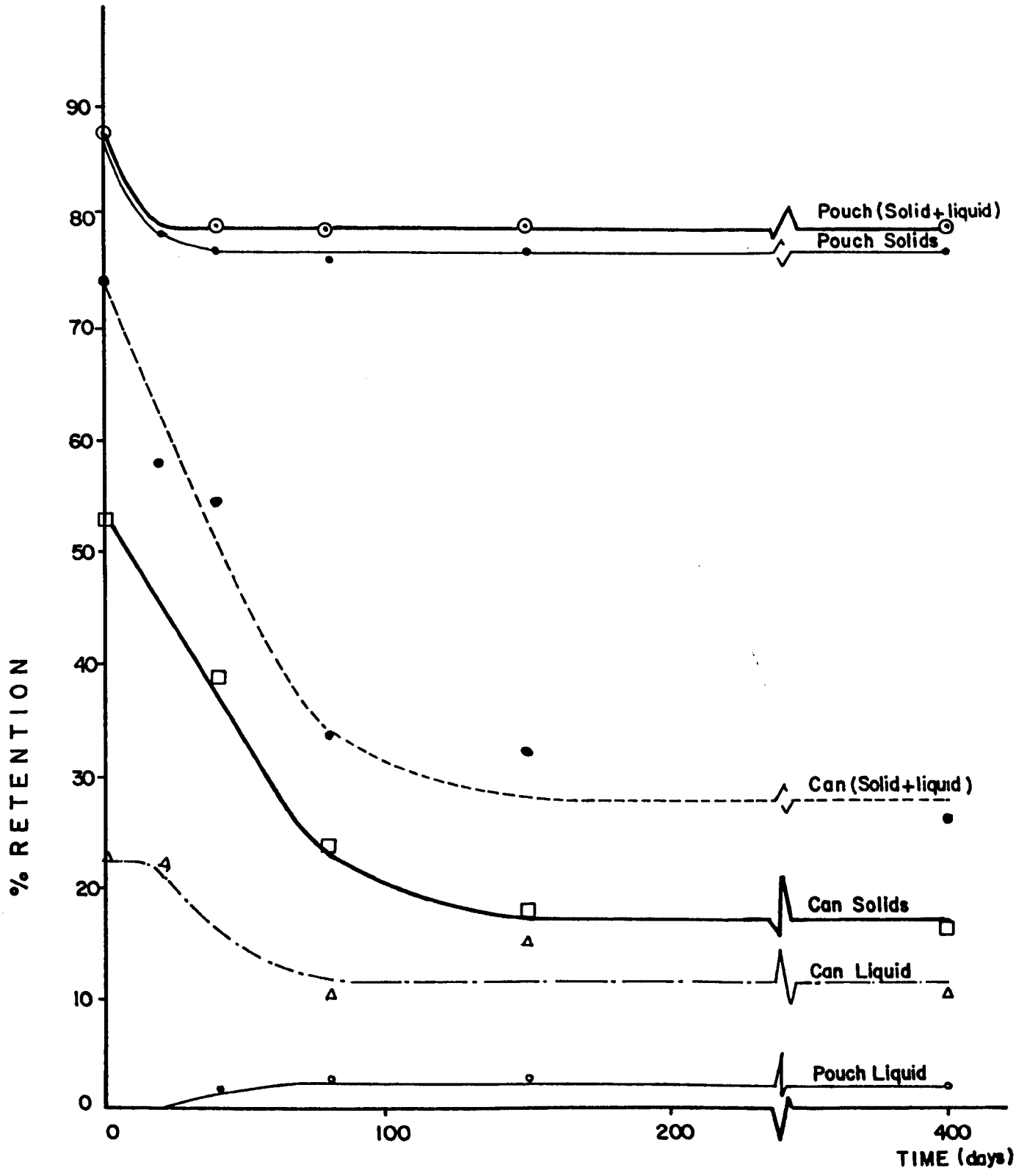


FIG. 5.16 ASCORBIC ACID RETENTION IN JERSEY NEW POTATOES AFTER STERILISATION AND DURING STORAGE AT +20°C

Table 5. 12 Ascorbic acid content in Jersey new potatoes stored at 20 °C, in milligrams per 100 grams of sample

Storage days	Pouched new potatoes			Canned new potatoes		
	Liquid*	Solid	Total	Liquid*	Solid	Total
B.P.	-	8.06 ± 0.21	8.06	-	8.06 ± 0.21	8.06
A.P.	-	7.06 ±0.225	7.06	2.09 ± 0.06	3.84 ± 0.19	5.93
20	-	6.41 ±0.24	6.41	1.69 ± 0.05	2.97 ± 0.12	4.67
42	0.17 ± 0.002	6.21± 0.28	6.38	1.67 ± 0.04	2.63 ± 0.05	4.30
81	0.21 ± 0.005	6.12 ± 0.12	6.33	1.65 ± 0.06	2.29 ± 0.11	4.14
159	0.18 ± 0.008	6.20 ± 0.18	6.38	1.56 ± 0.09	1.98 ± 0.09	3.54
402	0.15 ± 0.006	6.21 ± 0.16	6.36	0.85 ± 0.005	1.27 ± 0.05	2.12

B.P. Before processing
A.P. After Processing

Each value reported is the mean of six determinations (two per package). Each value is followed by ± 2σ (2 standard deviations).

* Ascorbic acid in the liquids refers to the ascorbic acid leached out from 100 grams of potatoes.

Total and solids ascorbic acid retention in the canned new potatoes were greatly reduced during the first 5½ months of storage, an extra ≃ 45.0% and ≃ 35.0% respectively. Most of the ascorbic acid loss in the brine took place during the first four months of storage with no detectable change in the following nine months. Also no change was detected in the first 20 days of storage maybe because the high rate of ascorbic acid leaching masked the destruction rate. Although there is a ≃ 28% total retention in the canned product, the solids only contain ≃ 17% of the original ascorbic acid present due to the damaging leaching effect.

(b) A-10 containers

The results obtained for vitamin C retention after processing in the A-10 containers are shown in Table 5.13. The A-10 pouch results are probably a minimum since the pouch burst during the cooling cycle and water from the retort entered the pouch. Therefore some loss of ascorbic acid may have occurred under those circumstances. Nevertheless the % retention due to pouch processing is far better, 64.28%. than ascorbic acid retention during canning, 34.4%. In the canned product an extra 11% is lost due to leaching of ascorbic acid into the brine.

The results obtained for this product indicate that leaching of ascorbic acid into the brine is a very important factor in ascorbic acid retention during processing and storage. Also the reduced time of pouch processing produces a greater ascorbic acid retention during the sterilization process and this difference is greatly increased in bigger containers in favour of the pouched product.

Table 5. 13 Ascorbic acid content before and after processing for A-10 containers, in milligrams per 100 grams of new potatoes

Storage days	Pouched new potatoes			Canned new potatoes		
	Liquid*	Solid	Total	Liquid*	Solid	Total
A-10 containers						
B.P.	-	8.27 ± 0.23	8.27	-	6.27 ± 0.22	6.27
%	-	100 ± 2.78	100	-	100 ± 3.51	100
A.P.	-	5.32 ± 0.20	5.32	0.74± 0.09	1.42 ± 0.12	2.16
%	-	64.28 ± 2.41	64.28	11.76 ± 1.43	22.66 ± 1.91	34.42

B.P. Before Processing
A.P. After Processing

* Ascorbic acid in liquid refers to the ascorbic acid leached out by 100 grams of potatoes.

Each value given is the mean of five determinations. Each value is followed by $\pm 2\sigma$.

5.5 Pork Casserole Evaluation

5.5.1 Experimental Procedure

Homogenization for this product was carried out under nitrogen as described in Appendix 1. Also three packages were used at each time, taking duplicate samples for the chemical analysis. These were for vitamin B, the thiochrome fluorimetric procedure (see Appendix 2.3); for ascorbic acid, the DCP visual titration (see Appendix 2.1) and moisture content as described in Appendix 2.8.

5.5.2 Results

(a) Ascorbic acid retention in A-1 containers

Ascorbic acid content values for pork casserole samples in both pouches and cans before and after processing, and during storage, at 20°C and 37°C are given in Table 5.14. Here the content is given as milligrams of ascorbic acid per 100 grams of wet sample. The mean moisture content values obtained were: 85.61, (41 d.f. and σ = 0.3614) for the canned samples.

The effect of heat treatment and storage at both 20° C and 37° C on ascorbic acid retention means ascorbic acid retention can be easily seen in Figure 5.17, where, 100% retention means ascorbic acid content immediately before processing.

Canning produced a larger destruction of ascorbic acid (15-17%) than the alternative pouch sterilization (6 - 8%).

During storage most of the ascorbic acid loss occurred during the first six weeks, an extra 17 -20% for the pouched products and 13 - 15% for the canned samples. No further decrease was detected in the following twelve months. As the ascorbic acid loss due to different storage temperatures produced the same effect on both types of packages, the initial processing

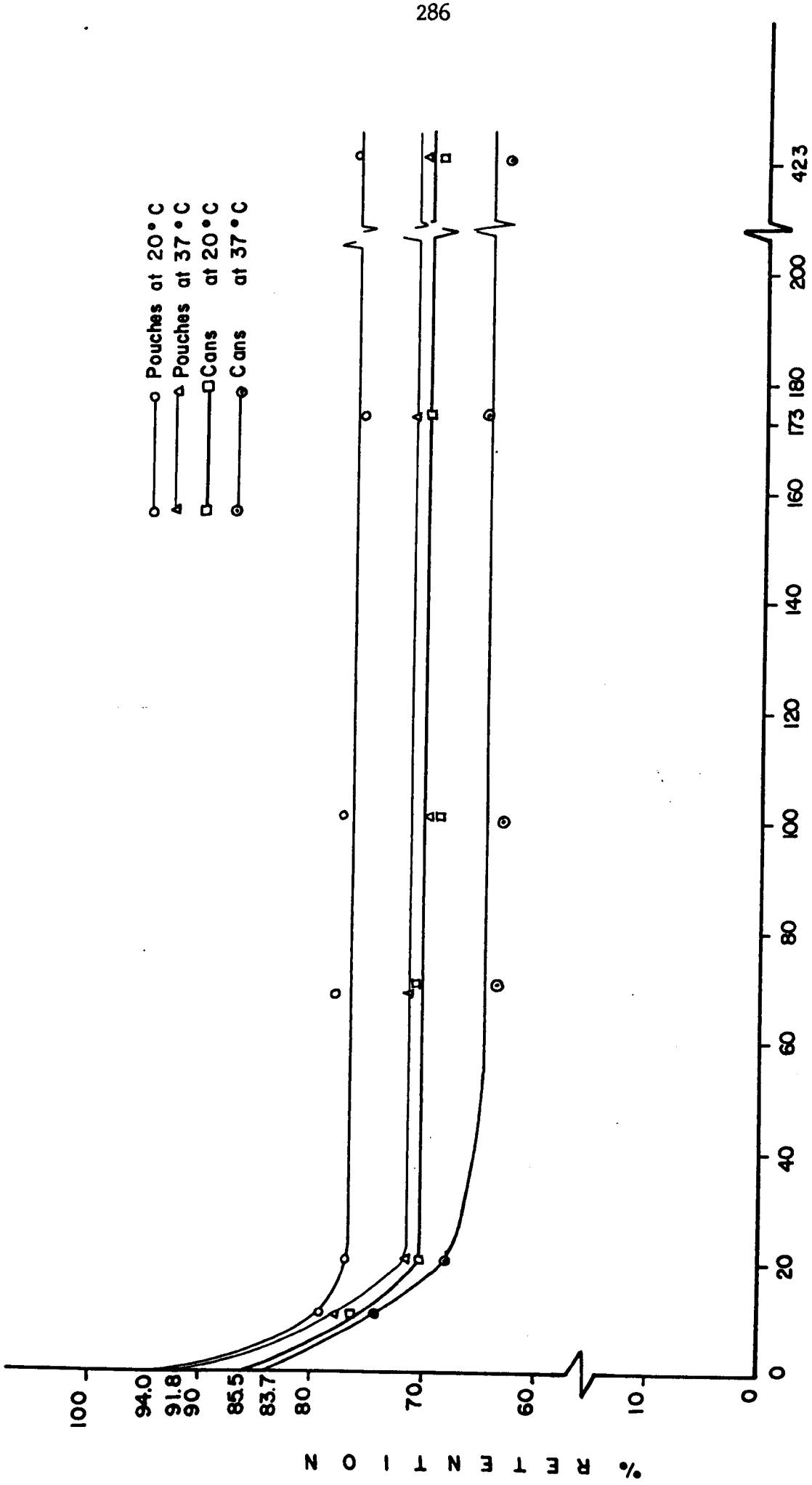


FIG. 5.17 ASCORBIC ACID RETENTION IN PORK CASSEROLE AFTER STERILISATION AND DURING STORAGE AT 20°C AND 37°C.

Table 5. 14 Vitamin C content as milligrams of ascorbic acid per 100 grams of pork casserole samples both in pouches and cans stored at 20°C and 37°C

Storage days	Pouched pork casserole A-1 containers		Canned pork casserole A-1 containers	
	Storage at 20°C	Storage at 37°C	Storage at 20°C	Storage at 37°C
B.P.	10.15 ± 0.47	10.15± 0.47	10.15 ± 0.47	10.15 ± 0.47
0 (A.P.)	9.57 ± 0.24	9.32 ± 0.29	8.71 ± 0.14	8.50 ± 0.14
9	8.05 ± 0.23	7.71 ± 0.22	7.66 ± 0.14	7.54 ± 0.05
20	7.81 ± 0.13	7.32 ± 0.27	7.27 ±0.17	7.08 ± 0.09
67	8.01 ± 0.18	7.25 ± 0.22	7.32 ± 0.04	6.53 ± 0.12
97	7.89 ± 0.24	7.01 ± 0.26	6.85 ± 0.16	6.29 ± 0.18
173	7.63 ± 0.21	7.21 ± 0.19	7.15 ± 0.05	6.59 ± 0.19
423	7.71 ± 0.13	7.13 ± 0.22	6.92 ± 0.18	6.27 ± 0.21

B.P. Before Processing
A.P. After Processing

±2 σ (± 2 standard deviations)

difference seems to be maintained during storage. Although ascorbic acid retention in the four groups of samples was very good, between 45% and 76%, pouched products were slightly better than the canned products, mainly due to the processing effect.

(b) Thiamine retention in A-1 containers

Vitamin B_1 content values of pork casserole samples in both pouches and cans before and after processing and during storage at 20 °C and 37 °C are given in Table 5.15 in milligrams of thiamine per 100 grams of wet sample. The results obtained were plotted as percentage retention of thiamine vs time of storage as shown in Figure 5.18 where 100% retention means thiamine content immediately before processing. In this Figure it can be seen that the effects of heat treatment during processing and storage at 20 °C and 37 °C on thiamine are very similar to the effects obtained for ascorbic acid which are shown in Figure 5.17. In the processing stage, the loss of thiamine in cans was 14 - 16% relative to the 6 - 8% in pouches. During the first 40 days of storage a further 20% loss was observed in both types of packages with no change detected in the following twelve months of storage. Thiamine retention during storage at both temperatures in pouches was slightly higher than thiamine retention at 37°C and 20°C in cans.

(c) Ascorbic Acid and Vitamin B_1 Retention in A-10 Containers

Ascorbic acid and thiamine content obtained before and after processing for the A-10 containers are given in Table 5.16, together with the results obtained for the A-1 size pouches and cans. Here, the effect of different heat treatments on vitamins retention can be seen. The difference in the heat treatments was

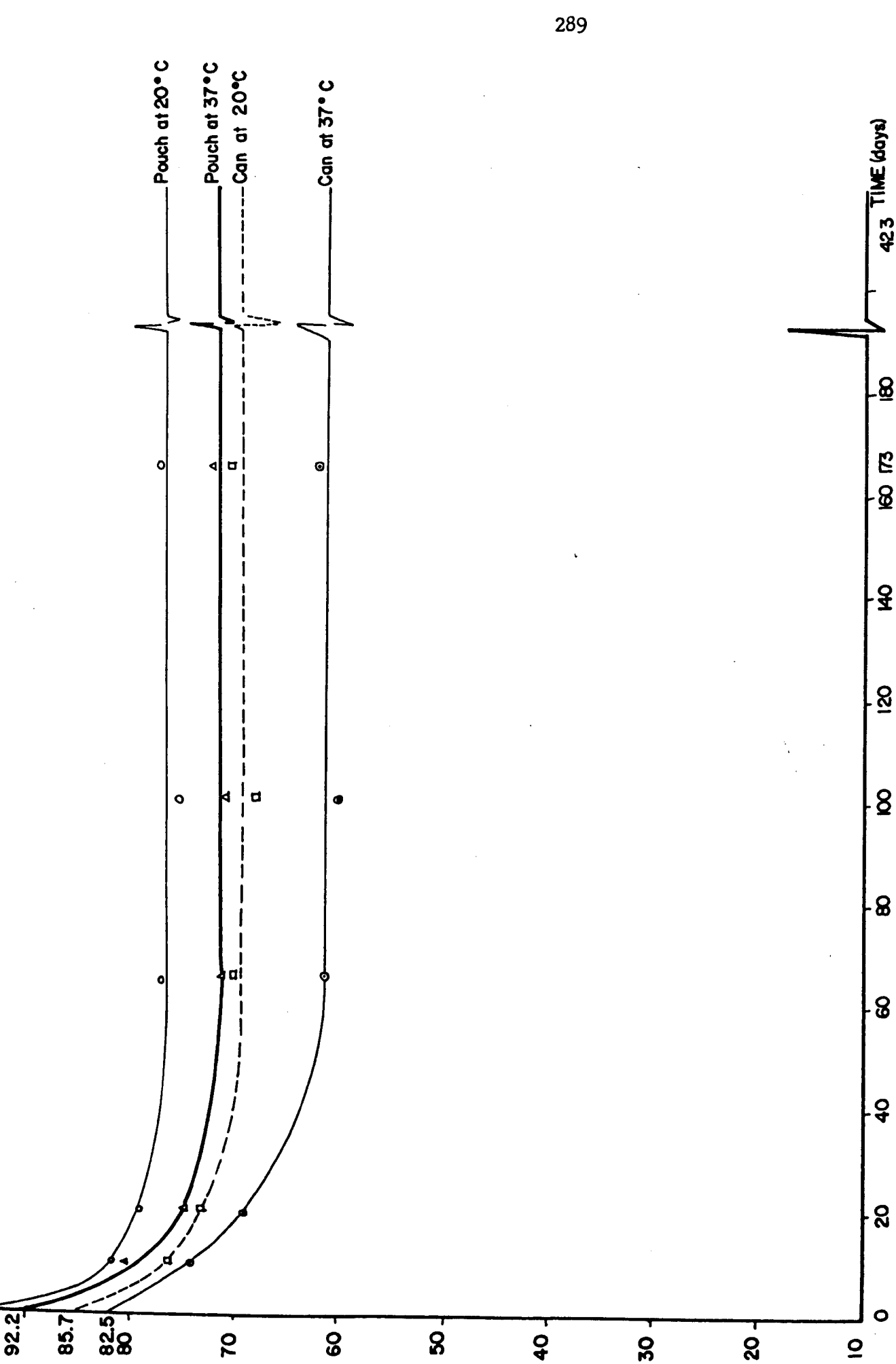


FIG. 5.18 THIAMINE RETENTION IN PORK CASSEROLE AFTER STERILISATION AND DURING STORAGE AT 20°C AND 37°C.

Storage days	Pouched samples		Canned samples	
	Stored at 20°C	Stored at 37°C	Stored at 20°C	Stored at 37°C
B.P.	4.35 ± 0.32	4.35 ± 0.32	4.35 ± 0.32	4.35 ± 0.32
(O) A.P.	4.11 ± 0.21	4.01 ± 0.18	3.73 ± 0.24	3.58 ± 0.20
9	3.55 ± 0.24	3.50 ± 0.21	3.32 ± 0.18	3.21 ± 0.11
20	3.44 ± 0.08	3.25 ± 0.13	3.17 ± 0.16	2.98 ± 0.19
67	3.33 ± 0.17	3.09 ± 0.12	3.06 ± 0.08	2.66 ± 0.04
97	3.26 ± 0.24	3.06 ± 0.14	2.94 ± 0.11	2.57 ± 0.07
173	3.35 ± 0.13	3.12 ± 0.15	3.08 ± 0.19	2.72 ± 0.10
423	3.46 ± 0.04	3.16 ± 0.08	3.00 ± 0.20	2.56 ± 0.14

B.P. Before Processing
A.P. After Proces sing
Mean of six values followed by ± 2 standard deviations

Table 5. 15 Vitamin B_1 content in pork casserole samples both in pouches and cans stored at 20 °C and 37 °C in milligrams of thiamine per 100 grams of sample

caused by the difference in size and type of package since all processes were designed to achieve the same sterilization value.

The results obtained show that pouch processing advantages in nutrient retention increase when compared to canning as the container size increases. Also the results on this product indicate that nutrient losses during storage at 20° C are slower than those at 37 °C, as expected and that similar storage conditions produce a similar effect on vitamins B_1 and C retention

	Type of container	Ascorbic acid content 1	%	Thiamine content 1	%
B.P.	all	10.15 ± 0.47	100	4.35 ± 0.32	100
A.P.	A-1 pouch $_1$	9.57 ± 0.24	97.28	4.11 ± 0.21	94.48
A.P.	A-1 pouch$_2$	9.32 ± 0.29	91.18	4.01 ± 0.18	92.18
A.P.	A-1 can$_1$	8.71 ± 0.14	85.81	3.73 ± 0.24	85.75
A.P.	A-1 can$_2$	8.50 ± 0.14	83.74	3.58 ± 0.2	82.29
A.P.	A-10 pouch	7.22 ± 0.91	71.13	2.72 ± 1.02	62.60
A.P.	A-10 can	5.099	50.24	1.76	37.86

B.P. Before process
A.P. After process
(1) Milligrams of vitamin per 100 g of sample
±2 standard deviations

Table 5.16 Effect of container type and size on ascorbic acid retention and thiamine retention during sterilization process

in both types of packages. This suggests that the difference in nutrient retention at the end of the storage period is basically the difference produced by the processing conditions.

(d) Thiamine content of flavouring ingredients

In Tables 5.15 and 5.1 it can be seen that the values obtained are too high for the range of values expected in a non-fortified thiamine product. Because pork meat was believed to be the major contributor to the thiamine content in the pork casserole product and the reported thiamine value for lean pork meat is 0.89 milligrams/100 grams of sample from McCance and Widdowson (1976)food composition tables.

Therefore some sort of interference with the analytical

technique was suspected in the product. The excitation and emission spectras were obtained for both the sample extraction solution and the standard thiamine hydrochloride solution. The spectras were found to be identical. Also, ion-exchange purification procedure used to separate the suspected interfering substance (s) showed no real difference. The thiamine content values obtained for the sample solutions remained the same whether or not the ion-exchange purification procedure was included in the analytical technique. Finally, thiamine content was determined for each one of the pork casserole ingredients, to find out the possible source of interference. The results obtained showed that the gravy was the ingredient containing the interfering substance (s). Therefore the gravy ingredients were analyzed separately to find out that the flavouring ingredients were the source of the interference, particularly H.P.P. type RF-P oil coated (see Appendix 3). As the spectra from the standard and sample solution were identical and because no improvement was achieved by using ion-exchange purification procedure, it was suspected that a high level of thiamine was present in this flavouring ingredient. The ingredient was described by the manufacturers (F.I.S.) as hydrolyzed plant protein.

Based on both literature reports of the "meaty" flavour of thiamine thermal decomposition products (Fenema, 1976, Farrer, 1955, and Tannenbaum, 1980) and the high thiamine content of thiamine in yeast products, enquiries about the origin of this flavouring ingredient were made. The manufacturer confirmed that it would be expected to have a high vitamin B_1 content. Consequently the pork casserole was accidently fortified with thiamine.

5.6 Rice Pudding Evaluation

5.6.1 Experimental Procedure

Rice pudding samples were homogenized as described in Appendix 1 and duplicate samples were taken, from each package, for available lysine content by the FDNB procedure (Appendix 2.5), nitrogen content (Appendix 2.7), moisture content (Appendix 2.8) and colour measurement as described in section 3.10.

5.6.2 Results and Discussion

Nitrogen content was determined for both formulations. The mean of 20 determinations for formulation 2 is 0.496% with a standard deviation (σ) of $\pm$ 0.01%. For 26 determinations on formulation 4 the mean is 0.484% with $\sigma = \pm$ 0.02%. The results for moisture content are for 19 determinations on formulation 2 76.32% with $\sigma = \pm$ 0.829 and for formulation 4, 25 determinations were carried out resulting in a mean moisture value of 78.42% and $\sigma = \pm$ 0.981%

(a) Available Lysine

The effects of both heat treatments during processing,and package dimensions on available lysine content in rice puddings of both formulations, 2 and 4, are given in Table 5.16. Here it can be seen that rice pudding in both A-1 pouches and cans showed higher available lysine values than their equivalent A-10 containers. Also that shorter heat treatments (F_o-value = 5 min) resulted in higher available lysine values than the longer heat treatments (F_o -value = 10 min). These findings can be easily explained by the difference in processing time obtained, (see Table 5.1).

The results in Table 5.1 also suggest a slight formulation

effect. For A-1 and A-10 pouches formulation 4 gave higher available lysine yields while for cans formulation 2 produced better results for both A-1 and A-10 sizes. These can be partially explained by the processing times obtained. Explained by an indirect effect of the rice pudding formulation on the processing times obtained due to changes in the viscosity of the product.

	Target F_o-value 5 min		Target F_o-value 10 min	
Container	Formulation 2	Formulation 4	Formulation 2	Formulation 4
A-1 pouch	86.99 ± 4.11	89.44 ± 3.8	-	86.96 ± 4.01
A-1 can	87.84 ± 2.7	84.87 ± 3.95	77.93 ± 3.37	-
A-10 pouch	-	75.75 ± 4.53	66.22 ± 3.34	73.13 ± 5.1
A-10 can	78.75 ± 2.7	73.72 ± 2.9	70.76 ± 3.19	-

All values are the mean of four determinations and are followed by ± 2 σ

Table 5.17 Effect of heat sterilization on available lysine content in rice pudding sample. Expressed as percentage of the total amount of lysine present in the samples.

Storage conditions

The effects of storage temperature and time on available lysine retention in both pouched and canned rice puddings are shown in Table 5.18 and Figures 5.19 to 5.23. In these figures 100% retention means the total lysine content of the sample since any difference between the total lysine and the available lysine after precooking was too small to be detected by the assay procedure.

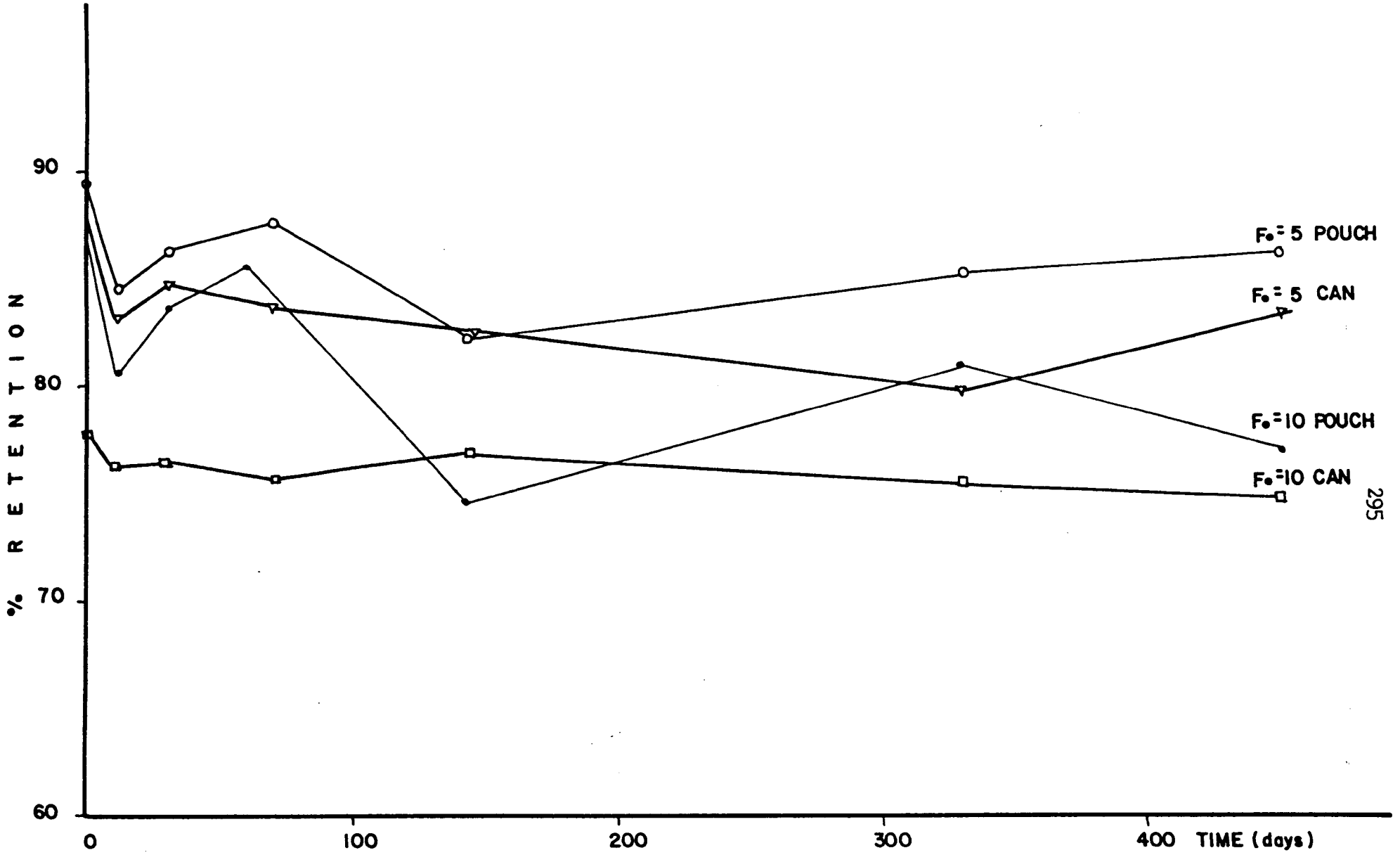


FIG. 5.19 AVAILABLE LYSINE RETENTION IN RICE PUDDING AFTER STERILISATION AND DURING STORAGE AT 5°C

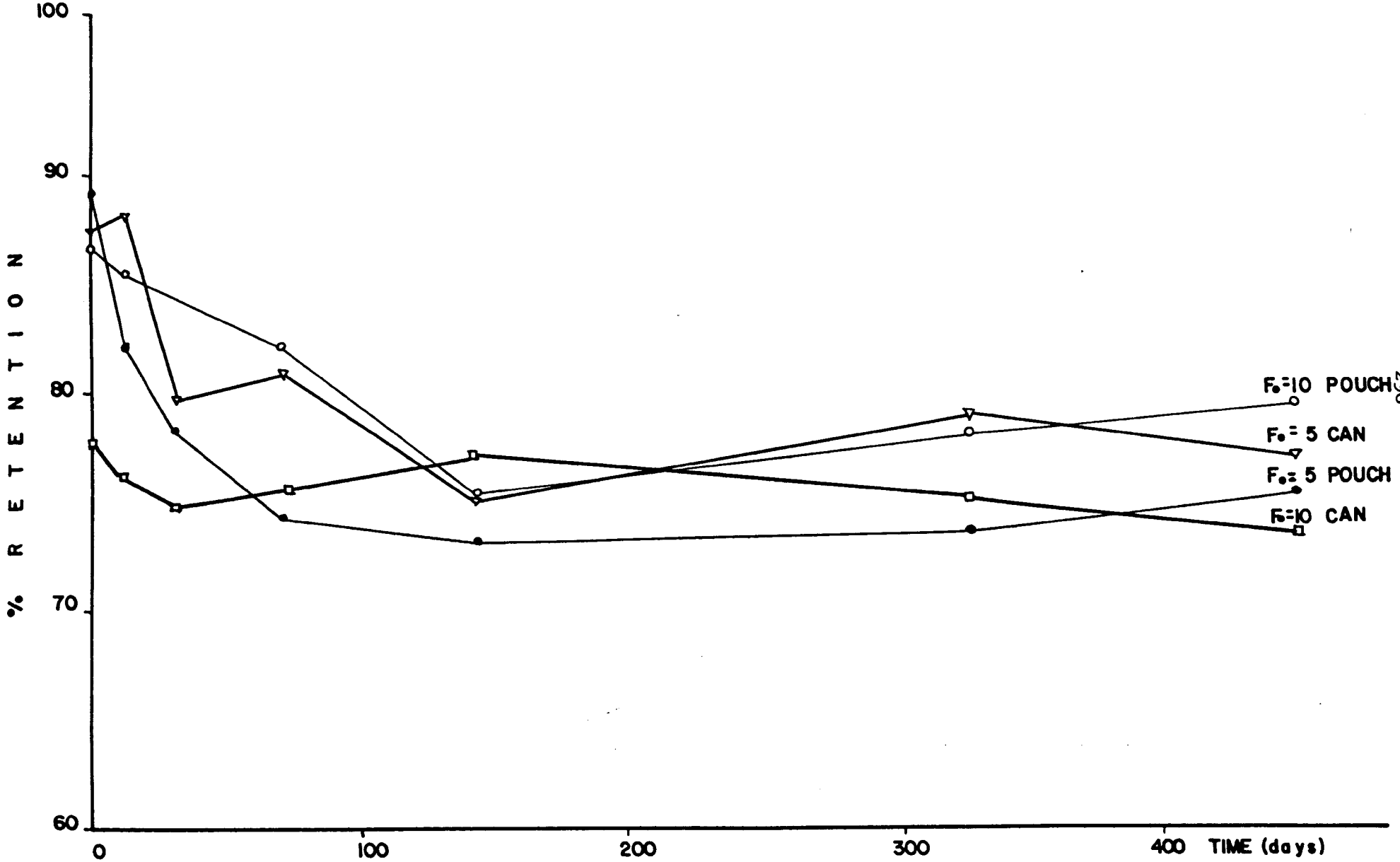


FIG. 5.20 AVAILABLE LYSINE RETENTION IN RICE PUDDING AFTER STERILISATION AND DURING STORAGE AT 20° C

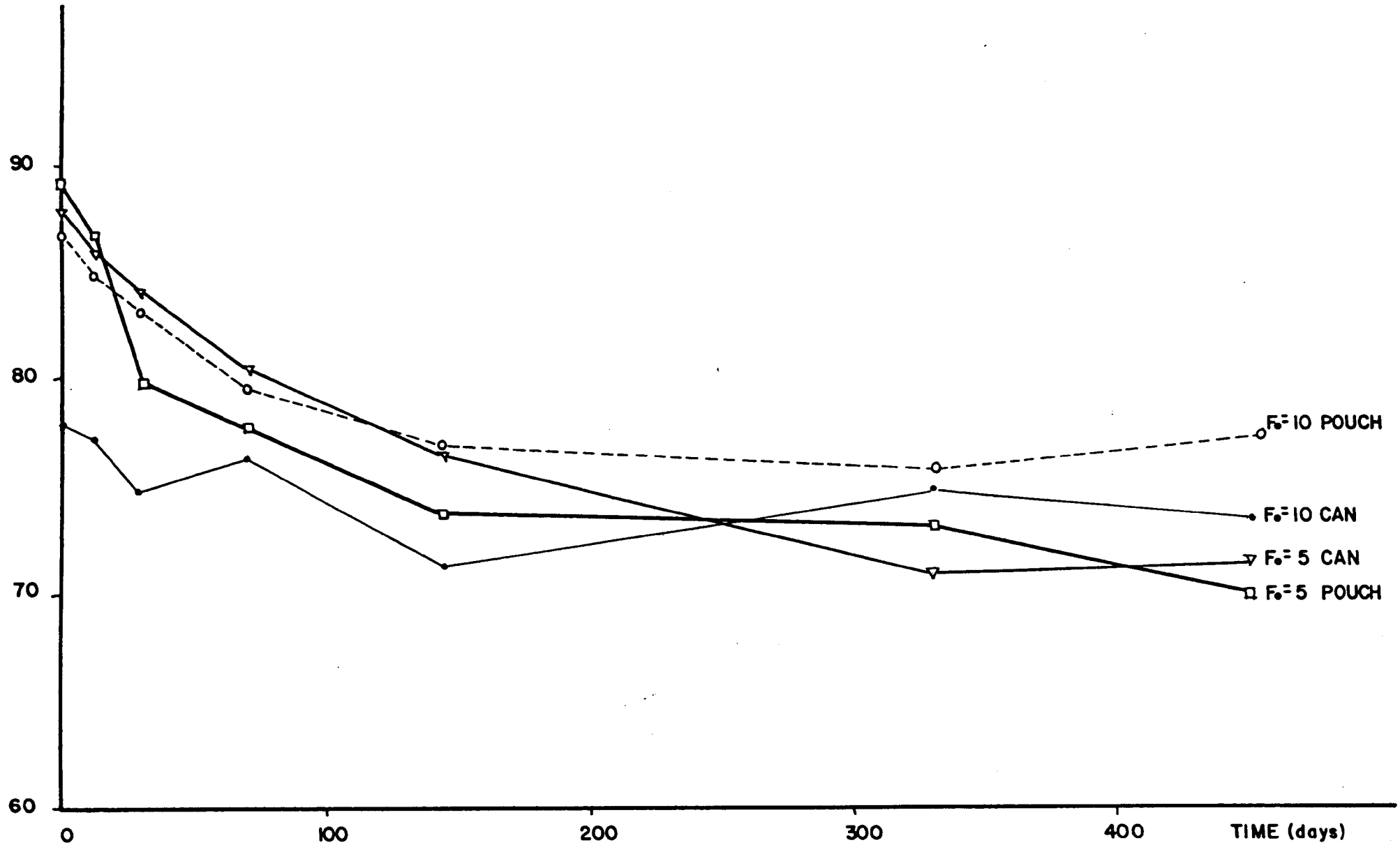


FIG. 5.21 AVAILABLE LYSINE RETENTION IN RICE PUDDING AFTER STERILISATION AND DURING STORAGE AT 37° C

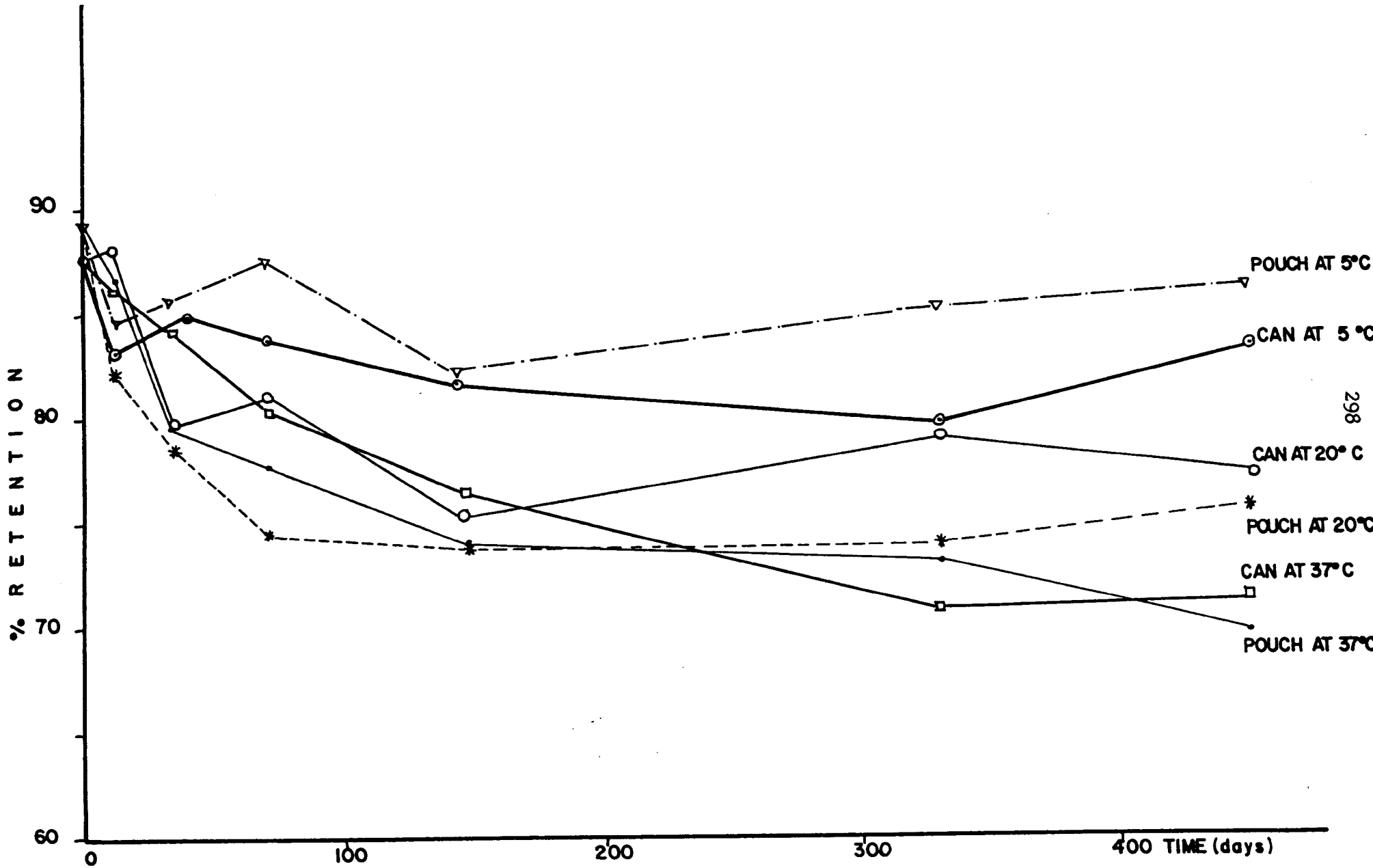


FIG.5.22 AVAILABLE LYSINE RETENTION IN RICE PUDDING. COMPARISON OF 3 STORAGE TEMPERATURES. $F_o \simeq 5$ min

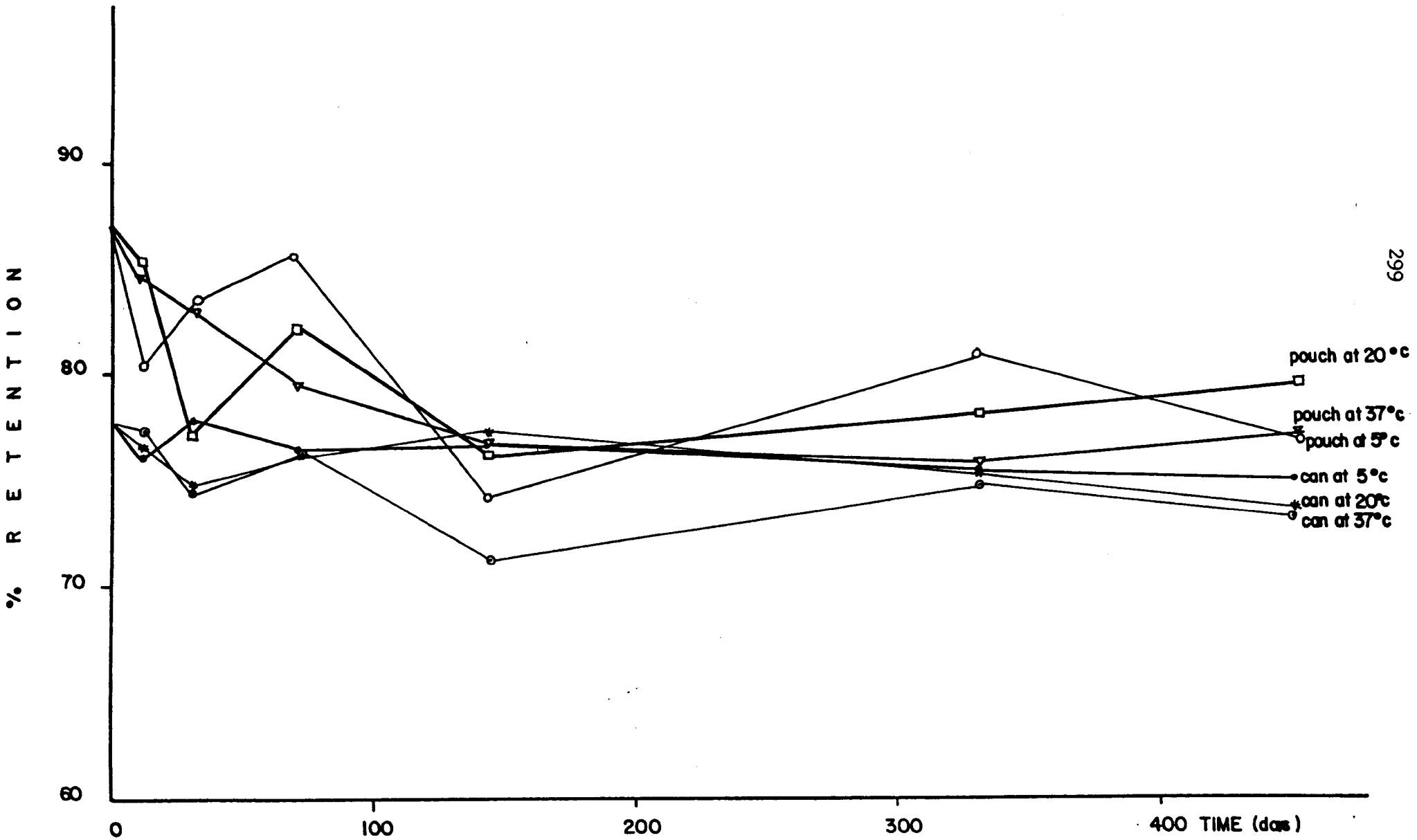


FIG. 5.23 AVAILABLE LYSINE RETENTION IN RICE PUDDING. COMPARISON OF 3 STORAGE TEMPERATURES. F_o = 10 min.

Table 5.18 Available lysine content in rice pudding samples in A-1 containers due to sterilization process and storage. In millimoles of available lysine per gram of nitrogen of sample.

Storage temp	Target sterilization value F_o = 5min						Target sterilization F_o = 10 min					
	5°C		20°C		37°C		5°C		20 °C		37°C	
Package storage / days	Pouch	Can	Pouch	Can	Pouch	Can	Pouch	Can	Pouch	Can	Pouch	Can
B.P	3.03	2.96	3.03	2.96	3.03	2.96	2.99	2.99	2.99	2.99	2.99	2.99
A.P	2.71	2.6	2.71	2.6	2.71	2.6	2.6	2.33	2.6	2.33	2.6	2.33
13	2.56	2.46	2.49	2.61	2.63	2.56	2.41	2.28	2.56	2.30	2.54	2.31
34	2.61	2.51	2.38	2.36	2.42	2.49	2.50	2.28	2.31	2.24	2.49	2.24
70	2.65	2.48	2.26	2.40	2.36	2.38	2.56	2.29	2.46	2.28	2.38	2.28
145	2.49	2.44	2 24	2.23	2.24	2.27	2.22	2.30	2.28	2.31	2.30	2.13
330	2.58	2.36	2.24	2.34	2.22	2.10	2.42	2.36	2.34	2.25	2.27	2.29
450	2.61	2.47	2.29	2.28	2.12	2.12	2.30	2.24	2.38	2.20	2.31	2.19

B.P. Before process
A.P. After process

Each value given is the mean of four determinations

The mean standard deviation of the samples is ± 0.04.

Storage at 5°C

Storage at 5°C (see Figure 5.19) produced the same small reduction of available lysine, (≃ 5%), in both pouches and cans processed to a target F_o -value of 5 min. But the losses from the pouched samples aiming at a higher heat treatment (F_o = 10 min) were larger, a further ≃ 9.0%, while, for the equivalent canned samples, no extra losses in available lysine were detected during storage. Most of the losses occurred during the first five months of storage with no significant change detected in the following ten months. The critical difference due to the processing effect in favour of the pouched samples was lost during storage. At the end of the storage period no difference existed between pouches and cans with equivalent heat treatments.

Storage at 20°C

The effect of storage at 20°C is shown in Figure 5.20 where it can be seen that pouched samples suffered larger losses during storage, ≃ 9% and ≃15% for F_o = 5 min and F_o = 10 min respectively, than canned examples, ≃ 2% and ≃ 9%. Thus at the end of the storage period there was no difference in the available lysine content of pouched and canned samples. Here, also, most of the losses took place during the first five months of storage.

Storage at 37°C

Storage at 37°C (see Figure 5.21) produced the same loss, (≃ 16%) in available lysine for pouches and cans processed to a target F -value of 5 min. But for pouched rice pudding with a target F -value of 10 min, the loss was higher (≃ 10%) than for the equivalent canned product (≃ 4%). Therefore the

advantages of pouch processing in available lysine retention were lost during storage and no significant difference was found at the end of the storage period. For this group of samples, the major part of the loss also occurred during the first five months of storage.

Storage Temperature Effect

The effect of storage temperature in the samples processed to a target F_o-value of 5 min is shown in Figure 5.22 where it can be seen that higher temperatures produced slightly larger available lysine losses than lower temperatures. Also it is shown that no real difference exists between pouches and cans stored under the same conditions. However, for samples processed to a target F_o-value of 10 min, (see Figure 5.23) no difference due to type of package or storage temperature was obtained. Nevertheless the quality of the product processed in pouches in a static retort is the same or possibly slightly better than when processed in one of the best (rotary retort) canning procedures available, in spite of longer process time and less efficient sterilizer.

(b) Colour difference

Reduction of available lysine through non-enzymatic browning has been widely studied and reviewed, (Anderson, 1980, Bender, 1972, Carpenter and Booth, 1973, Hodge, 1953). Furthermore, it has been suggested by Rhee and Rhee, (1981), that a significant correlation exists between browning index and available lysine. Therefore it was considered that a study of the colour change of the rice pudding samples could be useful to establish if a correlation with available lysine reduction existed.

Storage Conditions

The effects of storage temperature and storage time on colour development of the same are shown in Table 5.19 and Figures 5.24 to 5.28. Inspection of these Figures shows a tendency for the colour development to increase with storage time, particularly towards the end of the storage period, the effect being more pronounced at the higher storage temperatures. There is also some evidence that colour development during storage is greater for the canned product and greater for the more severe heating process.

These findings do not correlate well with the available lysine storage changes which were found to be greater at the beginning of the storage period. This may be explained by the proposed nonenzymatic browning mechanisms where lysine is damaged at the beginning of the chain of reactions (formation of sugar-amine complexes) with no colour evelopment but even if no further lysine is damaged the reation continues until in the intermediate state yellow compounds are formed and in the final stage, highly coloured compounds are produced.

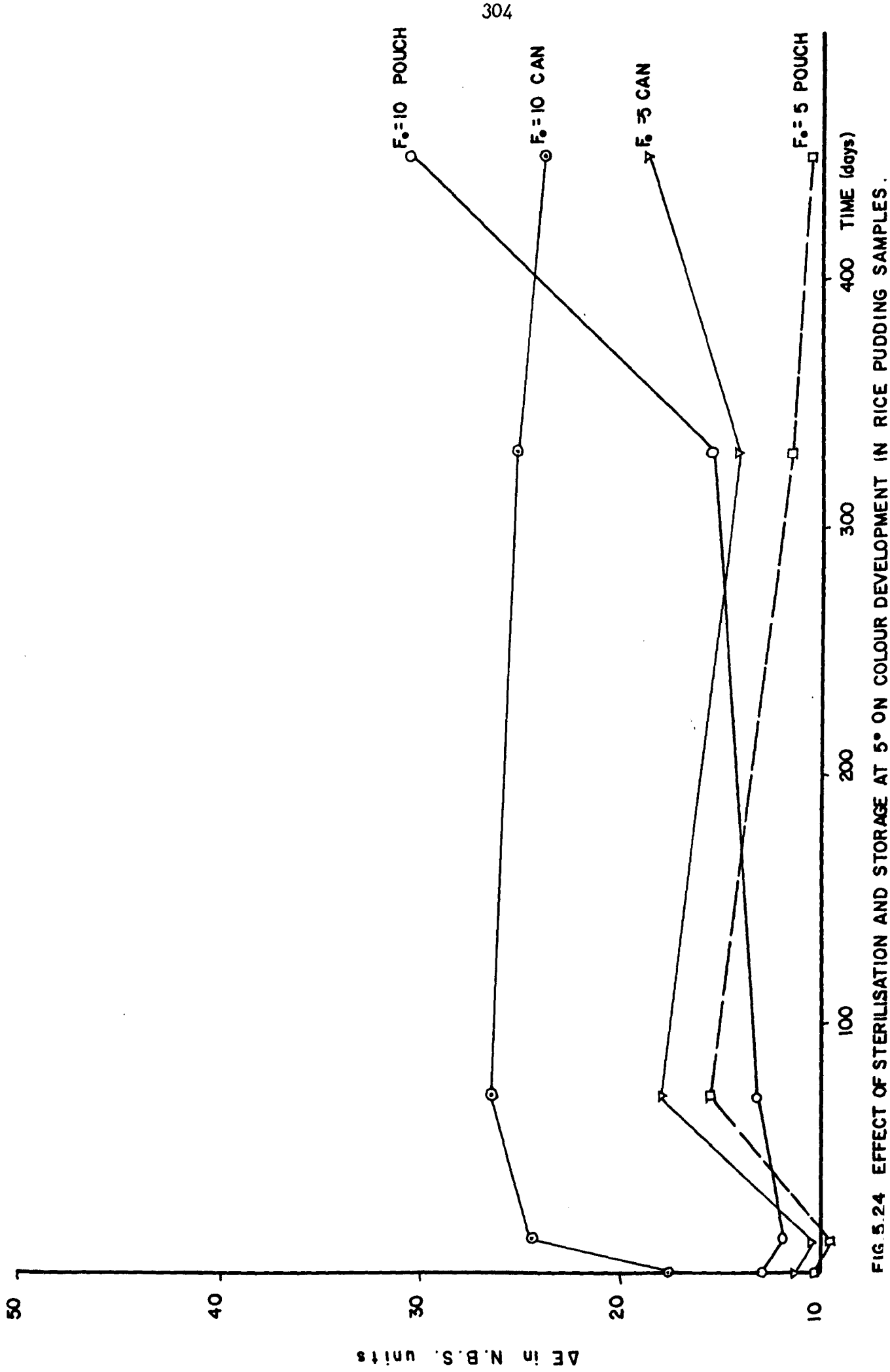


FIG. 5.24 EFFECT OF STERILISATION AND STORAGE AT 5° ON COLOUR DEVELOPMENT IN RICE PUDDING SAMPLES.

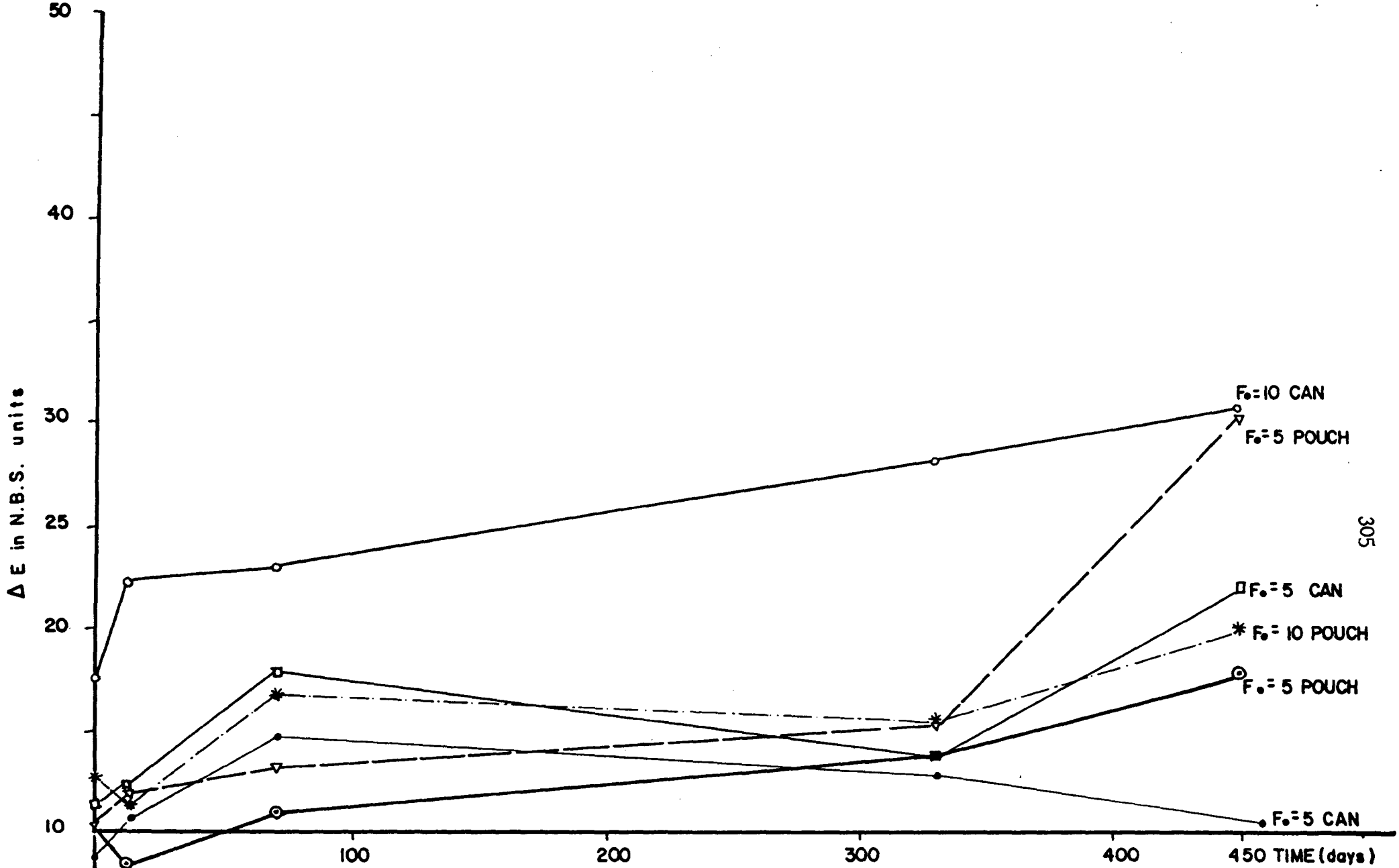


FIG. 5.25 EFFECT OF STERILISATION AND STORAGE AT 20° C ON COLOUR DEVELOPMENT IN RICE PUDDING SAMPLES.

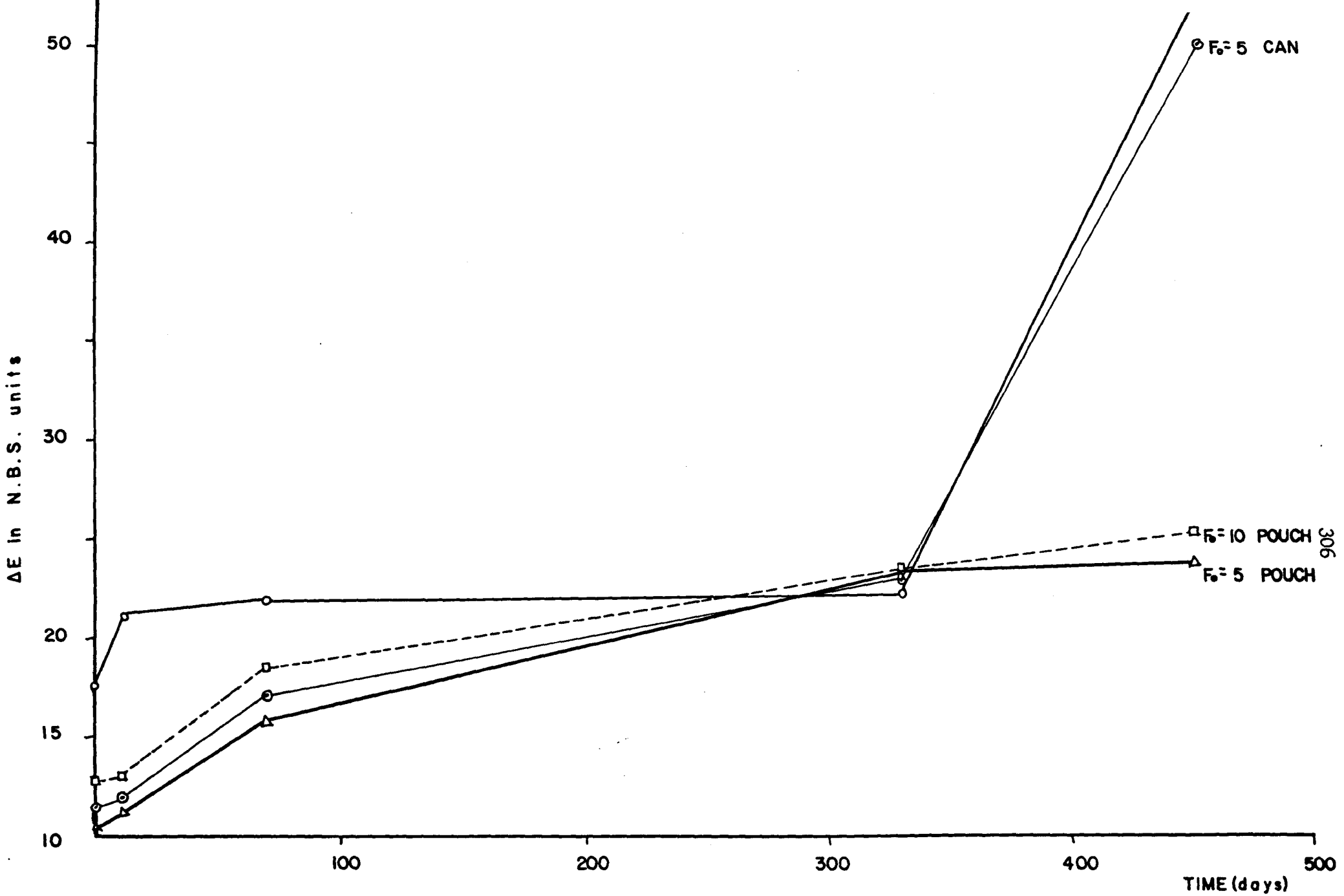


FIG. 5.26 EFFECT OF STERILISATION AND STORAGE AT 37°C ON COLOUR DEVELOPMENT IN RICE PUDDING SAMPLES.

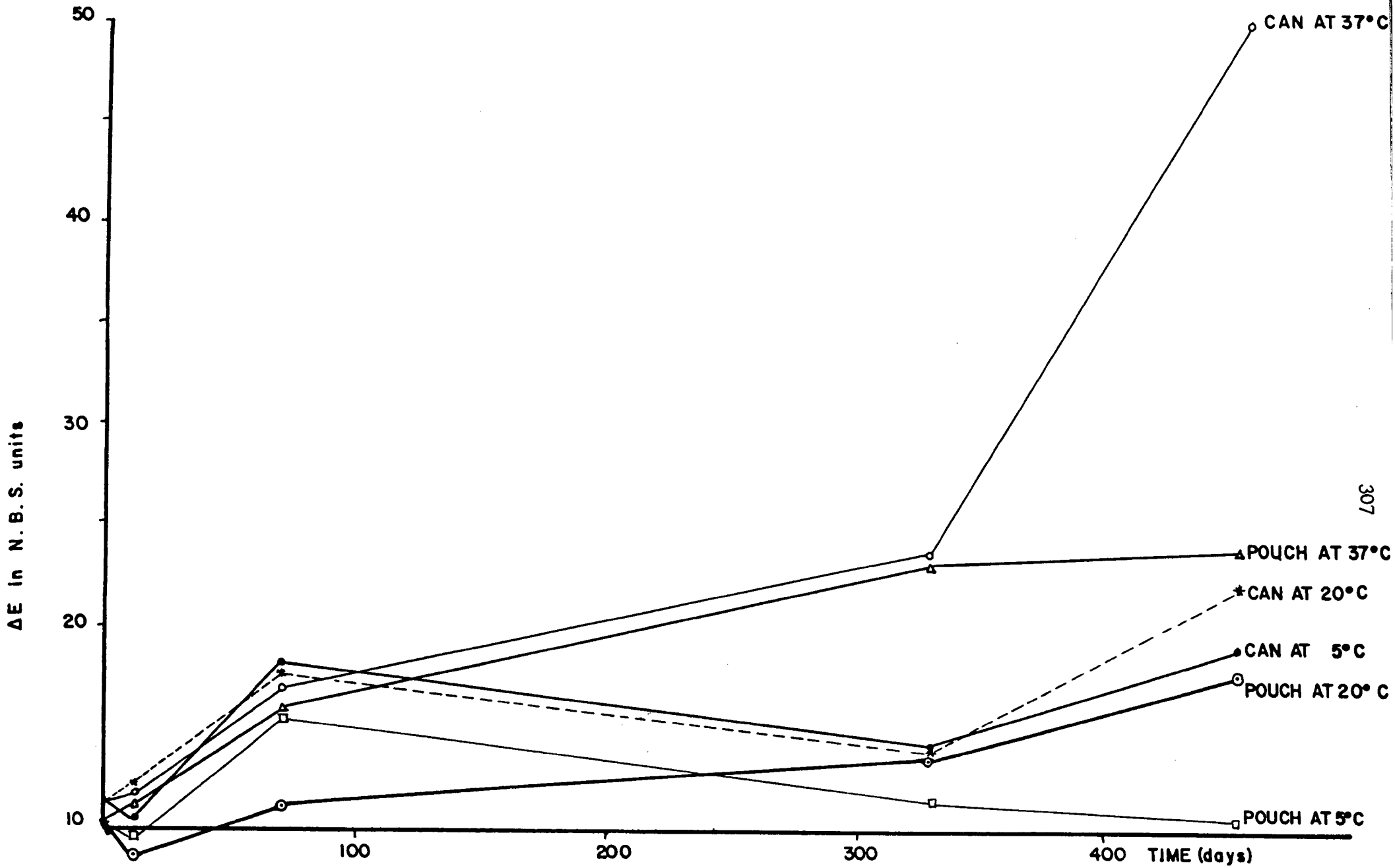


FIG. 5.27 EFFECT OF STERILISATION PROCESS ($^{z}F_{\bullet} = 5$ min) ON COLOUR DEVELOPMENT IN RICE PUDDING SAMPLES. COMPARISON OF THREE STORAGE TEMPERATURE .

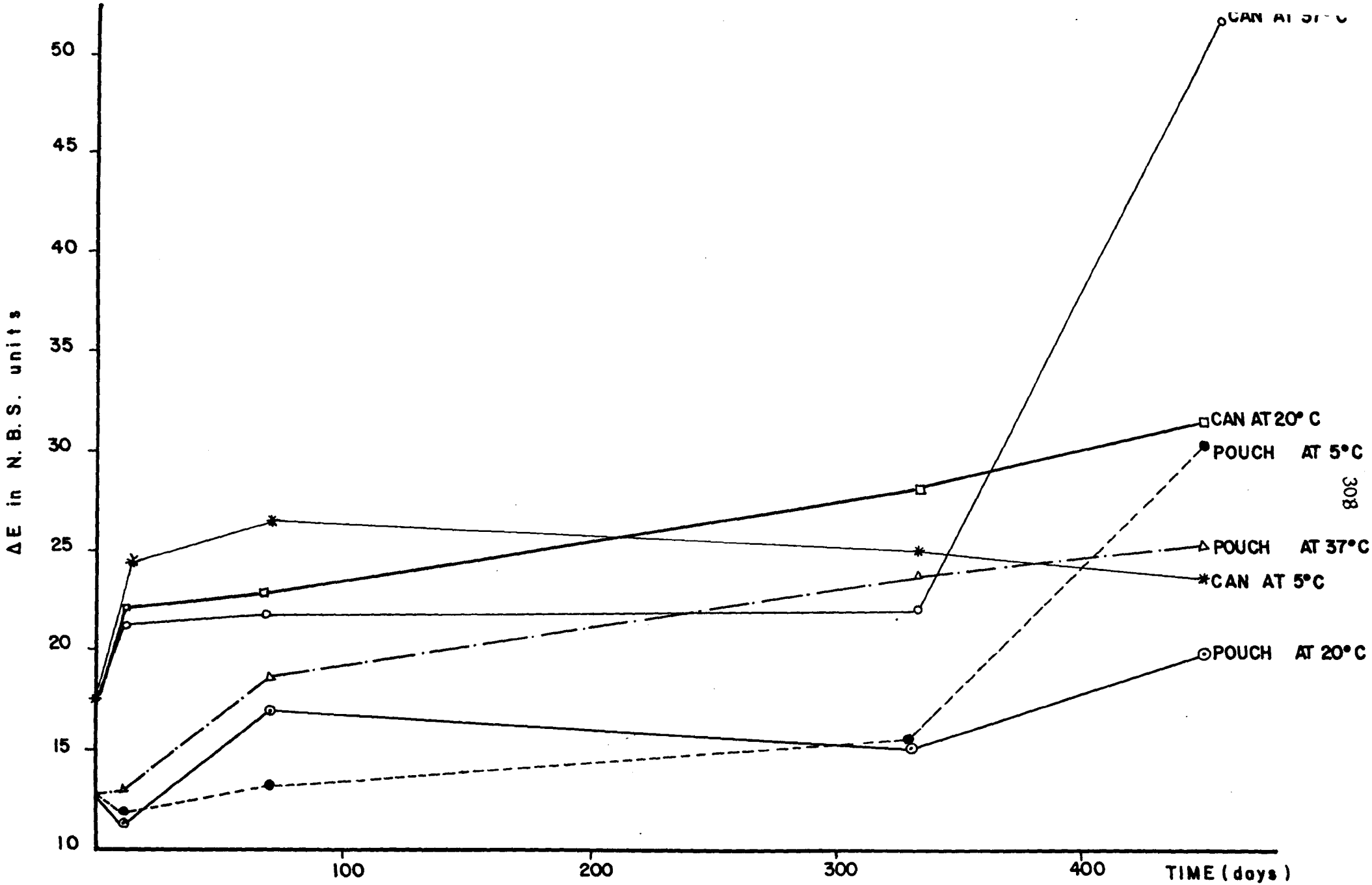


FIG.5.28 EFFECT OF STERILISATION PROCESS (F_o=10min) ON COLOUR DEVELOPMENT IN RICE PUDDING SAMPLES. COMPARISON OF THREE STORAGE TEMPERATURES.

Table 5.19 Total colour difference, (Δ E), of rice pudding samples in A-1 containers due to sterilization process and storage, in NBS units

Storage temp	Target sterilization value F_o = 5 min						Target sterilization value F_o = 10 min					
	5°C		20°C		37°C		5°C		20°C		37°C	
Package / Storage days	Pouch	Can	Pouch	Can	Pouch	Can	Pouch	Can	Pouch	Can	Pouch	Can
B.P.	0	0	0	0	0	0	0	0	0	0	0	0
A.P	10.3	11.4	10.3	11.4	10.3	11.4	12.7	17.6	12.7	17.6	12.7	17.6
13	9.6	10.5	8.6	12.2	11.2	11.8	11.9	24.6	11.6	22.2	13.0	21.1
70	15.5	18.1	11.1	17.8	15.8	17.0	13.3	26.4	16.8	22.9	18.6	21.9
330	11.4	14.1	13.7	13.8	23.2	23.1	15.6	25.1	15.5	28.1	23.8	22.1
450	10.4	18.7	17.8	22.0	23.7	50.0	30.4	23.8	20.0	31.6	25.4	51.8

B.P. Before Process
A.P. After Process

The percentage reflectance of green (G), red (R) and blue (B) obtained from the Colormaster readings, were treated according to the modified Adams chromatic value system proposed by Glasser and Troy(1952),to relate the instrument data to colour dimensons in the well known Munsell system of colour designation. The calculations were carried out using the colour co-ordinate tables computed by Glasser and Troy, (1952), to obtain the total colour difference, (ΔE).

The colour changes obtained in the rice pudding samples due to processing are shown in Table 5.20 where it can be seen that higher F_o-values (10 min) produced more colour development (larger ΔE) than lower F_o-values (5 min). Also rice puddings in A-1 containers showed less colour development than its equivalent in A-10 containers. These findings agree with the processing times obtained. Longer processing time produced larger colour development, and correlate well with the available lysine results (see Table 5.1).

Container	Target F_o-value 5 min		Target F_o-value 10 min	
	Formulation 2	Formulation 4	Formulation 2	Formulation 4
A-1 pouch	10.7	10.3	-	12
A-1 can	11.4	8.8	17.6	-
A-10 pouch	-	22.17	30.4	23.97
A-10 can	21.90	23.36	26.17	-

Table 5.20 Effect of heat sterilization on colour development in rice pudding samples. Colour is measured as the colour difference (ΔE) between sample and precooked sample, in N.B.S. units

CHAPTER 6

FURTHER STUDIES ON BRUSSELS SPROUTS SAMPLES

As a direct consequence of the results obtained from the studies carried out to evaluate ascorbic acid retention in the food samples produced (sections 5.3.2, 5.4.2, 5.5.2) and literature survey on the subject, an investigation was undertaken to study the enzyme regeneration in pouched brussels sprouts. Also the relationships between process temperature, F_o-value and vitamin C retention with residual peroxidase activity were investigated. The experimental procedures used and results obtained are described in this Chapter.

6.1 Experimental Procedure

6.1.1 Food sample production

Brussels sprouts were prepared and packaged as described in Appendices 4 and 5, on the day of purchase, stored at $0^{o}C$ overnight and processed the next day.

Processing

All processing conditions were as described in section 5.1.3 when samples were processed at $121.1^{o}C$, but when processing was carried out at $116^{o}C$ the pouch water retort working pressure was reduced to 137.89 KPa above the atmospheric pressure. All other conditions remained constant.

Three different batches of samples were prepared and processed, and two different processes were carried out, for each batch. The target F_o-values selected for these products were 6 min, 5 min and 3 min when the retort temperature was $121.1^{o}C$ and 8 min, 6 min and 3 min when the retort temperature was $116^{o}C$.

Storage

Samples were stored at 37 C and 20 C as shown in Table 6.1.

Retort temp	Target F_o-value	Storage Temperature
121°C	6 min	20°C
121°C	5 min	37°C
121°C	3 min	37°C
116°C	8 min	20°C
116°C	6 min	37°C
116°C	3 min	37°C

Samples stored at 20°C were the samples that presented no peroxidase activity immediately after processing.

Table 6.1 Storage temperatures for the brussels sprouts samples produced

6.1.2 Food sample evaluation

Vitamin C determination

Sampling was carried out as described in Appendix 1. Three pouches were opened for each determination and duplicate samples were taken from each package for vitamin C content which was determined using the OPDA fluorimetric procedure described in Appendix 2.2. Also duplicate samples were taken for moisture content (see Appendix 8).

Peroxidase activity determination

The method used was a modification to the Nagle and Hard (1975) procedure as described by Burnette and Flick (1977). It is a quantitative measurement using hydrogen peroxide and

o-dianisidine in phosphate buffer solution. A simplified description of the method is given in Appendix 2.6.

Standard curve

Before the method was applied to the samples, two standard curves were prepared using purified horseradish peroxidase. Several solutions of known enzyme concentration were prepared and their activity was measured as described in Appendix 2.6. One of the standard curves was obtained by measuring enzymic activity at 20°C while the second (using the same solutions) was obtained at 37°C.

Sample enzymic activity

Duplicate samples were taken from the homogenized brussels sprouts, (see Appendix 1), immediately before and after processing and after one week of storage. Peroxidase activity was determined as described in Appendix 2.6.

Original samples enzymic activity

From the original canned and pouched brussels sprouts (whose production and evaluation was described in section 5.3) duplicate samples were taken and residual peroxidase activity determined (Appendix 2.6) after 12 months of storage.

6.2 Results

6.2.1 Heating parameter

The semilogarithmic heating curves obtained for the six batches of samples produced are shown in Figures 6.1 and 6.2, where it can be seen that all samples presented straight line

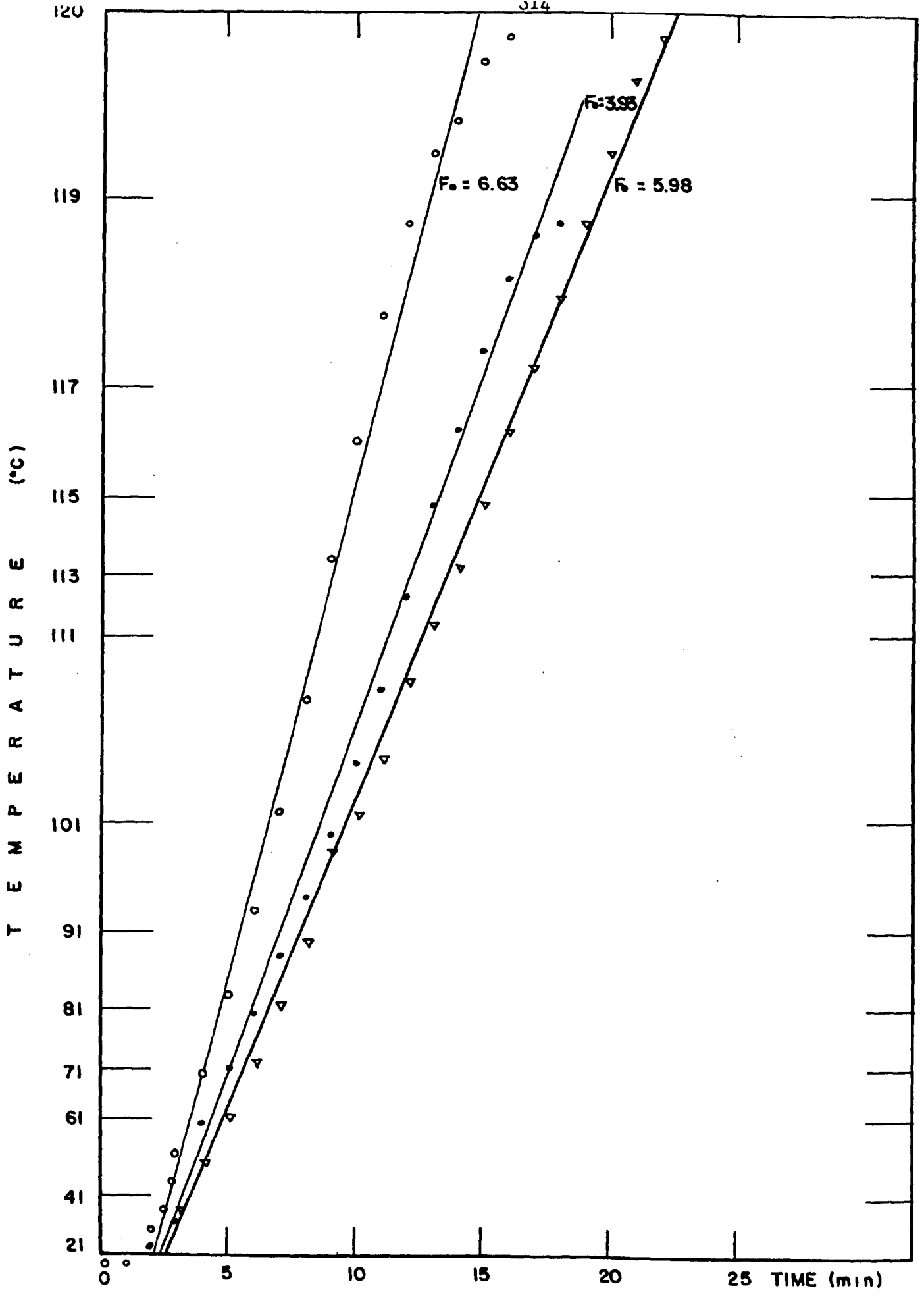


FIG. 6.1 HEAT PENETRATION CURVES BRUSSELS SPROUTS IN A-1 CONTAINERS POUCHES STERILISED AT 121° C

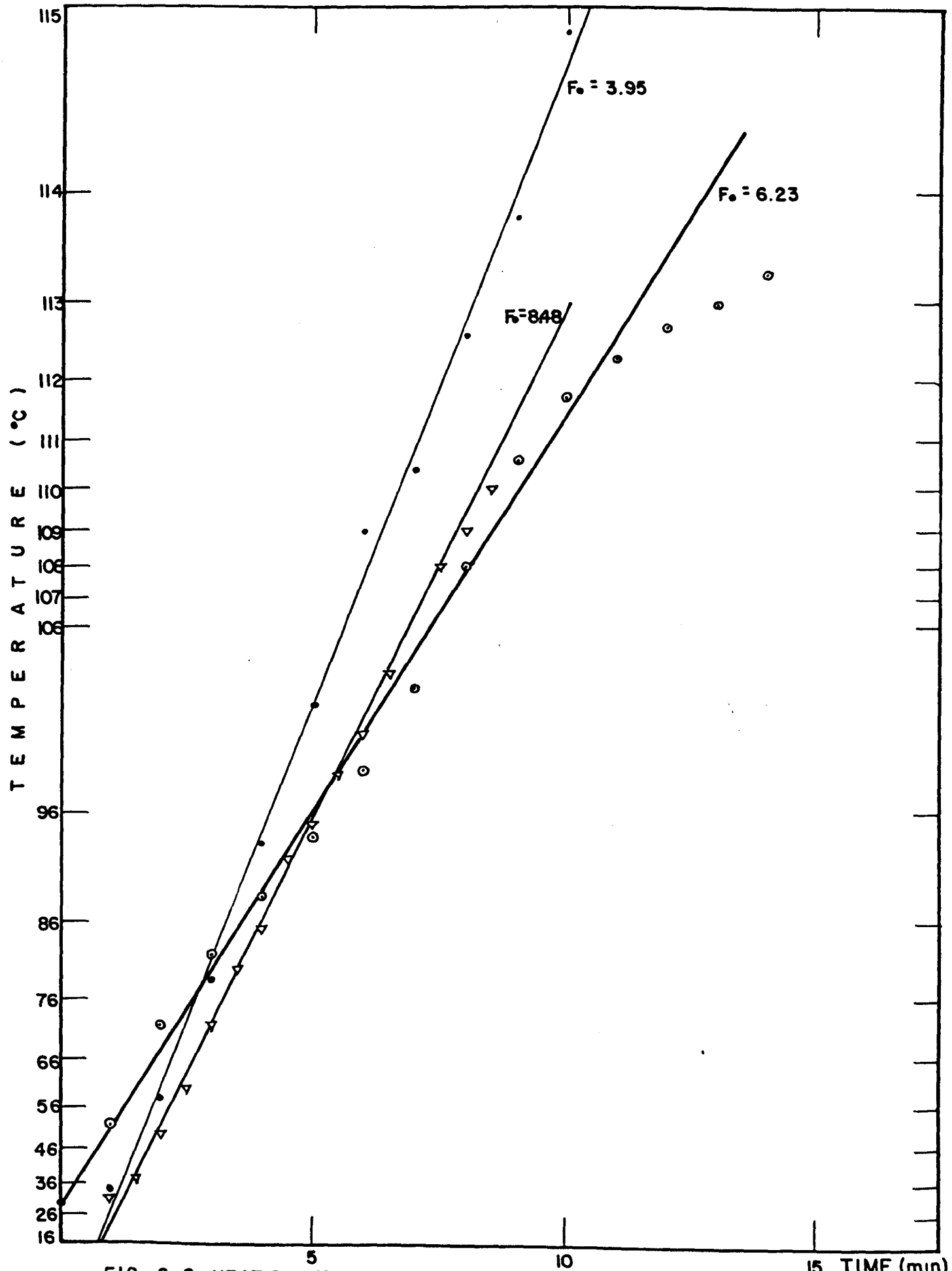


FIG. 6.2 HEAT PENETRATION CURVES - BRUSSELS SPROUTS IN A-1 CONTAINERS POUCHES STERILISED AT 116 °C.

heating curves with negligible deviations. The heating parameters obtained for these samples are given in Table 6.2. Here, it can be seen that the come-up time for all processes was the same (5 min). Therefore the corrected zero time was 2.9 min after the opening of the heating medium valve, for all cases.

Lag factor

The lag factor (J-value) variations between samples processed under the same conditions (same retort temperature) were very small (see Table 6.2). These results agree with the theoretical expectations (Hayakawa, 1972, Olson and Jackson, 1942, and Stumbo, 1965) since all packaging and processing conditions were maintained constant. The small variations obtained can be attributed to either the small initial temperature difference (I) variations, as suggested by Stumbo (1965), and/or the variations present in the food sample itself, such as leaf compactness, leaf composition, etc; which would affect the food heat transfer characteristics.

There is a significant difference between the lag factors of samples processed at 121.1° C and the lag factors of samples processed at 116 °C. This agrees with Stumbo's (1965) claim that a variation in initial temperature difference (I) would produce a variation on J-value, and there was an approximate 20°C difference in "I" between samples processed at 121° C and 116° C.

f_h-value

The f_h-value obtained (see Table 6.2) for the six batches of brussels sprouts produced showed large variations. Under ideal conditions all these f_h-values should be identical since f_h-value is known to depend on the nature and dimensions of the

food itself and the container dimensions, (Charm, 1978, Leniger and Beverloo, 1975, Mohsenin, 1980, and Stumbo, 1965). The container used was the same for all samples and the experimental procedure was designed to maintain the size and nature of the products as similar as possible. To explain the large f_h-value variation obtained, dissimilarities in both food sample dimensions and nature should be considered. As mentioned by Moshsenin (1980) thermal diffusivity varies with chemical composition and physical structure of the sample. Consequently, the f_h-value should also be affected. In addition to this, the shape of the brussels sprouts is not uniform, therefore, small variations in sample dimensions were present, affecting the heating rate of the food product.

Cooling cycle parameters

As mentioned in section 5.2, due to the variation of the cooling water temperature during the cooling cycle, the cooling cycle parameters such as cooling lag factor (J_c) and cooling rate (f_c) were not evaluated.

F_o-values

The actual F_o-values obtained for the samples processed are given in Table 6.3 together with the processing times. As before, heating processing time was considered from the corrected zero time (0.58 CUT) to the end of the heating cycle. Total processing time was taken from the corrected zero time until the temperature of the food fell below 87.7°C, when the lethal rate is negligible. The actual heating sterilization treatments obtained are very near to the selected target F_o-values, however, the variations in process time were large. For samples processed at

PARAMETERS	Target F_o-values in minutes					
	6	5	3	8	6	3
Retort temp (RT)	121.1°C	121.1°C	121.1°C	116°C	116°C	116°C
Initial temp (IT)	17.5 °C	10.7 °C	14.4 °C	26.6°C	22.7°C	25.0° C
Come-up time (CUT	5 min	5 min	5 min	5 min	5 min	5 min
Maximum food temp at end of heating (T_{MAX})	119.8° C	119.6°C	118.9°C	109.0° C	113.8°C	115.3°C
heating rate (f_{h1})	6.2 min	9.4 min	8.6 min	6.0 min	7.8 min	4.8 min
pseudo initial temp (JI)	76.1 °C	94.1°C	90.1°C	45 °C	37 °C	36°C
I = RT - IT	103.6 °C	110.7 °C	106.7°C	89.4° C	93.3° C	91 °C
Lag factor (J) J = JI/I	0.73	0.85	0.84	0.50	0.40	0.40
g = RT - T_{MAX}	1.3 °C	1.5 °C	2.2°C	7° C	2.2° C	0.7° C
Cooling water temp (CW)	24 °C	25°C	26°C	24° C	23° C	24°C
m = T_{MAX}-CW	95.8° C	94.6 °C	92.9°C	85 °C	90.8 °C	91.3 °C

Table 6.2 Heating parameters obtained for brussels sprouts samples

116 °C, as expected, larger times were obtained for higher sterilization treatments. But for samples processed at 121.1°C this did not happen, the sample with F_o-value of 6.63 min had a shorter processing time that the sample with an F_o-value of 5.99 min. This may be attributed to the larger f_h-value of the second sample, because it is well known that process time is dependent on the sample heating rate, (f_h-value) which as mentioned earlier was very variable. Therefore, these process time variations can also be attributed to variations of both sample dimensions and nature of the brussels sprouts.

Retort Temperature	Heating cycle (min)		Total process contribution (min)	
	F_o-value	time	F_o-value	time
121.1°C	6.02	16.24	6.63	19.78
121.1°C	5.01	20.33	5.99	24.58
121.1°C	3.05	16.00	3.89	18.69
116°C	7.94	24.60	8.48	28.10
116°C	5.89	17.71	6.23	19.59
116°C	3.02	10.16	3.96	16.00

Table 6.3 Actual F_o-values at the centre of the food in the geometrical centre of the container for the food samples produced.

6.2.2 Peroxidase Activity

Standard curves

The standard curves showed linear relationships as shown

in Figures 6.3 and 6.4. As no important difference was found using either 20°C or 37°C as the temperature for the peroxidase activity determination, all subsequent determinations were carried out at 20° C. The minimum amount of horseradish peroxidase detected with this assay was 1.5 ng/cm^3.

Peroxidase activity of the food samples

Three peroxidase fractions were separated from each sample. The mean values obtained of five determinations for each sample, are given in Table 6.4. Here it can be seen that for the blanched samples, between 89% - 97% of the total peroxidase activity corresponded to the soluble peroxidase fraction; between 5% and 11% was ionically bound peroxidase. Only one batch showed traces of covalently bound peroxidase (≈0.4%).

Peroxidase activity values for fresh brussels sprouts extracts were not determined in the present work but values reported by McLellan and Robinson (1982) indicate that over 90% of the peroxidase was inactivated during the blanching procedure McLellan's values were transformed to the same units as in Table 6.4 (Δ O.D.$_{460}$ per min per 100 g of sample) and are as follows: soluble fraction, 16 643.0, ionically bound fraction, 1320.0 and covalently bound fraction, 0.96.

For samples processed at 116°C with F_o-value of 8.48 min no residual peroxidase activity was detected. Samples processed at 121.1°C with F_o-values of 5.99 min and 6.63 min showed only traces of ionically bound and covalently bound peroxidase. However, the remaining three batches of processed samples showed moderate residual peroxidase activities, the highest value was obtained for samples processed at 116°C with F_o = 3.96 min,

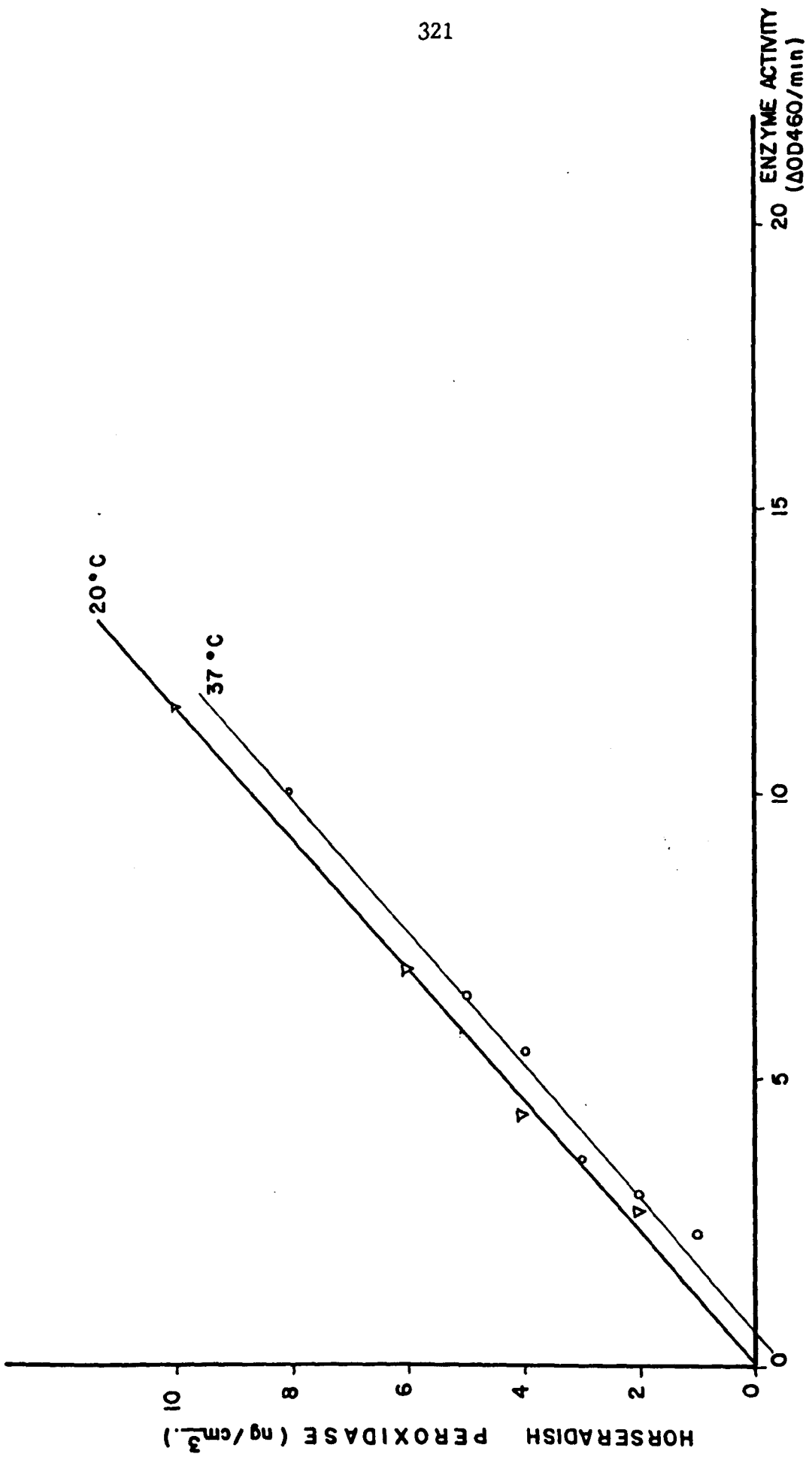


FIG. 6.3 STANDARD CURVE FOR HORSERADISH PEROXIDASE ACTIVITY.

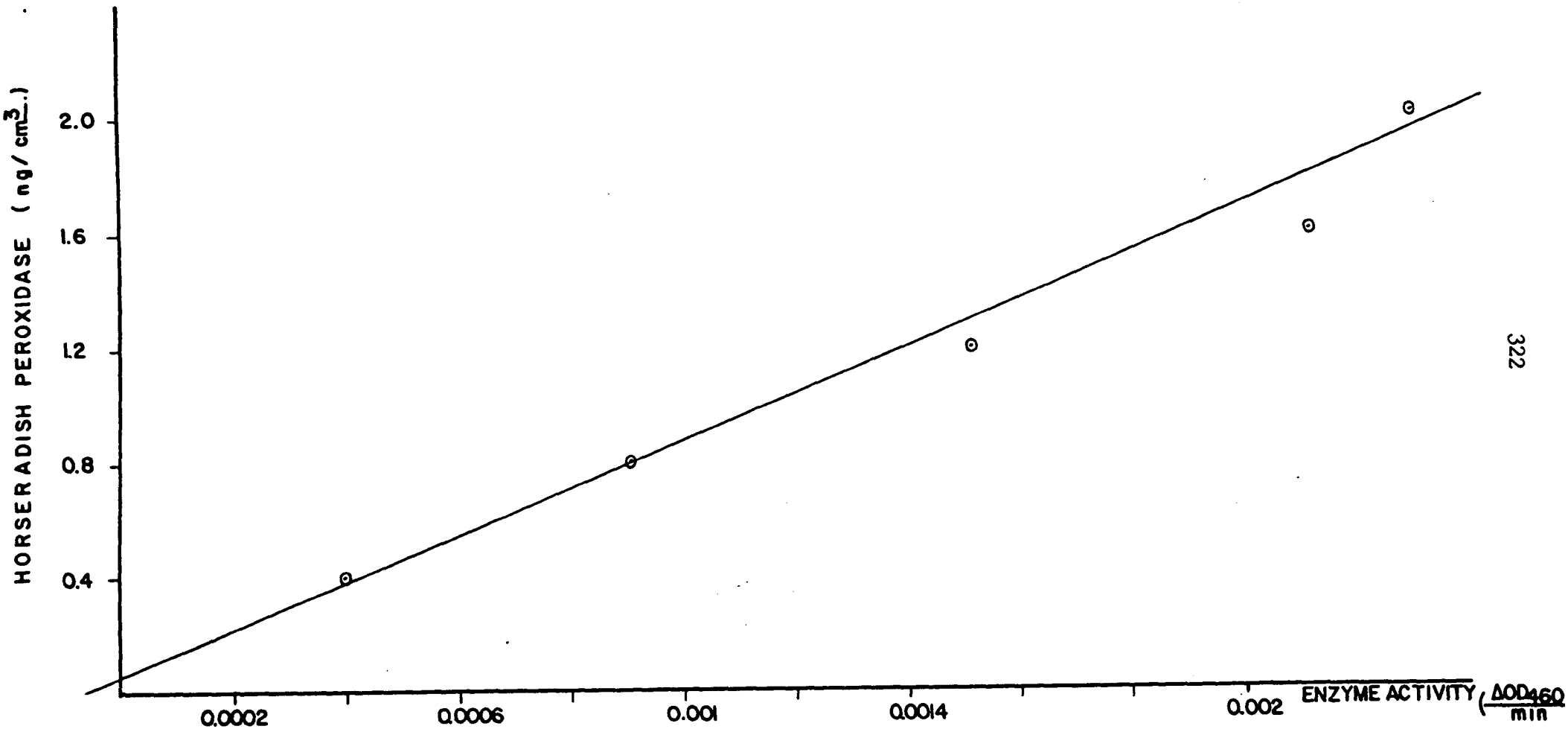


FIG. 6.4 STANDARD CURVE FOR HORSERADISH PEROXIDASE ACTIVITY. LOWER CONCENTRATION.

Storage time	Sample	Portion	Peroxidase activity in O.D./min per 100 g of sample		
			Soluble	Ionically bound	Covalentely bound
	BATCH I				
B.P.	Blanched sample	solid	1104.95	130.77	0
A.P.	121°C F_o=6,63	solid	0	0.81	0
		liquid	0	0	0
7 days		solid	0	0.75	0
A.P.		liquid	0	0.64	1.06
	116°C F_o=8.48	solid	0	0	0
		liquid	0	0	0
		solid	0	0	0
		liquid	0	0	0
	BATCH II				
B.P.	Blanched sample	solid	1249.50	74.97	0
A.P.	121°C F_o=5.99	solid	0	0	0
		liquid	0	0	0
7 days		solid	0	0	0
		liquid	0	2.64	1.66
A.P.	116°C F_o=6.23	solid	12.56	9.37	6.82
		liquid	7.08	3.21	0
7 days		solid	0	5.62	0
		liquid	18.60	9.37	9.66

Table 6.4

B.P. before processing
A.P. after processing

	BATCH III				
B.P.	Blanched sample	solid	2410.8	65.22	9.52
A.P.	121°C F_o=3.89	solid	20.0	3.64	1.69
		liquid	10.50	7.89	9.98
7 days		solid	23.74	8.49	2.14
		liquid	39.98	6.09	8.02
A.P.	116°C F_o=3.96	solid	78.25	2.64	0
		liquid	129.37	61.05	0
7 days		solid	49.00	1.29	2.03
		liquid	158.12	2.36	0

Table 6.4 Relative peroxidase activity of various processed samples as change in optical density per minute per 100 g of sample

followed by samples processed at 121.1°C to an F_o-value of 3.89 min and at 116 °C with F_o-value of 6.23 min, in that order. These findings support the view that at relatively high thermal processing temperatures the destruction rate of micro-organisms is greater than for enzymes, (Adams and Gawger, 1961, Burnette, 1977, Farkas et al, 1956, Gibriel et al, 1977, Harris and Karmas, 1977), mainly due to the activation energies which are larger for microbial destruction than for enzyme destruction. So, for a given increase in processing temperature, the rate of micro-organism destruction will increase more than the rate of enzyme destruction; this results in processing time becoming a more significant factor in enzyme inactivation than process temperature.

It is important to mention that a high degree of variability in residual peroxidase activity was obtained in different packages taken from the same retort load. It was considered that

this variability was caused by variations in both the heat treatment received by each package (as shown in section 4.3) and the peroxidase content in the food itself as mentioned by Nebesky et al (1950). Nevertheless the values reported in Table 6.4 are a good general indication of the levels of residual activity found in the processed samples.

In Table 6.4 it also can be seen that relatively large amounts of residual covalently bound peroxidase were detected in the processed samples, while no covalently bound peroxidase was detected in the blanched samples of batch II and even in batch III, where covalently bound peroxidase was detected in the blanched samples, the amount is large (9.52 $O.D_{460}$ min^{-1} per 100 g of sample) when compared to the report by McLellan and Robinson (1980) of 0.96 $O.D._{460}$ min^{-1} per 100 g of sample. To explain this apparent increase in covalently bound peroxidase only two explanations seem possible, either the intrinsic differences in peroxidase content and its binding state between brussels sprouts due to variation in season, maturity and location in the plant (Nebesky et al, 1950, Saunders et al, 1968) or due to conversion of peroxidase from one binding state to another (soluble and/or ionically bound to covalently bound) by similar mechanisms as the ones suggested by Gkinis and Fenema, (1978), for conversion of soluble to ionically bound peroxidase in green beans during frozen storage. Although no experimental or theoretical evidence can be given to support the second alternative, in this case the mention of Gkinis's findings leaves the subject open to further studies.

There are also several reports in the literature (Adams, 1978, Burnette, 1977, Farkas et al, 1956, McLellan and Robinson, 1982, and Schwimmer, 1944) on the regeneration of heat

inactivated peroxidases. Most of the reports agree that regeneration is complete after a few hours of storage when the sample is held between 6 °C and 40 °C. These conditions are present in these studies so there is reason to believe that some regeneration may have occurred in the samples with residual peroxidase activity but this was difficult to assess in view of the wide variations found in residual peroxidase activity in samples processed in the same retort run and taken at the same time. Nevertheless, if regeneration occurred, this was taken into consideration in the residual peroxidase activity determinations carried out after seven days of storage.

Peroxidase activity of the original brussels sprouts after 12 months storage

The residual peroxidase activity results obtained for the original brussels sprouts samples are shown in Table 6.5. Here, it can be seen that for the canned samples only traces or no residual peroxidase activity was detected after 12 months of storage, while for pouched samples significant amounts of residual activity were found, with the pouched brussels sprouts stored at 37 °C presenting the greatest residual peroxidase activity level. These findings suggest that for canned samples the heat treatment applied was adequate for the enzyme inactivation, as shown by the lack of peroxidase activity on samples stored at 5° C, but some regeneration may have occurred (Farkas et al, 1956, McLellan and Robinson, 1982, and Schwimmer, 1968) during storage at 20 °C and 37 °C. The heat treatment, according to these results, for the pouched samples was clearly insufficient for peroxidase inactivation although the sterilization treatments were the same in terms of microbiological inactivation, the processing

Container	Sample storage temperature	Portion	Peroxidase activity in $O.D._{460}$/min per 100 g of sample		
			Soluble bound	Ionically bound	Covalently bound
pouch	5°C	solid	0	13.25	0
		liquid	216.42	20.40	30.00
can	5°C	solid	0	0	0
		liquid	0	0	0
pouch	20°C	solid	0	14.33	35.07
		liquid	258.77	8.20	0
can	20°C	solid	0	1.41	1.56
		liquid	0	0	0
pouch	37°C	solid	18.71	7.55	3.71
		liquid	212.79	53.96	22.24
can	37°C	solid	0.21	4.69	0
		liquid	8.95	0	0

Table 6.5 Relative peroxidase activity of original brussels sprouts samples in both cans and pouches stored at 5°C, 20°C and 37°C

times were different, being larger for the canned samples (20 min) when compared to the 15.5 min of total processing time of the pouched samples. Therefore these findings agree with the generally held view that at high temperatures the rate of destruction of micro-organisms is greater than for enzymes, (as mentioned above).

Figure 6.5 was constructed by the combination of the results presented in Tables 6.3 and 6.4 and shows the percentage of inactivated peroxidase at different total processing times. In

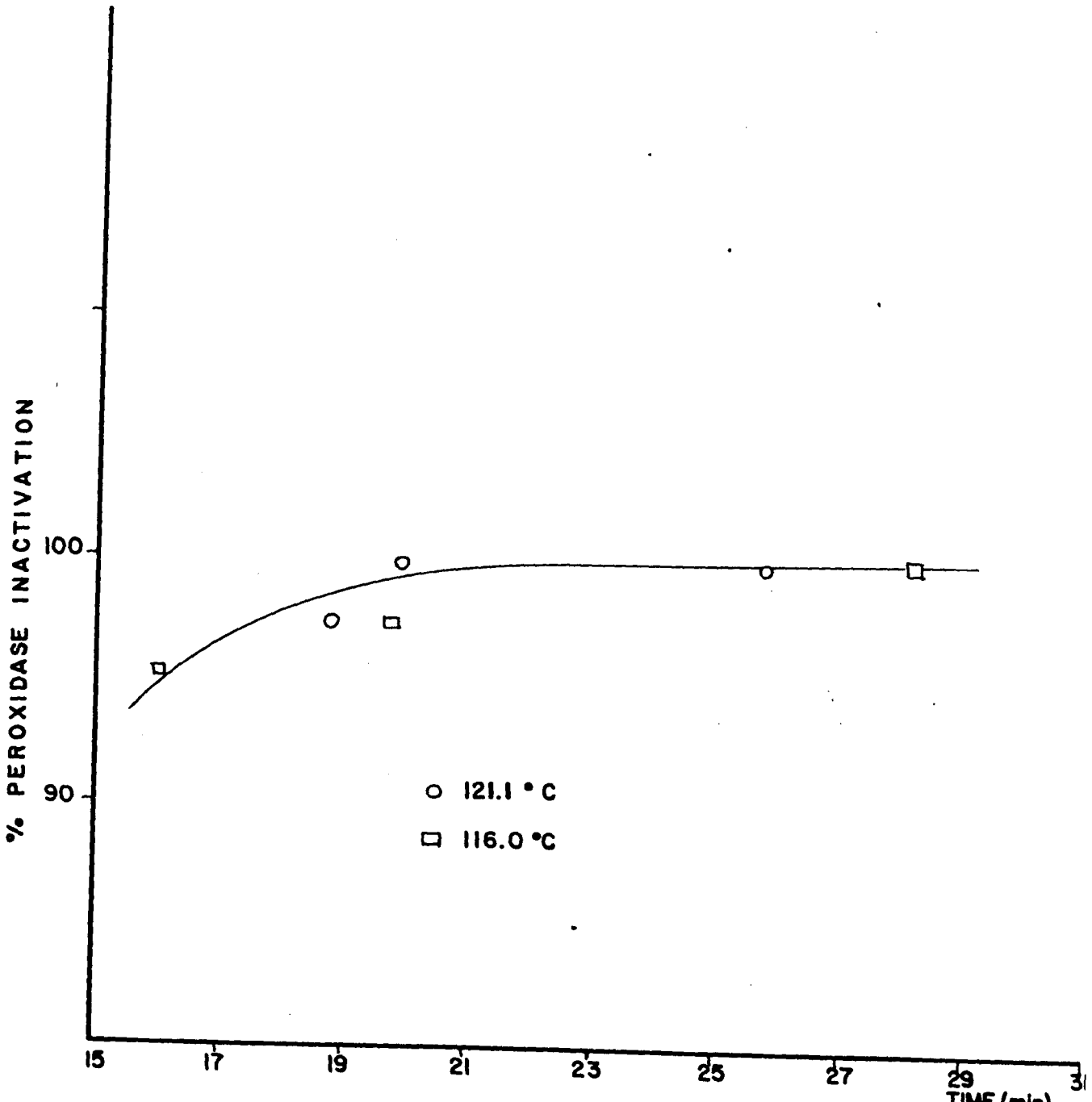


FIG. 6.5 EFFECT OF PROCESSING TIME ON PEROXIDASE ACTIVITY.

this Figure 0% inactivation means the residual peroxidase activity measured after blanching. Here it can be seen that the total processing time of the original canned samples, (20 min) produced adequate enzyme inactivation for brussels sprouts in pouches processed under similar conditions. Also it can be seen that the total processing time of the original pouched brussels sprouts (15.5 min) was not adequate for enzyme inactivation. Therefore good agreement was found for the enzyme inactivation as a processing time function between different batches of brussels sprouts.

6.2.3 Vitamin C content

The amount of both ascorbic acid and dehydroascorbic acid in all samples was determined immediately before and after processing and during the storage period. The effect of different sterilization processes on vitamin C retention is shown in Table 6.6, where Vitamin C values are given as percentage retention of the total vitamin C content of blanched brussels sprouts immediately before processing. The vitamin C percentage retention against processing time is shown in Figure 6.6 where it can be seen that generally smaller processing times produce higher vitamin C, ascorbic acid and dehydroascorbic acid retention. All total vitamin C retention values are high, (over 75%), even for the longer processing times, where large sterilization values were obtained (F_o = 8.48 min).

The effect of storage conditions on vitamin C retention for the six batches of samples processed followed two distinct behaviours which are exemplified in Figures 6.7 and 6.8, where the vitamin C content (ascorbic acid plus dehydroascorbic acid) in the blanched samples immediately before processing was considered 100%. All batches of brussels sprouts presented a very

Retort Temperature	F_o-value achived	Ascorbic acid %	Dehydro-ascorbic acid %	Total vitamin C %
121°C	6.63 min	76.35	6.73	83.08
121°C	5.99 min	72.17	5.72	77.89
121°C	3.89 min	76.98	7.24	84.22
116°C	8.48 min	76.14	2.54	78.68
116°C	6.23 min	76.99	6.75	83.74
116°C	3,96 min	80.41	11.41	91.82

Table 6.6 Effect of different sterilization processes on vitamin C retention in pouched brussels sprouts (Results immediately after processing)

small amount of dehydroascorbic acid content (under 10%), which showed a small decrease (under 5%) in the first 30 days with no further losses detected during the following 30 days of storage. Also for all samples the percentage of vitamin C content in the liquid, showed an increase during the first 10 days of storage which agrees with the results obtained for brussels sprouts in Chapter 5 (Figures 5.9, 5.10 and 5.11). Here the same considerations can apply; i.e., that the high rate of vitamin C leaching may have covered the destruction of vitamin C in the liquid. Nevertheless, the amount of total vitamin C content in the liquid is very small, with most of the remaining vitamin C in the edible portion of the samples (solids).

The three batches of samples that presented Figure 6.7 type of vitamin C retention curve were processed at 116°C to an F_o-value of 8.48 min and two batches processed at 121.1°C to

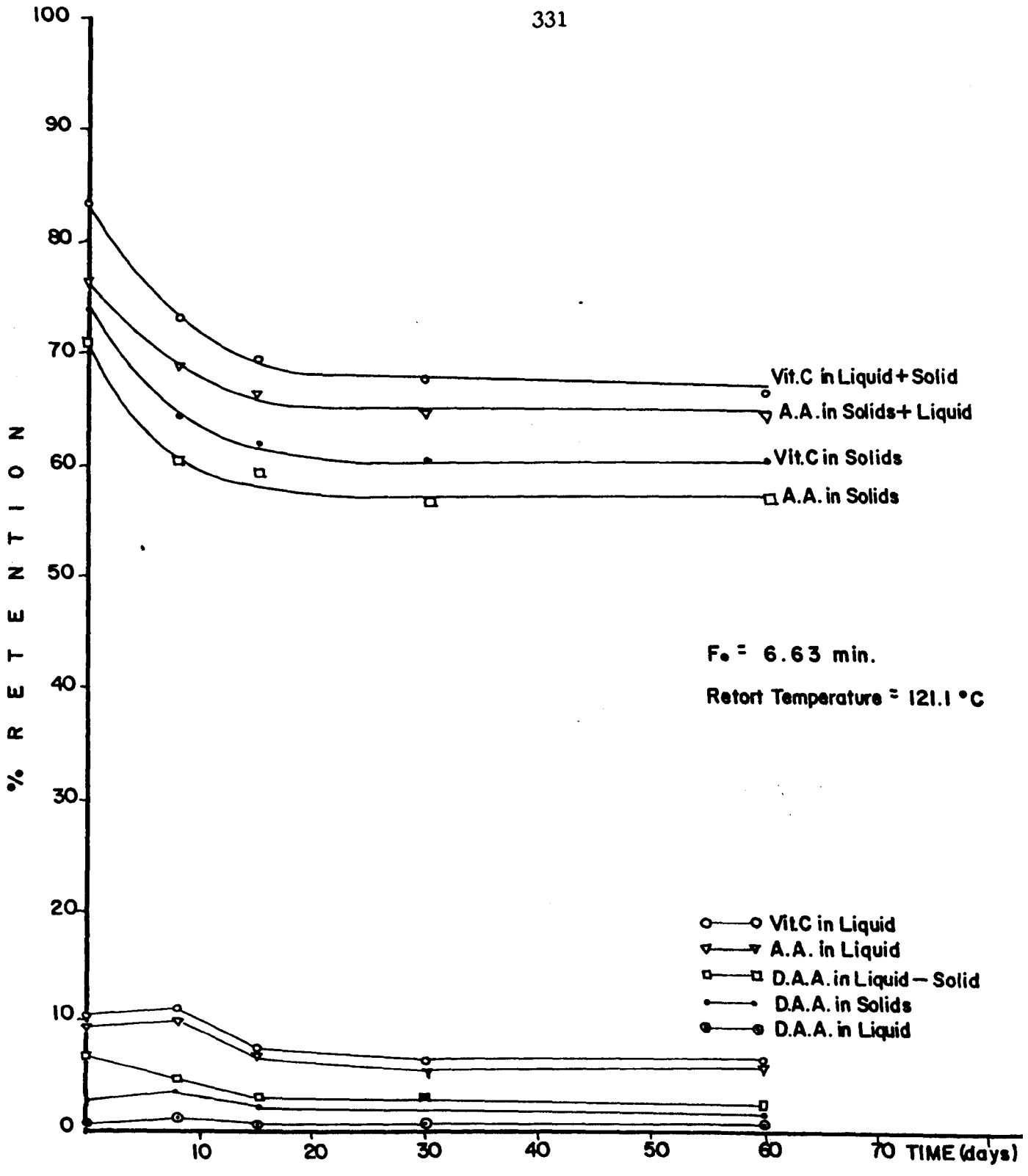


FIG. 6.7 VITAMIN C RETENTION IN BRUSSELS SPROUTS AFTER STERILISATION AND DURING STORAGE AT 37° C. PEROXIDASE ACTIVITY NOT DETECTABLE.

F_o-value of 5.99 min and 6.63 min. These batches of samples also showed only traces or no residual peroxidase activity. In these three batches of samples for both total and solids vitamin C content, most of the losses occurred during the first four weeks of storage with no further losses detected in the following six weeks. This resulted in very high total vitamin C retention(65% - 70%) in the package at the end of the storage period. Thus, ≃5% was in the liquid and between 65% and 60% remained in the brussels sprouts themselves.

Samples that presented Figure 6.8 type of vitamin C retention curves were: samples processed at 121.1 C to an F_o-value of 3.89 min and two batches of samples processed at 116°C to F_o-vlaues of 6.23 min at 3.96 min. These three batches of samples also presented common residual peroxidase activity (Table 6.4). For these samples, vitamin C losses continue throughout the 60 days of storage, resulting in lower total vitamin C retentions (42% to 55%) in the package than for samples with Figure 6.7 type of behaviour.

The effect of storage conditions on residual peroxidase activity and sterilization treatment are shown in Figure 6.9 for total vitamin C content in the pouches (solids plus liquid). Here it can be seen that the difference in vitamin C retention after sterilization treatment between batches is small (≃16%) when compared to differences exhibited under varying storage conditions in the presence of residual peroxidase activity (≃35%). Samples without significant residual peroxidase activity showed high vitamin C retention during storage (65% to 70%) irrespective of the after processing vitamin C retention level (76% to 84%). Samples with residual peroxidase activity showed larger vitamin C losses during storage, a further 30% to 40%. Therefore, although these samples were processed

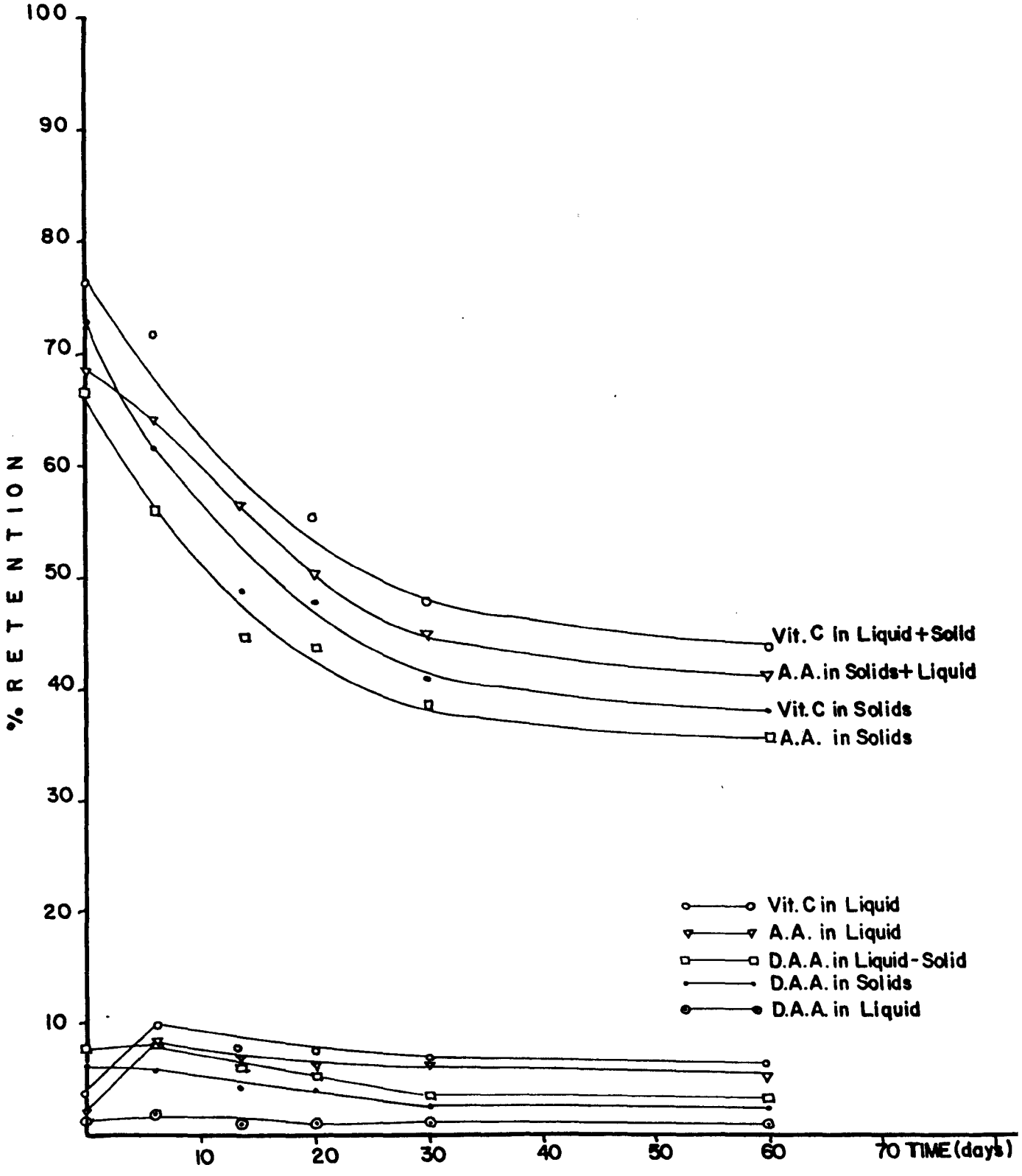


FIG. 6.8 VITAMIN C RETENTION IN BRUSSELS SPROUTS AFTER STERILISATION AND DURING STORAGE AT 20° C. PEROXIDASE ACTIVITY PRESENT.

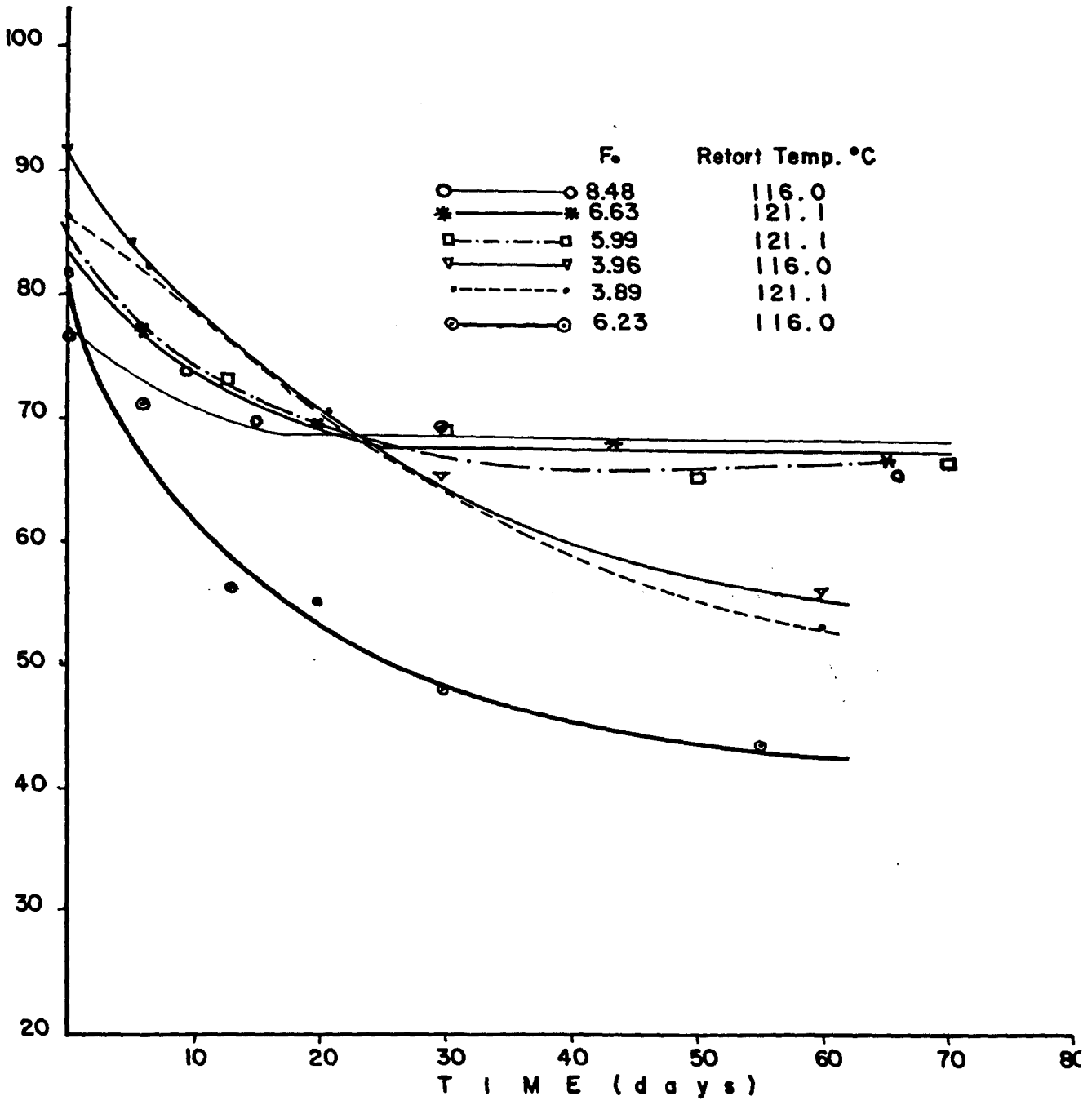


FIG. 6.9 TOTAL VITAMIN C RETENTION IN BRUSSELS SPROUTS AFTER STERILISATIO AND DURING STORAGE. EFFECT OF STERILISATION CONDITION.

for shorter periods of time and presented larger vitamin C retention after processing, the greater losses during storage produced a low vitamin C retention level (42% to 55%) at the end of the storage period.

Summarizing, the results obtained in this Chapter show that:

(i) The extremely large losses in the original brussels sprouts ocurred in pouches in which residual peroxidase activity was detected.

(ii) The small difference in total processing time between canned and pouched samples was the main cause for the importance difference in peroxidase activity, and

(iii) The generally held view that in canning heat sterilization processes that are sufficient to destroy microbiological life also are sufficient to inactivate enzymes was not true for pouch processing in this particular case.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

The main objective of this work was the comparative nutritional evaluation of real food systems sterilized in both pouches and cans, having as the only variable the type of container and the thermal treatment required to achieve the same sterilization treatment. Therefore systems for process control and evaluation and nutrient assay techniques needed to be assessed in order to achieve a better understanding of the significance of the results to be obtained.

7.1 Systems for Control, Monitoring and Evalutation of the Sterilization Process

The importance of accurate temperature measurements in sterilization processes has been mentioned by several authors. Basell and Ball, (1939) emphasized this by stating that precise knowledge of the time-temperature cycles is necessary to evaluate the sterilization effect.

Obtaining accurate temperature measurements depends on the position of the point measured as much as on the use of adequate equipment and adequate measuring techniques.

Therefore, several points were considered before the selection of the procedure and monitoring systems employed and the most important are mentioned below:

(i) It is well known that the use of very thin thermocouple wires helps to reduce conduction errors to a minimum in conduction heated packs. So in the present work then copper-constantan (0.315 mm diameter) thermocouples were selected.

(ii) Ecklund (1956) reported that the nut or thermocouple recep-

tacles used for thermocouple insertion affected the heating rate of small cans particularly when the nut extended into the can. Therefore in this work small brass glands were designed and constructed with minimal extension to the inside (see Fig. 3.6). For the pouch glands as the thickness of the pouch is minimal, any source of interference would have a greater effect than for cans. Consequently the pouch glands were positioned as far away as possible from the centre or measured point of the pouch (one of the top corners).

(iii) Leaking of air into the package through the thermocouple gland was particularly avoided with a silicone rubber product, since this would affect the heat penetration tests. This would be more important for pouches because of their extended surface. A layer of insulating air against the top wall of the pouch may reduce the effective heat transfer area by 50%.

(iv) The position of the thermocouple needs to be carefully chosen, so the instrument measures the temperature desired. It is generally agreed that the cold point or area of a container should be used for heat penetration tests leading to sterilization evaluation (Alstrand and Ecklund, 1952, Desrosier, 1970, and Ball, 1920), since it is the point more difficult to sterilize and the aim of the sterilization process is to render safe any point in the container.

In this work the geometrical centre of the containers was used for the thermocouple sensitive element location. This location is the accepted cold point for conduction heated foods in cans. However for convection heated foods the cold point position depends on the food packaged and size of the container. This point is normally located between the bottom and the centre of the can along the vertical axis. Jackson and Olson (1940) showed that the cold point was about 19 mm above the bottom for the A-1 cans

and about 38 mm for A-10 cans. Some other authors have reported that the cold point is not located in the central vertical axis but at different distances from the can wall (Fagerson et al, 1951) Obviously the cold point for a broken type heat penetration curve food product is variable. According to Alstrand and Ecklund,(1952,) for this type of product, experimental determination of the cold point is the only answer. Since firstly, this type of test requires extensive heat penetration data and highly sensitive and accurate equipment for temperature monitoring, and secondly because the present work is a comparative study, it was considered justified to use the geometrical centre of the container for the temperature measurement. Also it is believed that convection heated products reach uniform temperature fairly rapidly, consequently, the difference in the heating rate between the real cold point of the container and the geometric centre should be small.

(v) For pouched products heated by conduction, it has been shown that the slowest heating point is located on a flat plane through the long central axis of the pouch. The edge of the area of the cold plane is at least as far from the edges of the pouch as the thickness of the pouch, (Davis, et. al, 1972). However this assumption holdstrue only when the shape of the pouch remains similar to a rectangular parallelopiped during processing. This was achieved by the use of racks to support and restrict the volume expansion of the pouch, (see Figure 4.2). A pouch in a vertical position without constraining racks will present a drop like shape, while in horizontal position it will present a balloon type shape. It is obvious that these two shapes will not have the same heat penetration rate and their cold spots will be located in different positions. So it was considered essential for this work to use restraining racks during pouch sterilization.

(vi) The food selected for thermocouple insertion was always of the upper limit of size. For pork casserole after several tests potato was chosen as the temperature monitoring particle.

(vii) From the results obained in section 4.1 it is clear that the sensitivity of the back up monitoring systems (Phillips chart recorder) was limited to $\pm$0.5°C which is within the limits of the accuracy as claimed by the manufacturers. This accuracy was restricted to $\pm$0.35% of the temperature measured ($\pm$0.45°C for 130°C). The thermocouples claimed accuracy limits were $\pm$0.25% of the temperature measured ($\pm$0.33°C for 130°C), and for the digital thermometer was $\pm$0.2% ($\pm$0.26°C for 130°C). The variability encountered in the digital thermometer readings is within the cumulative effect of thermocouples and digital thermometer ($\pm$0.59°C). These variations in the temperature measurements and the accuracy limitations of the equipment available were considered adequate for the purposes of this work.

7.2 Process Evaluation

It has been mentioned (Holdworth, 1974) that the use of computerized lethal rate integrators reduce the errors involved in the graphical and/or numerical methods of process evaluation, due to human manipulation. Also prediction of process times requires extensive heat penetration studies and the precision with which a final specified sterilization treatment is achieved depends greatly on the reproducibility of the retort performance and food characteristics. Consequently, continuous microprocessor monitoring of temperature coupled with instantaneous read-out of the F_o - value was one of the best ways to achieve considerable precision in the retort operation and the sterilization treatment applied.

This type of process control system was selected and used for the present work. The computer program used was tested before adoption by comparison with the normal manual calculations.

7.3 Heat Treatment Variations

Since the heating data from the slowest heating container are required for the evaluation of the sterilization treatment, and also, because variations in the heat treatment received by different packages within a retort run would produce variations in the nutrient retention of the different packs, it was necessary to know the temperature distribution of the retorts.

For the two steam heated canning retorts it can be considered that since the heat transfer coefficient for condensing steam is sufficiently high, the resistance to heat transfer can be ignored. However problems arise from maldistribution of containers in a steam retort, such as overloading. It is well known that when the steam canning retorts are properly operated (particularly venting) the coldest area will be a function of the can load. During the present work, the centre of the can load distribution was selected as the cold area of the retort. Later, during the heat distribution studies within a retort load (section 4.4.2) this was found to be unnecessary since the results obtained showed negligible variations in F_o-values due to can position in the retort. This variation could be the effect of the variability of the temperature measurement system. It has been mentioned that no temperature distribution tests were carried out in the rotating retort due to equipment limitations.

Unfortunately when this work was finished, it was discovered that the commerical Ellab thermocouple used for process control in the rotating canning retort was badly constructed in that the

junction of the thermocouple wire was not located at the tip of the device. An evaluation of the error caused by this thermocouple construction on the process control evaluation was undertaken. It was found that the response was slower to temperature changes than the copper-constantan thermocouples used (see Figure 1 in Appendix 6). It is believed that this was a result of the sensitive element being buried in a plastic rod approximately 3 mm from the target measuring point. F_o-values were calculated assuming the corresponding average temperature given by the copper-constantan thermocouples. The F_o-values obtained with the use of the Ellab thermocouple readings were between 70% -79% of the F_o-values calculated with the copper-constantan thermocouples. The difference was larger at low F_o-values. These results mean that the canned rice pudding samples received a heat treatment resulting in F_o-values 25% higher than that actually received.

For the pouch water retort, experimental work to determine the coldzone of the retort was considered necessary since it is known that the heat transfer coefficient of water depends on factors such as the velocity of the flow of the medium. Consequently unless the water flow is the same at all points of the retort, different heat transfer conditions would exist. Some interference to the water flow was thought to be likely due to the pouch loading and the racks holding the containers. The results obtained showed statistically significant variations between retort runs and between pouch position within a retort load. The determination of the variation due to pouch position in the retort allowed the identification of the cold zone, as the middle shelf of the retort. These findings were later confirmed by the studies on heat treatment variation due to pouch position within a retort load. Furthermore, the left hand side of the middle shelf was

identified as the cold spot. In general the left hand side positions of the retort showed significant lower F_o-values than the right hand positions, and bottom and top shelves were significantly higher in heat transfer rate than the middle shelf (section 4.4.1).

This can be explained in terms of the pouch retort construction. Hot water input was located on the top right hand side of the retort while cool water input was located on the left and bottom side of the retort. This could create bias in the heat transfer conditions. During the time of filling the retort with hot water (between 1-2 minutes) the pouches on the right side received extra heating, since the water falls on top of the packages.

The difference between the shelves may be explained by a restriction in the water circulation, some restriction to the movement was likely due to both pouches and racks obstruction and, thus the heat transfer efficiency reduced.

The large variations obtained between runs could be affected by several factors such as differences between different batches of foods and retort operation. According to Millenville,(1980) it is more difficult to control and adjust pressure fluctuations during retort operation using hot water rather than steam.

The variations obtained for the static canning retort could be caused by fluctuations produced by the temperature measurement system as already mentioned. Particularly if it is considered that a variation of $\pm$0.5°C could produce a change in F_o-value of about 12% (Pflug, et al, 1980). But the large variations obtained for the pouch water retort are considered to be a combined effect of variations in both temperature distribution in the retort and the temperature monitoring system.

The small variations obtained for the static canning retort

as shown by the coefficients of variation obtained, 1.39% and 3.63% can be considered a measure of the maximum of variations produced by the temperature measurement system.

7.4 Assay Procedures

The assay procedures to determine nutrient retention were also evaluated since the significance of the results will depend largely on the sensitivity and accuracy of the method used.

The ascorbic acid determination procedures, (DCP visual titration and OPDA fluorometric procedure), show high level of reproducibility as indicated by the low standard errors obtained ($\pm$0.39% and $\pm$0.302%). These results compared favourably with variabilities reported by other authors (Dunmire, 1979, and Egberg, 1977). The accuracy of the procedures was also shown to be good with high percentage recoveries for the two techniques used (97.73% and 97.20%). So the performance of both techniques was considered satisfactory to detect the nutritional changes due to processing.

Comparison between the results obtained using both assay procedures was very good as shown by the coefficient of correlation of 0.9991 obtained. These results agreed with the comparison reported in the literature (Deutsch, 1967). These good agreements between both techniques justifies comparisons between the results obtained using both techniques.

Thiamine assay procedure was also assessed for reproducibility, as shown by the standard erros $\pm$0.78and $\pm$0.48 obtained in the recovery tests. The ion exchange purification procedure was omitted (as mentioned in section 4.8) because the samples presented very low blanks, the recovery of the standard was lower than the

recovery of the food samples and the variability of the technique was increased by including the ion exchange purification procedure. These findings are in agreement with reports of several other workers such as Andrews and Nordgren, 1941, Glick, 1944, Hennessy and Cerecedo, 1943, and Rice, 1945. All these workers reported that the determination of thiamine could be carried out in crude extracts.

Later when determining the thiamine content of the pork casserole sample, the high level of thiamine content present in this product was suspected to be due to interference and further tests were carried out using the ion exchange purification procedure. But as described in section 5.5, the sample was accidentally fortified with thiamine when using commercial flavourings in the production of the food sample.

In general it can be concluded that the methods for ascorbic acid and thiamine determinations presented high reproducibility sensitivity and accuracy.

In the assessment of the available lysine assay procedures various effects were determined.

The FDNB direct method was selected since it is one of the well established methods for available lysine determination and according to Hurrell and Carpenter (1975, 1981) and Mauron (1981) compares favourably with the A.O.A.C. recommended procedure. Furthermore, it is claimed to be more sensitive in detecting early Maillard damage that the A.O.A.C. recommended method. This was an important factor for the selection of this method, since early Maillard damage was expected to be one of the prime causes of lysine damage in the food systems studied.

However, when using this method one of the main drawbacks was the interference due to carbohydrates present in the sample.

To assess carbohydrate interference, tests with glucose and starch were carried out using levels similar to those expected in the rice pudding. The results indicated that starch was more damaging for ξ -DNP lysine recovery than glucose. These findings agree with El-Nockrascky (1965) and Mathenson, (1968b) reports. An attempt to improve ξ-DNP-lysine recovery was carried out by using thiodiglycollic (TDG) acid before hydrolysis as described by Lyman and Thomas (1965) who claimed that the additon of TDG reduces the damage by carbohydrates during hydolysis. However no significant improvement was obtained. Therefore as the recoveries of ξ-DNP-lysine and ξ-DNP lysine from added sodium caseinate were within the levels reported as acceptable in the literature (Booth, 1971) no further attempt was made to improve the recovery of the technique.

For the rice pudding samples it was preferred to use casein as a recovery factor since:

(i) According to several authors (Booth, 1971, Mathenson, 1968 and Ruderus and Kihlberg, 1970) ξ -DNP-lysine is more sensitive to damage during hydrolysis than indigenous ξ-DNP-lysine (lysine in a protein chain).

(ii) Better reproducilibity was obtained for the recovery tests with ξ-DNP-lysine from added sodium caseinate that for added ξ-DNP-lysine. These finding agree with reports from Ruderus and Kihlberg (1970) who reported also reduced variability when using sodium caseinate as a recovery factor. Booth (1971) did not agree with this finding. He concluded that the use of highly soluble and easily digested proteins such as casein and albumins were unsuitable recovery markers.

(iii) Booth (1971) suggested that an ideal recovery marker should resemble the protein being assayed. Therefore in this work casein

was very near to an ideal recovery factor for rice pudding samples.

The recovery results obtained after ether extraction (99.5%) and after hydrolysis and ether extraction (94.4%) clearly show that even for pure proteins, the major losses during the hydrolysis stage. These losses are greatly increased in the presence of glucose (90.88%) and starch (85.06%).

The new rapid and simple dye binding technique was assessed as an alternative to the tedious and time consuming FDNB direct assay method. The results obtained with this DBC method showed that careful standardization of the procedure is essential.

Variables such as time of shaking for the binding reaction and weight of sample used needed to be determined before applying the technique. Optimum time of shaking was found to be 5 hours which is well in excess of the period of time (1 to 2 hours) suggested by Hurrell and Carpenter (1979). These findings were later corroborated by Almas and Bender (1980) who reported that casein required longer time of shaking than vegetable proteins to reach equilibrium. They reported 8 hours as the shaking time for casein.

The relationship between dye bound and weight of sample used was found to be non-linear. These findings agree with most authors (Gullard, 1965, Walker,1979 and Hurrell et al 1979). The limits within which a linear response was obtained, agree with the limits suggested by Hurrell, et al, 1979. At this point it was decided that the dye binding technique was adequate for available lysine determination instead of the FDNB direct method. This was further corroborated by a comparison between FDNB direct procedure FDNB indirect procedure (A.O.A.C. recommended method) and DBC-method. The results suggested that DBC method was more suitable

due to its better agreement with the FDNB indirect procedure. However when the technique was applied to the rice pudding samples, the results obtained were unrealistically high; higher than the total lysine content of the sample. Starch was eliminated as a source of interference due to the small amount of dye bound for per gram of starch, and because being a different measurement starch would absorb due in both measurements, hopefully leaving the available lysine value unchanged. A possible explanation was found in the results of Mauron (1981) and Hurrell et al (1979). They reported that the Amadori compound was partialy propionylated (about 40 %). Hurrell et al (1979) explained that the Amadori compound was bound with the dye because it is basic.
In 1981 they found that 29% of the formyl fructosyl lysine and 42% of the formyl lactulosyl lysine reacted with the propionic anhyride. This partial propionylation of the Amadori compound would produce a higher DBC-lysine value. This should account for the high values obtained in the rice pudding.

Hurrell and Carpenter (1981) claimed that the Amadori compound would be totally destroyed after four weeks of storage. Obviously this would render this method as unsuitable for detecting changes in thermosterilized foods since the first weeks of storage are the most important in nutrient losses, as show by the results obtained in this work. More studies are needed on the improvement of this assay procedure, particularly since it offers a simple and rapid alternative to the more tedious and time consuming available lysine methods in common use.

Another finding interesting to point out is that when casein was used as a protein for analysis, the results were erratic with the DBC-procedure. Since white lumps of unreacted casein were found in the reaction flasks, the lack of dispersion of the casein

was suggested as the cause of the poor results obtained. This seems to indicate that methods to improve the dispersion of the protein in the dye solution will increase the applicability of the technique.

Colour measurement was assessed for reproducibility. The results show good reproducibility as claimed by Glasser and Troy (1952). The variation in the readings obtained corresponds to variations in ΔE of ± 2 units.

7.5 Food Sample Production

Brussels sprouts and new potatoes

The results obtain show that the processing time difference between A-1 pouches and cans for foods packaged in brine is small. However, this difference increases slightly for bigger packs. It has been generally assumed that in convection heated canned foods, all the food in the container receives practically the same sterilization treatment, since the heating of the packaging liquid is very fast. So, all the food particles in the liquid are surrounded by an homogeneous temperature heating medium very near to retort temperature. The factor controlling the heat penetration rate in this case would be only the food particle size and the thermal diffusivity of the food material.

For the pouched product as the package is "wrapped" around the food and since it has been demonstrated that the overall heat transfer coefficient from the heating medium to the food is governed mainly by the heat transfer from the inner surface of the pouch to the contents (Terajima, 1975 and Warnick, et al, 1970), it can be considered the food surface is also almost at retort temperature. Consequently the heat transfer will also depend mainly on the thermal diffusivity and size of the food.

Considering that for new potatoes and brussels sprouts the food packaged in both containers was the same and with similar size and shape, no real difference between pouched and canned foods should be expected. Therefore any significant difference found between canned and pouched conduction heated foods (brussels sprouts and new potatoes) f -values should be attributed to the brine delaying heating effect. It is possible that due to the high density of packing of the solid particles in the cans, restriction of the convection currents may occur thus decreasing the overall heat penetration rate as suggested by Wade's(1949) work and later demonstrated by Sarmad (1977).

These differences between pouch and can processing times cannot be accounted for by the food or thermocouple variability since either of them would present a random effect. The thermocouple variability is even less likely to affect these results since thermocouple variation will affect the largely F_o-value evaluation but not the heat penetration rate.

Pork Casserole and Rice Pudding

For mainly conduction heated packs (pork casserole), the differences in processing time between pouches and cans are greatly increased and are increased even further with the container size. These results agree with the theoretical expectations. So it seems clear that the advantages of processing time reduction due to pouch processing are mainly present when conduction heated foods are used. This is clearly shown in Figure 7.1 which shows the temperature-time profiles of brussels sprouts and pork casserole sterilized in both pouches and cans.

This reduction in processing time was considered to be important in improving retention of thermally sensitive nutrients.

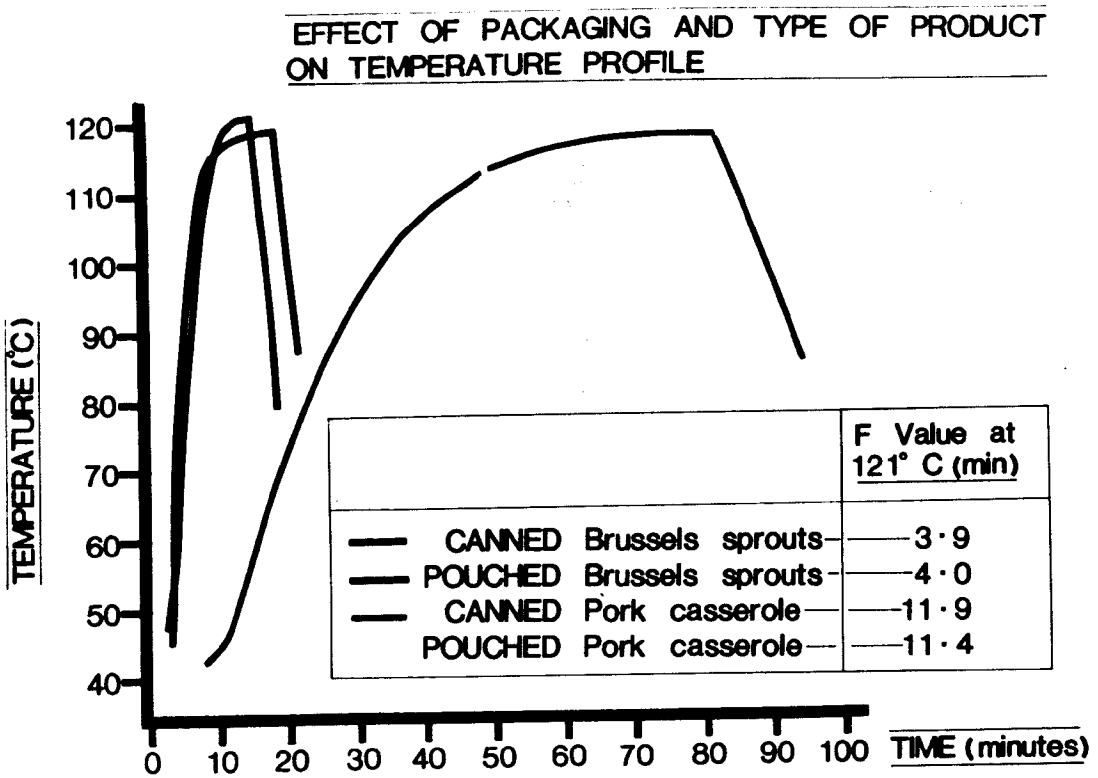

EFFECT OF PACKAGING AND TYPE OF PRODUCT ON TEMPERATURE PROFILE
TEMPERATURE (°C)
120
110
100
90
80
70
60
50
40
0
10
20
30
40
50
60
70
80
90
100
TIME (minutes)
F Value at 121° C (min)
CANNED Brussels sprouts — 3·9
POUCHED Brussels sprouts — 4·0
CANNED Pork casserole — 11·9
POUCHED Pork casserole — 11·4

Figure 7.1.

Rice pudding samples compared one of the best canning processes (rotating end-over-end sterilization) with pouch processing. It has been generally accepted that movement greatly increases the rate of heat penetration in slow heating fluid products such as in the case of rice pudding. The processing times reported for the sterilization of these products in the canning retort are not comparable to the pouch processing times, since the error introduced by the Ellab thermocouple construction produced a large sterilization treatment than intended. Thus process times for the canned product are larger than the equivalent process time for pouches.

However it seems that pouch processing and rotating retort can processing would be similar in processing times. Since the rice pudding in pouches have shorter process times than canned rice pudding before correction,it is expected that after correction the pouch processing times would be slightly higher than canning times.

It has been mentioned that products heating in cans by convection show a tendency to heat by conduction when packaged in pouches (Platt, 1975). This seems to be the case for the products packaged in this project.

The come-up times of the retorts show big differences, being slower for the pouch water retort. According to Masuo)1974) and Millenville (1980a and 1980b) the heat transfer in a steam retort will be faster since it takes longer to heat water to the desired sterilization temperature. This longer CUT may have a detrimental effect on nutrient retention since the thermal treatment applied to the foods during the CUT which is not important for microbiological lethality, may be damaging for heat sensitive nutrients.

The CUT is also an indirect measure of the efficiency of the

retort. The results obtained show that the pouch water retort used was less efficient than the canning processing equipment used.

The thermal diffusivity of the pork casserole product was calculated using Olson and Jackson (1942) equations for conduction heated foods. The results obtained show that the thermal diffusivities of the pouched products are smaller (about 50%) than the thermal diffusivities obtained for canned pork casserole. This can be explained by considering that the value obtained is an average thermal diffusivity value of the food packaged; the same piece of solid (a cubic 2.54 cm piece of potato) and gravy. The solid effect is greater in the thermal diffusivitiy of the pouched product, being probably a measure of the potato thermal diffusivity. For the thermal diffusivity of the canned product the gravy will be the main contributor. It is obvious why the pouched product should have a smaller thermal diffusivity, which means a slower heat penetration rate under the same conditions..

The thermal diffusivity obtained for A-1 and A-10 cans is very similar, as expected but the thermal diffusivity of the A-10 pouches is higher than the one of A-1 pouches. This may be attributed to an increase in the gravy effect for pouches in increasing the heat penetration rate.

The only product where thermal diffusivities were calculated was pork casserole since no model was available for the irregular pouch shapes presented by potatoes and brussels sprouts, and all other products heated by a combination of convection and conduction.

7.6 Food Sample Evaluation

7.6.1 Ascorbic acid

From the results obtain on ascorbic acid retention in brussels sprouts (section 5.3) and new potatoes (section 5.4) packaged in pouches and cans), it is clear that leaching is one of the main causes of water soluble nutrient loss in processed foods.

Leaching of ascorbic acid into the can brine accounted for about 30% of the losses due to processing and at the end of the storage period approximately half of the asco bic acid retained was in the brine. Leaching in the canned products caused 50% lost of ascorbic acid.

Canned brussels sprouts and new potatoes show a larger ascorbic acid loss in the solids than in the liquid during the first days of storage. Furthermore, for brussels sprouts, there was an increase in ascorbic acid content in the liquid during the first 10 days of storage. These results suggest that the rate of leaching of ascorbic acid into the brine is extremely high during the beginning of the storage. This effect covers any destruction taking place in the liquid.

Processing ascorbic acid losses are always larger for canning than for pouch processing as shown in Table 7.1., where it can be seen that processing ascorbic acid losses for brussels sprouts and new potatoes are similar but larger than for pork casserole ascorbic acid losses.

Pork casserole had a slightly/more acid pH (5.35) than potatoes (5.76) and brussels sprouts (5.82). This might have increased the ascorbic acid retention of pork casserole in relation to brussels sprouts as reported by Salib (1980a, 1980b) who reported that vitamin C and B_1 retention improved during blanching and processing when slightly acidic solutions were used as brine or

blanching water. However the reduction of ascorbic acid loss is significant and may not be totally accounted for by the small difference in pH value. It may also be caused by some other factors inherent to the food product itself such as protective naturally antioxidants.

Another factor that might have affected the rate of destruction is the combined leaching processing effect. According to Lathrop and Leugh (1981) leaching of ascorbic acid is mainly a diffusion mechanism. Therefore it should be affected by the concentration of solutes in the liquid. Gravy is a very viscous material so leaching of ascorbic acid to it should be slower than leaching to the brine solution. This effect could reduce the ascorbic acid destruction rate by reducing the probability of contact between oxygen and ascorbic acid molecules, since the oxygen present in solution and trapped in the tissues is expelled during sterilization into the head space. Also since the concentration of solids is larger in the gravy than in the brine and oxygen solubility is known to be inversely affected by the solute concentration in the liquid, a reduction in the possibility of contact between ascorbic acid and oxygen will result with the subsequent reduction in ascorbic acid destruction. As ascorbic acid (AA) destruction rate depends largely on oxygen availability, it was thought that any effect reducing the possibility of contact between oxygen and AA will reduce ascorbic acid destruction rate. Consquently the factors just mentioned could be some of the reasons for the improved ascorbic acid retention in this product during sterilization.

Storage conditions seem to produce the same effect in AA losses for food packaged in both pouches and cans for pork

casserole products. However AA losses in new potatoes and brussels sprouts due to storage seem to be larger.

These seem to be a side effect of leaching since the destruction of AA is larger in the canned samples that contain brine. The difference in AA losses during storage between canned and pouched samples is very large. It is clear that the main cause was leaching but potatoes did not present a exudate with high content of vitamin C during the beginning of the storage period.

Also the rate of loss of AA in new potatoes was very much slower than brussels sprouts and pork casserole. While these two canned products reached a stable vitamin level after one or two months of storage, canned potato continue to decrease in vitamin C content even after five months of storage.

These considerations seem to indicate that there is a mechanism in new potatoes which reduces the rate of leaching. A possible explanation for this was proposed by Boushell and Potter (1980) who suggested that at temperatures above 80°C there may be some starch gelatinisation with time, thus possibly protecting against leaching by "sealing" the surfaces of the potato pieces. Another explanation can be a higher cellular membrane resistance to damage during processing which would reduce the leaching rate. This second effect was observed by Selman (1982) in the blanching of peas. He mentioned that intact peas leached less ascorbic acid into the blanch water than slightly bruised peas.

For all products the rate of ascorbic acid loss is greater at the beginning of the storage and the AA destruction rate decreases with storage time until no detectable rate of AA loss is reached. This can be explained as a function of oxygen presence. At the beginning of the storage period some residual oxygen may be present in the packages. This residual oxygen will greatly

increase the rate of destruction because as proposed by Kurata, et al (1975), AA destruction in the presence of oxygen follows two parallel reactions pathways, anaerobic and aerobic destruction. As oxygen is consumed in ascorbic acid destruction and some other process such as corrosion of the tin surface of the can during the storage period; a reduction in the rate of destruction occurs until no oxygen or a very low level of oxygen is left in the package, thus leaving only the slow anaerobic AA destruction taking place.

It seems possible that the rate of AA destruction caused by the anaerobic mechanism is too low to be detected by the analytical techniques employed.

The anomalous results obtained with pouched brussels sprouts will be discussed later.

7.6.2 Thiamine

Thiamine retention follows the same general behaviour as ascorbic acid retention in pork casserole. The same general factors that affect ascorbic acid retention affect thiamine retention but to different extents.

No effect due to presence of residual oxygen should be expected since the destruction of thiamine is known to be thermal, not oxidative (Mulley et al, 1975).

Therefore the initial heavy losses of thiamine during storage and the reduction in thiamine destruction rate cannot be explained in terms of oxygen consumption. It may be possible that reactions with other active food components produce or "activated" during the sterilization process are responsible for the large losses of thiamine at the beginning of the storage and the consumption of these damaging compounds with time would produce the same effect

as oxygen consumption for ascorbic acid retention.

One type of compound that can be suggested as an interacting group is sugars. As suggested by Hurrell, (1980) they can react with the imido group of the thiazol group. Also it is known that thiamine solutions at pH 5 decompose slowly so it may be possible also that further losses during prolonged storage might be expected, although during the later stages of the storage period investiated the thiaminelevel appeared to be constant.

7.6.3 Available lysine

Available lysine retention in rice pudding samples shows a slight difference between pouch processing and canning. This difference is favourable to pouch processing but purely on the basis of smaller processing times, since the sterilization processes were not equivalent (Appendix 6).

No evidence was obtained that either of the two processes studied produced better nutrient retention. However differences in available lysine retention were found between A-1 containers and A-10 containers after sterilization. This can be easily explained by the shorter process times obtained for the A-1 containers.

Storage conditions produced a better available lysine retention at low temperatures than at high temperatures. Losses for pouched products during storage are larger than cans processed for a similar length of time. There is no explanation that can account for this effect unless an increase in the reducing sugars content during processing took place.

A similar curve was reported by Wolf et al (1977) when studying heating of soya protein in the presence of glucose. They divided the curve in three phases. An initial first order reaction rate, a second phase showing an apparent increase in DNP-lysine

and a third phase of no change. They reported that different proteins may react differently to processing and storage conditions.

7.6.4 Colour difference

The colour difference results due to the sterilization proces obtained agree with the availably lysine results where a longer processing time produced higher colour development than shorter processing times. Rice pudding in A-1 containers showed less colour development than A-10 containers.

Changes in colour development during storage show a tendency to increase with storage time particularly to the end of the storage time. This effect is more pronounced at high temperatures. These findings do not correlate well with the available lysine changes which were found to be greater during the beginning of the storage period.

Rhee and Rhee (1981) reported a significant correlation between browning index and available lysine in heated soya bean protein with glucose. However Kato et al (1978) found no correlation between rate of colour development and loss of free amino groups. Labuza and Saltamach (1981 a, 1981 b) and Mizraki et al (1970) proposed zero order reaction rates for brown pigment formation and first order reaction rates were proposed for available lysine loss by Wolf and Thompson (1977) and Labuza and Saltamach (1981). These two different reaction rates reported, could explain the difference between colour development and available lysine destruction rates obtained during storage or rice pudding.

7.7 Peroxidase Activity

Extremely large losses of ascorbic acid during storage were obtained in the pouched brussels sprouts. Also AA losses in this product were greatly increased when the temperature of storage was 37°C. The comparison of these findings with the results obtained for ascorbic acid retention in pouched new potatoes and pork casserole indicated that a different mechanism of destruction was present in the pouched brussels sprouts.

A series of experiments wee undertaken to investigate this effect. Peroxidase activity was found to be present in the pouched brussels sprouts but not in the canned samples. Therefore it was considered that residual peroxidase activity was responsible for the extremely large losses of ascorbic acid in the pouched product. Several new batches of brussels sprouts were produced to assess the validity of this statement. The results obtained showed several important considerations:

(i) A moderately high sterilization value is needed to reduce the enzyme activity to an adequate level (traces only). F_o-values of 6 min were found to be the limit for process at 116°C and 121.1°C.

(ii) The results suggest that processing time is a more important variable than sterilization treatment (F_o-values) for enzyme inactivation. Processing times over 20 min at 121.1°C and 116°C were found to be necessary to inactivate the enzymes.

(iii) Batches of thermosterilizedbrussels sprouts in pouches with residual peroxidase activity presented large and continuous losses throughout ten weeks of storage. However when no peroxidase activity was present in the samples, ascorbic acid losses were reduced and occurred only during the first four weeks of storage after which no ascorbic acid changes were detected.

This results confirmed the hypothesis that residual peroxidase activity was the main cause of ascorbic acid destruction in the pouched brussels sprouts samples. However in conventional canning processes it has been generally accepted that blanching prior to canning does not have as primary objective the inactivation of enzymes since the product generally receives a heat treatment severe enough to inactivate enzymes (Karel, et al, 1975, Svensson, 1977, Harris and Karmas, 1977).

The regeneration of peroxidase activity has been reported by several authors. Schwimmer (1944) reported that the effect of time and temperature on turnip and cabbage juices. He showed that the shorter the time of exposure to heat the greater the portion of enzyme regenerates. Farkas, 1954, suggested that heat resistant peroxidase should be considered at processing temperatures above 124°F, and that although peroxidase regeneration occurs, it becomes an important factor only when the required sterilization treatment is carried out above 124 °C. He suggested a Z value of 26.6°C for peroxidase activity.

Guyer and Holmquist (1954) reported that residual peroxidase activity was regenerated in canned peas sterilized in a rotary retort at 126.6°C for 5 min after two weeks of storage and when processed in a still retort at 121.1°C for 10 min after four months of storage. Shorter times at those temperatures presented enzyme regeneration, longer processing times showed no enxyme regeneration. However most authors agree that regeneration usually occurs after a few hours when the product is stored at room temperature or it may take several months if the product is stored at freezing temperatures (-18°C).

For HTST processes heat resistant peroxidases have always

been regarded as a problem, and have been used as an index of adequacy for HTST processes, (Ling and Lund, 1978, Gebriel, et al 1977). The results obtained in the present work and the information available in the literature seem to suggest that the improved heat penetration rate of processing in pouches caused conditions similar to the HTST processes where peroxidase activity was either not totally inactivated or regenerated during storage.

7.8 Conclusions

Several considerations emerge from the analysis of the results:

(i) Pouch processing can be a major contributor to increased nutrient retention in thermal sterilization processing. The results presented generally agree with the theoretical predictions of Teixeira, et at (1975) and Lund, (1977) who suggested that a modification of the container geometry to approximate a flat disc shape could be an effective way to improve nutrient retention in conduction heated foods, due to the extended surface for heat transfer with relation to the volume of food. This would be the case for the pouch and as predicted by them the nutrient retention is better for conduction heated foods (pouched pork casserole). The results obtained also extended the validity of these predictions to combined convection and conduction heated foods such as brussels sprouts and new potatoes.

(ii) Large reductions in process time can be obtained during pouch processing relative to canning. The main processing factor affected by the change in geometry is the processing time, which is greatly reduced for conduction heated packs and only slightly reduced for convection heated packs.

(ii) The results presented also show that this advantage of reducing processing time, thus increasing thermally sensitive nutrient retention is greatly increased as the container increases in size. However care must be taken not to overfil the pouches since the heat penetration factor is known to be dependant on the pouch thickness. This can be deduced from Jackson and Olson formulae for conduction heated products (equation 2.12) by assuming thermal diffusivity length and width constant and varying only the thickness of the pouch.

(iv) Pouch processing can greatly improve the retention of water soluble nutrients, since it is possible to pack foods without or with small amounts of liquid thus reducing the leaching losses. The results show that up to 50% of water soluble nutrients can be saved. This same effect has been tried in high vacuum packaged cans (Wade et al 1950) where the use of high vacuum permits the use of a minimum amount of water or brine in the can thus reducing the loss of water soluble nutrients. However due to the difficulties in controlling the heat transfer in these types of products their use at the present is limited. Similar results were reported by Rizvi and Acton (1982). They used small volumes of packaging liquids in vegetables in pouches and reported higher retention of ascorbic acid in several foods relative to canned foods. Thorne (1976) also reported improved ascorbic acid retention in pouched processed potatoes.

(v) According to Komatsu, et al (1975) the nutrient retention can be further increased by the use of higher temperatures of processing. They suggested temperatures between 130°C and 160°C which would be in the HTST type of sterilization process. This type of process cannot be applied to rigid containers since temperatures in excess of 125°C may damage the product and/or the container. Optimization of thiamine retention in canned foods

resulted in the conclusion that for conduction heated foods HTST does not provide maximum nutrient retention, due to over processing of the outside layer of the food in the can (Saguy and Karel, 1979)

Most of the work done on nutrient prediction of nutrients during sterilization treatments have been limited to thiamine and in model systems or pureed foods due to the relative simplicity of its kinetics (Castillo, 1979, 1980 Mulley, 1975, Saguy and Karel, 1979, Teixeira, et al, 1975).

Ascorbic acid presents more obvious difficulties since two mechanisms can affect its destruction and can occur as parallel reactions. It is also greatly influenced by environmental factors such as oxygen, pH, temperature etc. Consequently prediction of ascorbic acid retention is still an unsolved problem.

(vi) The results obtain for ascorbic acid and thiamine retention can be affected by the process control limitations and the variability of the heat treatment received by the pouched processed foods. However the changes in nutrient content due to sterilization treatment and storage conditions are large enough to cover any heat treatment variation also since assay techniques were shown to be highly reproducible, the results obtained can be considered of significance.

(vii) The assay for available lysine damage was more variable and losses obtained were small consequently only the general trend of the destruction can be considered of importance. The percentage of available lysine lost during storage and sterilization process is low which indicates that in canned products losses of available lysine are minor, when compared with the dehydration and concentration processes damage or in losses intermediate

moisture foods. However it must be pointed out that rotary thermO-sterilization has been proved to increase nutrient retention when compared to stationary sterilization (Sorman and Zajac, 1974). Soit seems possible that losses due to normal canning practices for similar products may be larger than those obtained in this work. Nevertheless they are within the range of reported losses for canning.

The results obtained bring out the need for additional studies on several topics:

(i) To investigate the use of concentrated packaging solutions to improve water soluble nutrient retention by the reduction of leaching rate.

(ii) The use of oxygen scavenging films to investigate the effect of oxygen in nutrients like ascorbic acid in foods during sterilization without the variable of leaching, which is normally introduced in canning.

(iii) An investigation of the effect of starch in the reduction of leaching rate to increase nutrient retention.

(iv) Investigate the possibility of using HTST processes for conduction heated food in pouches and their nutrient retention effect.

(v) To evaluate peroxidase activity as a possible source of problems in pouch processirg.

APPENDIX 1

SAMPLING PROCEDURES

The different sampling procedures were designed to cause the least possible damage to nutrients and to ensure a homogeneous sample.

Brussels sprouts and new potatoes

Blanched samples. Immediately before processing, randomly taken blanched samples were cut into quarters with a sharp kitchen knife and mixed thoroughly. Four different samples of 40 grams were weighed out and stabilized by refrigeration in 350 cm^3 of 5% H_3 solution until the analysis was carried out. This was achieved within 24 hours of the samples being taken.

Processes samples. The processed packages used for evaluation were withdrawn from the retort batch in a random manner and analysed immediately after opening. The solid and the liqid were separated and the liquid volume measured and retained for analysis. Solids were prepared for analysis in the same way as blanched samples.

For brussels sprouts one pouch/can was used for chemical analysis at each time with the exception of analysis immediately after processing when two packages were used. The total volume of liquid collected plus the package washings were stabilized with 5% HPO_3 solution (250 cm^3 for pouch liquid and 350 cm^3 for can liquids) and refrigerated. Solids were weighted (40 grams) and stabilized in HPO_3 solution (350 cm^3) while waiting for the analysis to be completed within 8 hours of samples being taken.

For he Jersey new potatoes, sampling was carried out in the same way as for brussels sprouts. The only difference being that instead of using one package for each determination, three packages were used.

Pork Casserole

Blanched samples. Three unprocessed packages were taken in a random manner from the unprocessed batch immediately prior to processing. Samples were treated in the same way as processed samples with a maceration time of 6 minutes.

Proces sed samples. In the case of processed materials, three randomly selected packages were analyzed at each time interval for each storage temperature and immediately after processing. Each package was opened and the content transferred to an LSE-ATO mix blender flask. Nitrogen was passed through the sample for 5 minutes to expel any oxygen that could be present. The sample was then homogenized under nitrogen for 3 minutes and immediately weighted for ascorbic acid and moisture content analysis. The remainder of these samples were stored at -20°C for thiamine assay. Frozen homogenized samples were allowed to thaw under refrigeration overnight and were thoroughly mixed before weighing analytical samples. Each A-1 package was analyzed in duplicate. Each A-10 package was assayed by taking 5 different samples for analysis. Samples immediately after weighing were stabilized om acid extractant solution, either 5% HPO_3 for ascorbic acid or 0.1 N HCL for thiamine analysis.

Rice Pudding

One package was opened at each time and the whole contents were homogenized in an ATO-mixer for 5 minutes in the case of processed samples or pre-cooked samples. The raw materials were not analyzed. The homogenized samples were stored at -20°C while waiting for the analysis.

All samples were weighed and used according to the nutrient assay procedure employed. The analytical techniques are discussed in the next appendix.

APPENDIX 2

CHEMICAL METHODS AND CALCULATIONS EMPLOYED

All the chemicals used were Analar grade from BDH Chemicals Ltd., Poole, England, unless a special grade is stated or a different company mentioned.

2.1 2,6 Dichlorophenol Indophenol Visual Titration

Reagents Used

1. .01 M EDTA in 20% metaphosphoric acid. 2.6 grams of disodium ethylenediaminetetraacetate (EDTA) and 200 grams of metaphosphoric acid sticks (HPO_3) were dissolved and diluted to one dm^3 with glass distilled water. It was prepared fresh weekly and stored, under refrigeration, in a dark bottle.
2. 5% metaphosphoric acid (HPO_3) solution (0.0025 M EDTA). 250 cm^3 of the above solution (1) were diluted to one dm^3 with distilled water. It was prepared fresh daily.
3. Acetate buffer solution (pH 4.0). 125 grams of sodium acetate trihydrate were dissolved in glass distilled water and diluted to 250 cm^3. 250 cm^3 of glacial acetic acid were added and mixed.
4. 50% sulphuric acid solution V/V.
5. 37-41% formaldehyde solution, ordered as such.
6. Ascorbic acid standard solution. 0.1 grams of ascorbic acid were dissolved in 5% HPO_3 solution and diluted to 100 cm^3.
7. Working standard ascorbic acid solution. 10 cm^3 of the above solution (6) were diluted to 100 cm^3 with 5% HPO_3 solution.

8. 2,6 dichlorophenol indophenol solution. Dye was dissolved in 200 cm^3 of distilled hot boiled water, filtered and diluted to one dm^3 with cold water. The concentration was dependant on the ascorbic acid content of the samples. 0.04% and 0.06% for brussels sprouts and 0.02% for potatoes and pork casserole. It was prepared fresh weekly and standardized daily before use.

A Standardization of the Dye

1. 5 cm^3 of the working standard ascorbic acid solution were pipetted into 25 cm^3 conical flasks.
2. Ascorbic acid aliquots were rapidly titrated with the dye solution until a faint pink colour persisted for 5-10 seconds.
3. 5 cm^3 of 5% HPO_3 solution were titrated to determine the dye blank.

B Extraction

1. The homogenized sample was stabilised with the 5% HPO_3 extraction solution
   (a) For pork casserole, after homogenizing under N_2 the samples were immediately weighed and stabilized with 5% HPO_3 solution
   (b) For brussels sprouts and new potatoes the can or pouch liquid volume was measured and transferred to a conical flask. The measuring cylinder used and the package were washed with 5% HPO_3 solution until a total of 350 cm^3 were added to

to the can liquid or 250 cm^3 to the pouch liquid. The conical flasks were kept under refrigeration until the titration stage. Solids were weighed and stabilised with HPO_3 solution

2. 40 grams of solid sample were blended in 350 cm^3 of 5% HPO_3 in a top drive macerator Mark III for 3 minutes
3. The extract was centrifuged for 15 minutes at 3,000 rpm
4. Four 25 cm^3 aliquots of clear extract solution were pipetted into 50 cm^3 conical flasks, which were covered and stored under refrigeration while waiting for titration. Sample extraction and titration were completed as soon as it was possible

C Titration of Ascorbic Acid

1. 1 cm^3 of 50% sulfuric acid was added to one of the solutions obtained in B.4, to reduce the pH to 0.6
2. 3 cm^3 of formaldehyde solution were added. The resulting solution was mixed and three 5 cm^3 aliquots were transferred to 25 cm^3 conical flasks
3. Exactly 8 minutes after the addition of formaldehyde, the three aliquots were titrated with the dye solution
4. The procedure was repeated with 25 cm^3 of 5% HPO_3 solution to determine the dye blank titration value

D Titration for Reductones

1. 15 cm^3 of acetate buffer were added to a second 25 cm^3 aliquot of clear extract solution obtained in B.4 to adjust the pH to 3.5

2. The resultant solution was mixed and two aliquots of 10 cm^3 wre transferred to 50 cm^3 conical flasks
3. 10 cm^3 of formaldehyde solution were added to the above solutions (2)
4 Exactly 10 minutes after the addition of formaldehyde the reductions were titrated with standardized dye until a faint pink colour persisted for 10 seconds
5. The procedure was repeated with 25 cm^3 of 5% HPO_3 solution to determine the dye blank

E Calculations

A simple calculation was made to obtain millgrams of ascorbic acid in 100 grams of original samples using the following equations

W weight of sample taken, in grams

S total volume of extract (volume of 5% HPO_3 solution used plus moisture content of the sample) in cubic centimetres

V_1 volume of extract aliquot, in cubic centimetres (25 cm^3)

V_2 volume, in cubic centimetres, of extractant titrated (29 cm^3)

T_1 volume of dye used for titration minus dye blank

T_2 volume, in cubic centimetres, of dye used for reductones titration minus dye blank

V_3 volume, in cubic centimeters, of extractant and acetate buffer (40 cm^3)

F dye factor of dye used. Milligrams of ascorbic acid equivalent to one cubic centimetre of dye

1. Without reductones

$$\text{milligrams of ascorbic acid per 100 gms of sample} = \frac{F \times T_1 \times V_2 \times S \times 100}{V_1 \times W}$$

When reductones were present

$$\text{milligrams of ascorbic acid} = \left(\frac{T_1 \times V_2 \times F}{10} - \frac{2T_2 \times V_3 \times F}{10}\right) \frac{S \times 100}{V_1 \times W}$$

2.2 O-phenylenediamine Fluorimetric Procedure

Reagents

1. Extraction solution. 3% metaphosphoric acid -8% acetic acid - EDTA 0.0025 M. 60 grams of metaphosphoric acid sticks and 1.8 grams of EDTA were dissolved in 80 cm^3 of acetic acid glacial and 200 cm^3 of distilled water. The solution was diluted to 2 dm^3 . It was prepared fresh weekly and stored in a dark bottle under refrigeration
2. Standard ascorbic acid solution. 0.1 grams of ascorbic acid were dissolved and diluted in extraction solution to 100 cm^3 . It was prepared fresh daily
3. Working standard ascorbic acid solution. 10 cm^3 of the above solution were diluted to 100 cm^3 with extraction solution. It was prepared fresh daily
4. Sodium acetate solution. 500 grams of sodium acetate trihydrate were dissolved and diluted up to 1 dm^3

5. Boric acid - sodium acetate solution. 3 grams of boric acid were dissolved and diluted to 100 cm^3 with the above solution
6. O-phenylenediamine solution. 0.03 grams of o-phenylenediamine were dissolved in 65 cm^3 of 0.1 N HCl and made up to 250 cm^3 with distilled water
7. Acid washed norit

A Extraction

1. 40 grams of sample were weighed in a macerator flask and 350 cm^3 of extraction solution were added. Sample liquids were measured with a measuring cylinder. Measuring cylinder and package were washed until 250 cm^3 of HPO_3 were used and added to the liquid samples which were saved in conical flasks under refrigeration until total (ascorbic acid and dehydroascorbic acid) and dehydroascorbic acid determinations procedures
2. Samples were macerated for 3 minutes in a MSE top drive macerator
3. All the extract solution was centrifuged for 15 minutes at 3000 rpm
4. The clear resultant solutions were collected and saved in conical flasks

B Determination of Ascorbic Acid and Dehydroascorbic Acid

1. 100 cm^3 of extract solutions were pipetted into round bottom flasks

2. 100 cm^3 of working standard ascorbic acid solution were pipetted into a round bottom flask
3. 2 grams of acid washed norit were added to each of the round flasks and shaken vigorously for 20 minutes with a Gallenkamp flask shaker
4. The contents of each flask were centrifuged for 30 minutes at 3,000 rpm
5. 5 cm^3 of the centrifuged clear solutions were pipetted into 100 cm^3 volumetric flasks containing 5 cm^3 of boric acid - sodium acetate solution. The flasks were prepared and numbered while centrifugation was taking place
6. The flasks were left to stand for 15 minutes with occasional swirling, and made up to volume with distilled water
7. Another 5 cm^3 of centrifuged solutions were pipetted into 100 cm^3 volumetric flasks containing 5 cm^3 of sodium acetate solution. The solution was made up immediately with distilled water
8. 4 cm^3 of each solution in B.6 and B.7 were pipetted into light protected glass tubes, and
9. 5 cm^3 of o-phenylenediamine solution were added to each tube with an automatic pipetting unit and shaken immediately with a vortex mixer
10. The tubes were kept in a dark place for 35 minutes before fluorescence measurements

C Dehydroascorbic Acid Determination

1. 5 cm^3 of the extract solutions obtained in A.4 (untreated extracts) were pipetted into 100 cm^3 volumetric flasks containing 5 cm^3 of boric acid-sodium acetate solution
2. The flasks were allowed to stand for 15 minutes with occasional stirring
3. 5 cm^3 of the extract solutions obtained in A.4 were pipetted into 100 cm^3 volumetric flasks containing 5 cm^3 of sodium acetate solution and made up to volume with distilled water
4. 5 cm^3 aliquots of the solutions obtained from C.2 and C.3 were pipetted into light protected glass tubes
5. 5 cm^3 of o-phenylenediamine solutions were added to glass tubes. They were kept in a dark place during 35 minutes before measurement of fluorescence

D Measurement

1. A fluorescence spectrophotometer 204 Perkin - Elmer was set at 365 nm for the excitation wavelength and 435 nm for the emission wavelength
2. A cuvette was filled with distilled water and used to zero the apparatus
3. A second cuvette was filled with working ascorbic acid solution treated for total vitamin C determination (according to B.1-B.4, B.7-B.8). This solution was used to set the apparatus at 80% of scale deflection

4. All other solutions were measured always checking against the standard and zero points
5. All measurements were done within 15 minutes

E Calculations

W weight of sample, 40 grams

UA reading of total vitamin C solutions obtained from B.7 (sodium acetate treated)

BUA reading of blank solutions obtained from B.6 (boric acid-sodium acetate treated)

UDA reading of DAA solutions obtained from C.3 (sodium acetate treated)

BUDA reading of blank solutions obtained from C.2 (boric acid-sodium acetate treated)

B reading of ascorbic acid working standard solution treated with boric acid - sodium acetate

S volume of extraction solution plus moisture content of the sample, in cubic centimetres

SC concentration of working ascorbic acid standard solution. In mg. of AA/cm^3

1. Ascorbic acid plus dehydroascorbic acid as:

$$\text{milligrams of ascorbic acid per 100 g of sample} = \frac{SC(UA-BUA)}{(80-B)} \times \frac{2.5xS}{W}$$

2. Dehydroascorbic acid as:

$$\text{milligrams of ascorbic acid per 100 g of sample} = \frac{SC(UDA-BUDA)}{(80-B)} \times \frac{400xS}{4.5}$$

2.3 Thiochrome Fluorimetric Assay Method

Reagents

1. 15% (w/v) sodium hydroxide solution
2. 1% (w/v) potassium ferricyanide. It was kept in a brown bottle under refrigeration
3. Alkaline potassium ferricyanide. 3 cm^3 of solution, 2 cm^3 were diluted to a 100 cm^3 with solution 1. It was prepared fresh daily
4. 0.2 N hydrochloric acid solution
5. 2.5 M sodium acetate solution
6. Isobutyl alcohol, special grade for thiamine determination, free of fluorescence
7. Taka-diastase solution. Enzyme was obtained from Parke Davis and Co., Mitchigan, U.S.A. 6 grams of enzyme were dissolved and diluted to 100 cm^3 with solution 5 (2.5 M sodium acetate). It was prepared fresh daily
8. 0.1 N sulphuric acid solution
9. Anhydrous sodium sulphate, granular. Fired and kept in a desiccator
10. 25% (w/v) potassium chloride solution
11. 25% (w/v) potassium chloride acid solution. 8.5 cm^3 of concentrated HCl were diluted to 1 dm^3 with 25% (w/v) potassium chloride solution
12. 3% (v/v) acetic acid solution
13. 1% (w/v) silver nitrate solution
14. Activated decalso F ion exchange resin obtained from Permutit Co. Ltd., Hopkin and Williams Ltd., Essex, England

15. Stock thiamine hydrochloride solution - 1.0 gram of thiamine hydrochloride, dried overnight in a phosphorus pentoxide (P_2O_5) dessicator was dissolved in 25% ethanol and diluted to 1 dm^3
16. Standard thiamine hydrochloride solution - 10 cm^3 of the above solution were diluted to 100 cm^3 with 25% ethanol
17. Working standard thiamine hydrochloride solution. 2cm^3 of the above solution were diluted with 38 cm^3 of 0.2 N HCl and distilled water to 100cm^3

Activation of Decalso

1. 100 grams of decalso were immersed in hot 3% acetic acid (250 cm^3) for 15 minutes with occasional stirring
2. Acid was drained off using a buchner funnel under mild vacuum
3. This procedure was repeated once more with hot 3% acetic acid, and then
4. Once with 25% potassium chloride solution (250cm^3)
5. Once with hot 3% acetic acid (250cm^3)
6. Several times with hot distilled water until washing water was free of chlorides. This was assumed true when no precipitate was formed with 1% silver nitrate solution
7. It was dried overnight in an oven at 105°C before use. 6 grams of dried decalso were used to fill the columns

Procedure

A Extraction

1. Between 4-7 grams of homogeneous sample were weighed in a conical flask
2. 38 cm^3 of 0.2 N HCl and 30 m of distilled water were added to the sample
3. The flasks were kept in a boiling water bath for 30 minutes with occasional stirring
4. The extracts were cooled below 50°C and 5 cm^3 of freshly prepared enzyme solution were added
5. Sample extracts were incubated at 50°C for 3 hours
6. The extracts were cooled to room temperature and transferred to 100 cm^3 volumetric flasks and diluted with distilled water
7. Half of the sample was centrifuged for 15 minutes at 3,000 rpm. The rest was stored under refrigeration until analysis was completed
8. The clear solution obtained was transferred to conical flasks

B Ion Exchange Purification

1. Several ion exchange columns were prepared with activated decalso. 6 grams were packed for each column which was free of air pockets
2. 10 cm^3 of extract solutions (obtained from A.8) were pipetted into the column. The solutions were passed slowly through the columns. The solutions obtained were discarded
3. Three successive portions of distilled hot water were used to wash the columns. The washings were discarded

4. Two separate portions of 10 cm^3 of KCl solution were placed on the columns and the solutions obtained were collected in 25cm^3 flasks
5. The solutions were diluted to 25 cm^3 with 25% KCl solution
6. The procedure was repeated using working standard thiamine solution

C Oxidation to Thiochrome

1. 5cm^3 of the solutions obtained above were pipetted into glass quickfit centrifuge tubes (a) if purification step (section B) was not required the solutions taken were then obtained from A.8 and standard working thiamine chloride
2. 3 cm^3 of alkaline ferricyanide solution were pipetted into the tubes and 15 cm^3 of isobutanol were delivered with an automatic pipetting device
3. Tubes were vigorously shaken for 90 seconds with a specially designed manually operated shaker. The main features in the design of this shaker were to apply pressure on the tube tops to avoid leakages during shaking and to produce an end-over-end rotation. This shaker could take up to 8 tubes at one time
4. Procedure was repeated (C.1-C.3) using 15% NaOH solution instead of alkaline ferricyanide solution to obtain the (standard and samples) blank readings

D Thichrome Separation

1. Tubes were centrifuged for 2 minutes at 2,000 rpm to separate the two layers
2. Most of the aqueous layer was pipetted off with pasteur pipettes
3. 2 grams of fired sodium sulphate, previously weighed, were added to the tubes to eliminate the remaining water
4. The tubes were shaken and centrifuged for 3 minutes at 2,000 rpm

E Measurement

1. A fluorescence spectrophotometer Perkin-Elmer 204, coupled with a Perkin-Elmer 150 Xenon Power supply, was used. Excitation and emission wavelengths were set at 375 nm and 435 nm respectively
2. Four optical-grade quartz matched cuvettes were used. One fluorimeter cell was filled with distilled water to adjust the zero reading, and the other was filled with the standard to adjust the standard reading
3. All sample solutions were measured against standard and zero readings

F Calculation

U reading from the sample

UB blank reading from sample

SR standard reading

SB standard blank reading

V volume passed through the decalso column (10cm^3) in cubic centimetres

W weight of the sample, in grams

SC standard concentration in milligrams of thiamine/ cubic centimetre

1. When purification steps was used (section B)

$$\text{milligrams of thiamine per 100 g of sample} = \frac{U-UB}{SR-SB} \quad \frac{SC \times 250}{W}$$

2. When no purification step was used

$$\text{milligrams of thiamine per 100 g of sample} = \frac{(U-UB)}{(SR-SB)} \times \frac{SC \times 100}{W}$$

2.4 2,4 Dinitrofluorobenzene Direct Determination of Available Lysine

Reagents

1. Fluoro - 2,4 dinitrobenzene (FDNB). It was kept under refrigeration
2. Absolute ethanol
3. Hydrochloric acid (HC1) 8.1 N
4. Methoxycarbonyl chloride (MCC). It was kept under refrigeration
5. FDNB solution. About 0.4 ml of FDNB were measured with an automatic pipetting device and added to 15cm^3 of absolute ethanol, shaken and prepared fresh immediately before use

6. Sodium bicarbonate ($NaH\ CO_3$) solution 8% (w/v)
7. Diethyl ether, free of peroxides
8. Phenolphthalein solution. 0.4 grams were diluted to 1 dm^3 with 60% (v/v) ethanol solution
9. 12% (w/v) sodium hydroxide (Na OH) solution
10. Carbonate buffer solution (pH 8.5). 78 grams of sodium bicarbonate ($NaH\ CO_3$) and 4 grams of sodium carbonate (Na_2CO_3) were dissolved and diluted to 1 dm^3 with distilled water
11. 0.1 N HCl solution
12. Mono- ε -N-dinitrophenyl-lysine hydrochloride monohydrate (ε -DNP-lysine.HCl, H_2O) concentrated standard solution. 785 milligrams of ε -DNP-lysine.HCl.H_2O were dissolved and diluted to 250 cm^3 with 8.1 N HCl. Solution was kept in the fume cupboard
13. ε-DNP-lysine.HCl.H_2O standard solution 2. 314 milligrams of ε -DNP-lysine.HCl.H_2O were dissolved and diluted to 250 cm^3 with 8.1 N HCl. It was kept under refrigeration
14. Working standard ε -DNP-lysine.HCl.H_2O solution. 2 cm^3 of the above solution were diluted to 1 dm^3 with distilled water

Procedure

A. Reaction with FDNB

1. 2 grams of homogenized samples were placed in long neck round bottom flasks

2. Two pieces of washed porcelain and 10 cm^3 of $NaHCO_3$ solution were added, shaken gently by hand
3. 15 cm^3 of FDNB solution were added to each flask
4. Flasks were shaken in a Gallenkamp flask shaker for 3 hours
5. Ethanol was evaporated from the flasks in a boiling water bath until 12.5 grams on weight were lost
6. Flasks were cooled to room temperature in a cold water bath

B. Hydrolysis

1. 30 cm^3 of concentrated HCl (8.1 N) were added to the dinitrophenylated samples (DNP-samples)
2. DNP-samples were refluxed for 16 hours (overnight)
3. Heat was turned off and condenser was washed with distilled water
4. Sample hydrolyzates were filtered through Whatman paper 541 and collected in a 250 cm^3 flask. Washings from the hydrolysis flask were also saved and several washings were carried out to recover all DNP hydrolysate until 250 cm^3 was collected
5. Samples were cooled and volume was adjusted to 250cm^3. The precipitate was allowed to settle
6. 2 cm^3 of hydrolyzate samples were pipetted into 10 cm^3 volumetric flasks - two aliquots for each sample (one for blank determination)

C. Sample Extraction

1. 5cm^3 of diethylether were added to sample flasks
2. Flasks were shaken vigorously and left to stand
3. Most of the ether layer was extracted with pasteur pipettes and discarded
4. Remaining diethylether was evaporated in a water bath at about 80°C. Samples were left to cool to room temperature
5. Procedure was repeated twice more and the contents were made up to volume with 0.1 N HCl

D. Blank Extraction

1. Hydrolyzates (B.5) were extracted with ether as described in section C (C.1-C.4)
2. A drop of phenolphthalein was added and then, drop by drop, NaOH solution was added until a pink colouration appeared
3. 2 cm^3 of buffer solution were added to the sample hydrolyzates
4. Five drops of MCC were carefully added. Sample hydrolyzates were shaken vigorously for exactly 8 minutes releasing pressure carefully
5. 0.75 cm^3 of concentrated HCL were, dropwise, added to the flasks which were shaken until all frothing disappeared
6. Three diethylether extractions were done as described in section C
7. Blank solutions were made up to volume with distilled water

E. Second Hydrolysis

1. Filter papers with all the residues were placed back in the same flasks used during the first hydrolysis
2. 10 cm^3 of distilled water and 30 cm^3 of concentrated HCL were added
3. Samples were refluxed during 7 hours
4. The same procedure was followed as with the first hydrolyzates

Measurement

The absorbances of sample (U) and sample blank (UB) were read at 435 nm against distilled water. A reading of U-UB was taken as the net absorbance of the sample or ϵ-DNP-lysine absorbance. Concentration was obtained from a standard curve.

Calculations

W = weight of sample in grams

C = concentration obtained from the standard curve in milligrams of lysine per cubic centimetre

MW = molecular weight of lysine

% N = percentage of nitrogen in the sample

$$\text{milligrams of available lysine per 100 grams of sample} = \frac{C \times 125{,}00}{W}$$

$$\text{millimoles of available lysine per gram of } N_2 = \frac{C \times 125{,}000}{W \times MW \times \% N}$$

2.5 Acid Orange-12 Dye Binding Capacity for Available Lysine

Reagents

1. Buffer solution (pH 1.25) - 20.0 grams of oxalic acid dihydrate and 3.4 grams of potassium dihydrogen phosphate were dissolved in distilled water, 60 cm^3 of glacial acetic acid were added and dissolved to 1 dm^3
2. Acid orange-12 solution. The dye solids were purchased from Foss Electric, The Chantry, Bishop-thorpe, York, England. A fixed amount was provided by the manufacturers which diluted to 10 Kg with the above buffer solution. It contained 3.89 mmol/dm^3 of acid orange -12
3. 15% (w/v) sodium acetate solution
4. Propionic anhydride

Procedure

1. Finely homogenized samples were weighed in round bottom flasks
2. Glass beads and 2 cm^3 or 4 cm^3 of sodium acetate solution were added. The amount was dependent on the sample size. 2 cm^3 for samples below one gram and 4 cm^3 for larger samples

A. Histidine, Arginine and Lysine (HARL) Determination

3. Sample was shaken on a laboratory Gallenkamp shaker for 30 minutes

4. 40 cm^3 of Dye solution were added to the samples and flasks were shaken for 5 hours to complete reaction
5. Solutions were filtered through glass fibre paper to obtain a clear solution for subsequent readings
6. 2 cm^3 of clear sample solutions were pipetted into 100 cm^3 volumetric flasks and diluted with buffer solution

B. Histidine and Arginine (HAR) Determination

1. Propionic anhydride was added to the round bottom flasks prepared from A.2. The amount varied with sample size 0.2 cm^3 for samples below one gram and 0.4 cm^3 samples above 1 gram
2. The sample was shaken for 30 minutes, the dye solution was added and shaken, the solution obtained was filtered and diluted as described in section A

Measurement

1. A Cecil CE 292 digital spectrophotometer was set at 482 nm, and distilled water was used to zero the instrument
2. A glass cuvette was filled with the sample solution and the reading taken
3. Diluted dye solution was used to check the instrument as a standard
4. Concentration was obtained from a standard curve

Calculations

C_d original dye concentration, 3.89 mmol/ dm^3

C_b concentration of dye bound to the sample, in millimoles per cubic decimetre

C_u concentration of dye unbound, in millimoles per dm^3 , from standard curve

W weight of sample in grams

total volume used to react the sample (volume of dye solution, propionic anhydride, sodium acetate solution and moisture of sample) in cubic centimetres

%N nitrogen per cent in the sample

D dilution factor

Cb $(Cd \times V/40) - (D\ C_u)$

$$\text{Millimoles of available lysine per 100 grams of sample} = \frac{C\ b \times 100}{W}$$

$$\text{Millimoles of available lysine per gram of } N_2 = \frac{C\ b \times 100}{W \times \%N}$$

2.6 o-dianisidine, Peroxidase Activity Determination

Reagents

1. 1% (w/v) o-dianisidine solution in methanol. This solution was prepared fresh daily
2. 0.1 M phosphate buffer solution pH 6. This solution was prepared fresh monthly to avoid microbiological contamination

3. 0.8% (v/v) hydrogen peroxide (H_2O_2) solution in 0.1 M phosphate buffer
4. 1M sodium chloride solution (NaCl)
5. Fine muslin material

All solutions were kept under refrigeration at 5°C ± 2°C.

A. Sample Extraction

1. 20 grams of homogenized sample were weighed or liquid in pouch measured
2. 80 cm^3 of 0.1 M phosphate buffer solution at 5°C were added to the sample in a homogenizer flask
3. Sample was homogenized in a MSE top drive bench homogenizer for 6 minutes
4. Extract solution was filtered through a double layer of muslin. The clear solution obtained was saved in a conical flask
5. Sample solutions were stored at -18°C until separation and measurement of enzyme activity could take place

B. Enzyme Separation

1. Extract was thawed and divided between 4 centrifuge plastic tubes
2. Tubes were centrifuged for 20 minutes at 11,000 rpm and 4°C in a MSE refrigerated ultracentrifuge unit
3. Supernatant fluids were collected in a sample flask and defined as soluble peroxidase. It was retained for assay

4. Solids in centrifuge tubes were suspended in 80 cm^3 of distilled water at 5°C, 20 ml each, using a vortex mixer
5. Tubes were centrifuged for 20 minutes at 4°C and 11,000 rpm
6. Supernatant fluids were discarded
7. Washing procedure was repeated twice more
8. Solids were resuspended with 1 M NaCL solution, 20 cm^3 for each tube using a vortex mixer
9. They were centrifuged at 11,000 rpm and 4°C during 20 minutes
10. Fluids were collected in a conical flask and defined as ionically bound peroxidase
11. Solids were washed as described before B.4-B.7
12. Solids were resuspended with 0.1 M phosphate buffer solution and properly mixed
13. Centrifugation was carried out as before
14. Fluids were collected for assay and defined as covalently bound peroxidase
15. Sample crude extracts, soluble, ionically and covalently bound fractions were stored at -18°C before enzimatic assay

C. Enzymatic Assay

1. Reaction mixture was prepared in a test tube containing 2.6 cm^3 of 0.1 M phosphate buffer and 0.1 cm^3 of 0.8% of H_2O_2 solution

2. All solutions were kept in a water bath at 20°C or 37°C before measurement
3. 0.2 cm^3 of enzyme extract were added to the tube with an automatic pipetting device
4. The reaction was started by the addition of 0.1 cm^3 of 1% o-dianisidine solution

D. Measurement

1. A SP 1800 ultraviolet spectrophotometer with a Unicam AR 25 linear recorder incorporated was used. It was set to zero at 460 nm using distilled water as reference solution
2. The initial rate of enzyme reaction was measured by transferring the reaction solution into a quartz cuvette and
3, Absorbance was recorded during the 30 seconds immediately after the addition of o-dianisidine solution. Enzymatic activity was measured as absorbance development per unit of time at 460 nm

For the blanched samples it was necessary to dilute the assay solutions before measurements.

Cuvettes were washed in between readings with distilled water and acetone.

2.7 Crude Protein Determination

Protein content was determined using the Macro Kjeldahl method . The product was carefully weighed on filter paper and digested with concentrated sulphuric acid using copper sulphate as catalyst to convert organic nitrogen to ammonium ions. Sodium hydroxide was added and the liberated amonic was distilled into an excess of 4% boric acid solution. The distillate was titrated with standard hydrochloric acid solution, 0.1 N, to determine the ammonia absorbed in the boric acid.

A four unit Buchi 425 digestor was used to digest the samples for one hour after the sample solution was clear. Each flask was coupled in turn with a single Buchi 315 distillation unit. Distillation was then carried out until 150 cm^3 of distillate were collected. All samples were subjected to this procedure by triplicate and a blank test was conducted for every sample. Nitrogen content for each sample was calculated using the following equation:

$$\% \text{ total nitrogen} = \frac{V_t \times N \times 1.407}{W}$$

where

W = weight of sample digested in grams

V_t = titration value - blank value cubic centimetres

N = normality of hydrochloric acid

The crude protein value was calculated from the total nitrogen value using a factor of 6.25 for brussels sprouts, new

potatoes and pork casserole, while a factor of 6.38 was used for rice pudding.

APPENDIX 3

Additive ingredients used in the various recipes.

Ingredient and Source of Supply	Use
Sodium bicarbonate	Analar grade
BDH Chemicals Ltd., England	Used to stabilize the milk in rice pudding during processing
Calcium chloride	Analar grade
BDH Chemicals Ltd., England	Used to produce calcium pectate bonds in the cell walls, reducing softening during processing
Calcium lactate	Analar grade
BDH Chemicals Ltd., England	Used to produce the same effect as calcium chloride. It was preferred because there had been reports mentioning changes in flavour by calcium chloride in vegetables, particularly for potatoes
Citric acid	Used to prevent browning of vegetables
BDH Chemicals Ltd., England	Analar grade
Saromex garlic powder HF/03950, Bush Boake Allen, London	Flavouring
Saromex onion powder HF/14890, Bush Boake Allen, London	Flavouring

Ingredient and Source of Supply	Use
Ground white pepper Unicol Products, England	Flavouring
Ground celery Schwartz Spices Ltd., England	Flavouring
Purity HPC starch, Laing National Ltd., Manchester	Modified starch. It has been claimed that it increases the rate of heat penetration, relative to conventional starches during heating
Hydrolysed plant protein (HPP) type 245 oil coated, Food Ingredients Specialists (FIS) S.A., London	Suitable for highly processed foods. It enhances overall flavour of seafood, poultry and meat products. Stable at high temperatures
HPP type promac 20, FIS, London	Develops a mild pork flavour when heat is applied
HPP type RF-P oil coated FIS, London	Reproduces flavour and odour of roast pork. It is heat stable and possesses excellent storage stability

APPENDIX 4

PREPARATION AND BLANCHING OF FOOD SAMPLES

It was considered essential to maintain identical compositions for both canned and pouched products. Consequently, all food preparation, blanching and filling conditions were maintained constant in both pouches and cans.

4.1 Brussels Sprouts

Fresh brussels sprouts (*brassica oleracea var gemmifera*) grade one, were cleaned by removing the outer leaves and cutting the stalks before they were size graded to 1.5 to 2.5 cm diameter. This standard size was selected to ensure uniformity during heat treatment.

A solution was prepared containing 0.2% (w/v) of calcium chloride (see Appendix 3) and 2.5% (w/v) of sodium chloride in distilled water. Selected brussels sprouts, in a ratio 75% (w/v), were immersed in this solution for two hours in order to achieve adequate diffusion of solutes into the vegetable. After the two hours soaking, the treated brussels sprouts were taken out from the solution and both solids and brine were saved for blanching and filling procedures. The brine solution was then heated to boiling point for blanching. The brussels sprouts were blanched for three minutes in boiling brine. The vegetables were extracted from the brine immediately after blanching and cooled under running chlorinated cold tap water during 10 minutes. The brine solution was cooled to room temperature and used for filling during packaging when required.

4.2 Jersey new potatoes

A total of 25 Kg of jersey new potatoes (*Solanum tuberosum*) were purchased in the local market. All potatoes were cleaned with cold chlorinated water and size graded to 2.0 - 2.5 cm diameter. Steam was applied to the potato skins and for blanching. The blanched potatoes were peeled by mild hand abrasion to minimize cell damage during this operation, since that could reduce vitamin C content.

Peeled potatoes were steeped for one hour before packaging in a solution containing 0.25% (w/v) of sodium chloride in distilled water. This solution was later used to fill the cans during packaging.

4.3 Pork casserole

The preparation of this type of sample required several raw ingredients which were prepared and treated separately.

Potatoes *(Solanum tuberosum)* A total of 12 Kg of potatoes were peeled in a Hobart abrasive potato peeler using a residence time of 5 minutes. They were cut with a specially modified potato chips cutter to a standard size of 2.54 cm square section and 1.5 cm wide. The potato pieces were then treated for 2 hours by immersion in a solution containing 0.25% (w/v) calcium lactate. No blanching was performed on this material to avoid over processing problems.

Carrots *(Daucus carota)* Five kilogrammes of carrots were hand peeled and cut to standard size slices of 2.0 - 2.5 cm diameter and 1 cm thick, with a specially designed guillotine device.

The carrot slices were then steeped for two hours in 0.25% (w/v) calcium lactate solution. This same solution was used for blanching, which was performed at boiling point during 1 minute. Cooling was carried out under running chlorinated cold water

immediately after blanching.

Celery *(Apium graveolens)* Celery stems were cleaned with cold water and cut to 0.5 cm thick slices with a guillotine device. The slices were then kept in a 1% (w/v) citric acid solution (to avoid browning) until all the celery was prepared and ready for blanching. The same citric acid solution was also used for blanching at boiling point for 2 minutes. After blanching, cooling was carried out with cold water.

Onions *(Allium cepa)* A total of 5 Kg of onions were cleaned by removing the outer leaves and cut into small pieces with a sharp kitchen knife. A 1% (w/v) citric acid solution was used for storing and later blanching the onion pieces at boiling point for 2 minutes. Cooling was performed as for the other samples.

Green Beans *(Phaseolus namus)* Several catering (2.3 Kg/bag) bags of commercial frozen sliced green beans were purchased in a local supermarket. All the packages were opened and mixed in a plastic container, to ensure homogenity, before storage at -20 °C.

Meat. Two whole fresh English pork shoulders were purchased in the local market. They were cleaned by removing bones and most of the fat before they were cut into 1 cm^3 pieces.

Gravy It was prepared using the following ingredients (see Appendix 3):

770 g	tomato puree
32.5 g	HPC starch
25.0 g	fat
150 g	white plain flour
75 g	HPP type RF-P
75 g	salt
45 g	onion powder
30 g	HPP type Promac 20
30 g	white granulated sugar
17 g	colouring
9 g	garlic powder
7.5 g	ground white pepper
5 g	ground celery
6.675 dm^3	distilled water.

All solid ingredients were added into a pan and dispersed in the cold water. The pan was heated with continuous stirring until boiling point and then left to cool before packaging.

4.4 Rice Pudding

Four rice pudding formulations were developed which are described in section 4.5. The formulations selected for production and processing of food samples were Formulation 2 and Formulation 4.

APPENDIX 5

PACKAGING AND FILLING OF FOOD SAMPLES IN BOTH POUCHES AND CANS

The amount of each component in the packages was carefully controlled by weight up to ± 1.0 gramme, or by volume up to ± 2 cm^3. For pork casserole and rice pudding formulations, the volumes or weights used for each package are given in Table A.1. It is important to mention that for pork casserole the gravy was placed into the package first and the solids were carefully immersed intoit to avoid air bubble formation.

Table A.1

Amounts of pork casserole and rice pudding ingredients in A-1 and A-10 size pouches and cans

Ingredients	Containers size A-1	A-10
Pork Casserole		
Gravy	130 g	1300 g
Pork meat	65 g	650 g
Potatoes	55 g	555 g
Carrots	20 g	200 g
Green Beans	25 g	250 g
Celery	10 g	100 g
Onions	10 g	100 g
Rice Pudding		
Formulation 2		
Raw rice	26 g	260 g
Treated milk*	230 g	2300 g
Sugar	13 g	130 g
Formulation 4		
Precooked rice	110 g	1100 g
Treated milk*	170 g	1700 g
Sugar	15 g	150 g

* For treatment of milk see section 4.5 in Rice Pudding Development.

For brussels sprouts and Jersey new potatoes the weights were similar and a record with the weight of each package was kept. This was necessary because the potatoes and brussels sprouts were packaged as whole vegetables, although the brine volume, when used, was the same for all packages. The target weights for each package are given in Table A.2.

Table A.2

Target weights and volumes used for brussels sprouts and Jersey new potatoes packaging

Ingredients	Containers			
	A-1 can	A-1 pouch	A-10 can	A-10 pouch
Brussels sprouts				
Brine	170 cm^3	-	-	-
Brussels sprouts	130 g	130 g	-	-
New potatoes				
Brine	120 cm^3	-	1200 cm^3	-
Potatoes	175 g	175 g	1750 g	1750 g

Sealing for A-1 cans, A-1 pouches and A-10 pouches was carried out under vacuum, while A-10 cans were exhausted at 90 °C before seaming as described in section 3.1.2.

APPENDIX 6

Assessment of Ellab Thermocouple

At the end of this work, it was discovered that the Ellab thermocouple used for temperature monitoring in the rotating canning retort (see section 3.5) was badly constructed.

It was found that the thermocouple sensitive element was not located at the end of the device, furthermore, it was buried in a plastic rod approximately 3 mm deep.

As a result of these findings an evaluation of the error caused in the temperature measurement and sterilization evaluation (F_o-value) was undertaken.

Experimental procedure

Since the thermocouple was used for temperature monitoring during the sterilization process of the rice pudding samples, it was decided to test the thermocouple under the same conditions. Consequently three cans with homogenized rice pudding were prepared and three thermocouples were inserted, i.e., two twisted wire copper constantant thermocouples (section 2.3) and the Ellab thermocouple. They were tied together and positioned in the geometrical center of the can. The response of the three thermocouples was recorded using a highly sensitive temperature measuring system. This system was implemented by P. Lappo, which consisted of a high impedance potentiometer interfaced to a micro computer for accuracy and precision. The temperature readings were taken every 20 seconds.

Results

The Ellab thermocouple always presented a slower response

than the twisted wire thermocouples as shown in Figure 1. The temperature reading was always lower. To evaluate the effect of this variation on the F_o-value of the process the lethal rate integration was carried out using both temperature time curves, i.e., the Ellab thermocouple curve and the twisted wire thermocouple curves. The results are presented in Table 1.

	Ellab thermocouple	twisted wire thermocouple	% agreement
1	21.45	27.23	78.92
2	6.11	8.00	76.34
3	5.45	7.27	74.88
4	5.30	7.29	72.70
5	4.74	6.57	72.05
			74.98

Table 1: F -value variation caused by two different thermocouple measuring systems.

From these results it can be seen that the Ellab thermocouple underestimated the sterilization effect by approximately 25.0%. Also some thermal arrests with melting ice were carried out using both thermocouples which gave the same reading. No thermal arrest with urea were carried out to prevent possible damage of the Ellab thermocouple.

These results show that the process control carried out with the Ellab thermocouple resulted in the overprocessing of the canned rice pudding samples. So it was considered that any comparison between pouched and canned samples was of limited significance.

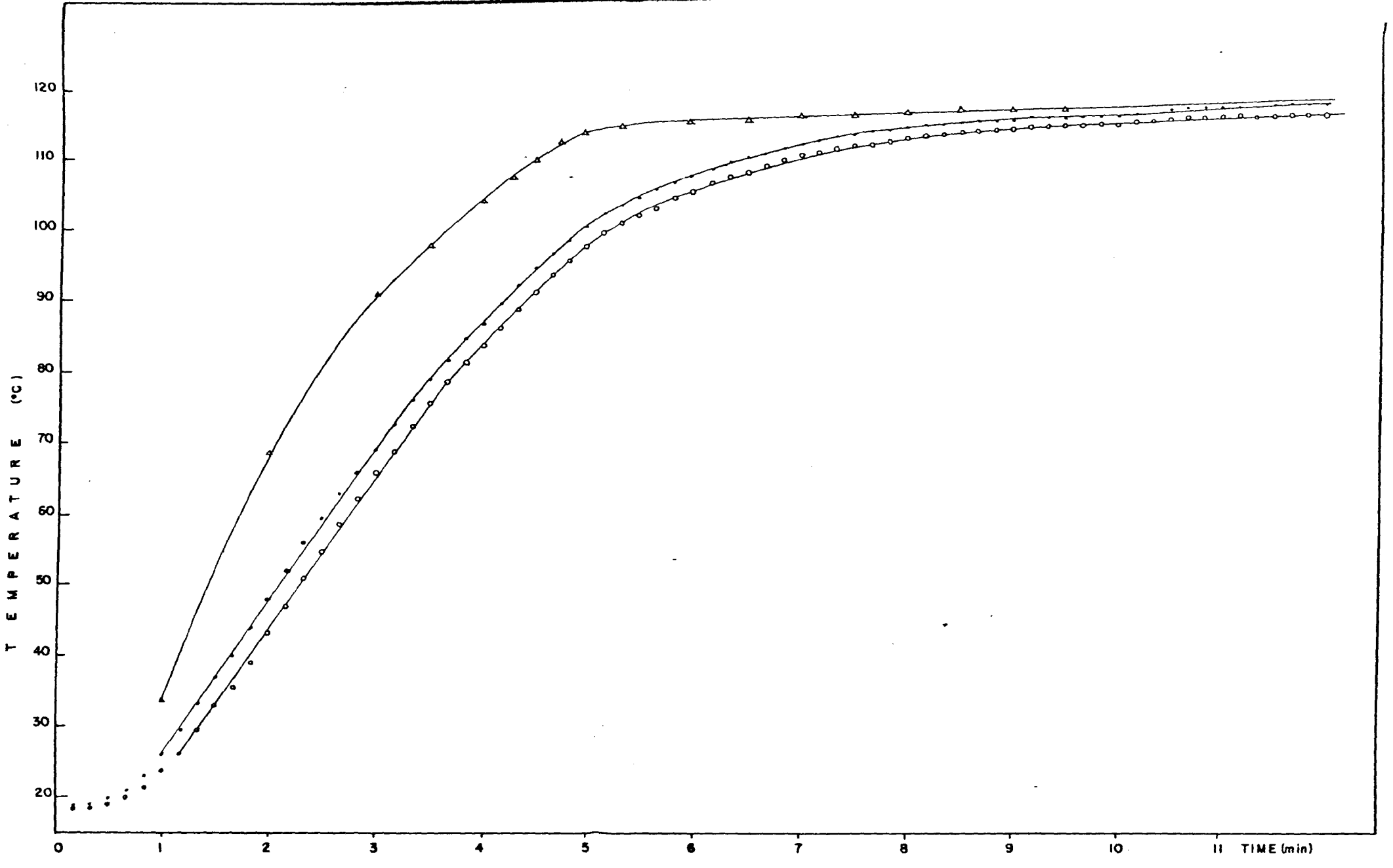

TEMPERATURE (°C)
120
110
100
90
80
70
60
50
40
30
20
0
1
2
3
4
5
6
7
8
9
10
11
TIME (min)

FIG. 1

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