# Colloidal Properties of Sugar Particle Dispersions in Food Oils with Relevance to Chocolate Processing

By
Hélène Babin

Submitted in accordance with the requirements for the degree of
Doctor of Philosophy

The University of Leeds
Procter Department of Food Science
March 2005

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

This copy has been supplied on the understanding that is copyright material and that no quotation from the thesis may be published without proper acknowledgment.

Everyday you make progress. Every step
may be fruitful. Yet there will stretch out
before you an ever-improving path. You know
you will never get to the end of the journey.
But this, so far from discouraging, only adds to
the joy and glory of the climb.

Sir Winston Churchill (1875 – 1965)

# *Acknowledgements*

I take this opportunity to extend my gratitude to many people, all of them of whom have helped me in various ways to achieve this goal:

I am indebted to Prof. E. Dickinson for selecting me in this project, for his invaluable supervision, his precious advices, and suggestions during this PhD; and for the provision of research facilities in the Procter Department of Food Science.

I am grateful to the BBSRC who allows me to work on this project and in the University of Leeds.

I am grateful to Nestlé Ltd UK, and particularly to Dr Steve Beckett and Dr Helen Chisholm for allowing me to be part of this partnership and to realise some investigations for their company. I would like to thank Angel Manez and Kevin Carr for their directives and commitments during the industrial scale applications.

I am grateful to Nestlé Research Centre and particularly Dr Heribert Watzke and his lipid team directed by Dr Philippe Rousset with Veronique Mooser for their support and allowing me to work and use their laboratory facilities.

I would like to thank the permanent staff of the Procter Department of Food Science for their invaluable support over the last three years, and specifically, Catherine Roberts, Nicola Bhuller, Dave & Betty Borrill, Ian Hardy, Paul Kajda, Carl Whitaker, Rammile Ettelaie, Mike Morgan, Bronek Wezicha.

I am grateful to my friends who have been walking beside me over the years for their invaluable support they have given to me, and particularly, Alice Foot, Leyla Villette, Marie-Helene Le-Breton, Maria Herrero Eggart, Sandrine Lhoste, Martina Roick, Jude Haigh, Sarah Austick, Katie Betts, Lindsay Bullivant, Sofie

Yag, Angela Routledge, Paola Sanna, Karine Parenteau, Valérie Parenteau, Frederic Vergnoux Bianucci, Heidi Roche, Fanny Boumekla, Arwa Mustafa, Nicole Schweizer, Emma Parkinson, Yoko Shishikura, Zoe Stec, Lisa Lang, Ka Lau, Janine Wagner, Laureline Jourdain, Sandra Mundt, Cathrine Karlsson, Diane Freeman, Susan Servais, Sebastien Populaire, Julien Vallet, Jerome Elhaik, Olivier Dubert, Terry Kee, Ben Scott, Ben Jost, Jason Rawding, Richard Rymer, Dario Santorelli, Aristomenis Maganas, Roberto Castro Sanudo, Chris Fowler, Rakesh Modi, Richard Rymer, Jon james, Joseph Rothwell, Luis Pugnaloni, Rassoul Kadkhodaee, Stefan Meyer, Thomas Kostaskis, Thomas Moschakis, Dimitrios Kriebardis, Tristan Dew, Yichao Xu, Stewart Radford, Frank Podd, Sven Kerstens, Nick Smith, James Woffenden, Eren Kotan, Nick Bletzer, Wayne Medhurst, Ozlem & Hubert Chazelle, Tim Nash, Cecile & Philippe Chevalier, Alexandre Chauvet & Karine Guion, Virginie & Laurent Fortin, Laurence & Jean Marc, Cyril & Veronique Morel, and many others…

I wish to make a special mention of the precious help, understanding and strength from Virginie Couton, Fiona Thorley, Blandine Clique, Peggy Courtois, Becky Clarke, Marcela Krcmova, Ally Johnston, and also from my Family they have brought to me during difficult times.

Finally I would like to thank my Sister, Albane, my Brother, Aymeric, and my Mum, Monique, for their support, for their unwavering faith in my work and for their strength in life which they will always bring to me.

# *Abstract*

The influence of oil-soluble surface-active components on the interactions between fine sugar particles dispersed in different triglyceride oils has been investigated at a range of volume fractions. Four techniques were applied: rheology, sedimentation, light microscopy and adsorption. Eight different types of food oil were used: sunflower oil, soybean oil, milk fat, lauric fat (palm kernel oil), cocoa butter, pure stearin, pure olein, and a combined fats mixture. Low concentration additions of the emulsifiers, soya lecithin (phosphatidylcholine), polyglycerol polyricinoleate (PGPR) or monoolein, were used to alter the interactions between the hydrophilic sugar particles.

In a dispersion of 70 wt % sugar and 0.3 % of lecithin, approximately 30–40 % of the emulsifier present was found to be adsorbed at the surface of the sugar particles.

The viscosities of the dispersions in the different oils were found to be similar up to 30 wt % sugar content. At high sugar contents (30–60 wt %), however, differences between the oil samples were evident. Lauric fat generated the highest viscosity amongst the different oils whereas cocoa butter exhibited the lowest. The presence of the emulsifier lowered the viscosity of the dispersions to differing extents. The maximum effect was achieved in presence of soya lecithin.

Gravity settling observations were made for dispersions of 10 wt % sugar particles in the different oils in the presence and absence of emulsifier. The emulsifiers were effective in reducing the volume of sediment and hence in increasing the sediment particle packing density. This implies that the adsorbed surface-active species reduce the strength of the attractive interactions between the sedimenting sugar particles, in good agreement with the rheological data at high sugar contents.

Confocal laser microscopy was used to image the different mixtures, but it was not possible to quantify the sugar particle interactions with this technique.

# *Table of content*

# *Figures and Tables*

## *List of Figures*

## *List of Tables*

# *Chapter I Background to Chocolate Ingredients and Manufacture*

## *I.1. Aim of the thesis*

This thesis relates the use of palm kernel oil or different oils for the full or partial replacement of cocoa butter. This was originally initiated by the idea that lauric fat and cocoa butter exhibit similar interactions with sugar particles. Therefore we have been investigating the sugar particles' interactions in oil involved in our systems and we have compared them with each other's. We also introduced the addition of surfactants of different origins (e.g., emulsifiers, free fatty acid) in order to check the effect on sugar-oil interactions produced by these components. The comparisons between the different oils were carried out with several physico-chemical techniques in order to probe the colloidal interactions of sugar particles in the different systems: surface adsorption, sedimentation, light microscopy and especially rheology. In addition, the nature of the particle dispersion process has been investigated during this project, leading to an improved applicability of laboratory results to factory chocolate, at different levels of interactions, from the most diluted one, via sedimentation, to the highly concentrated one, via rheology.

### *I.1.1. Plan of thesis*

This thesis is constructed as follows:

The first chapter will give a brief introduction on the history of the chocolate followed by a review of the general colloid science used in the present system, and finally further details regarding the oils' properties will be given.

The second chapter will mention all the common ingredients involved in this work.

The third chapter will relate to the interactions encountered between sugar particles in oils at a molecular scale. It will involve the comparison of strength and surface adsorption of surfactants and minor oil components within oils at different sugar concentrations.

The fourth chapter will investigate the interaction of sugar particles at aggregate levels. Results based on two separate techniques, via sedimentation and confocal microscopy, and involving two different sugar concentrations will be disclosed.

The fifth chapter will discuss the interactions encountered at highly concentrated sugar in oil mixtures and will study these interactions via rheology technique. In order to achieve closer samples to manufacturing ones, evolution of the process occurred and interactions between sugar particles and oils have been compared between the two processes.

The sixth chapter will discuss the results obtained with the different techniques and will develop similarities and correlations between the techniques involved.

Finally, our laboratory experiments, leading us to testify our hypotheses relating the strength of interactions of sugar particles in oils and the chemistry of the main ingredients at a chocolate trial scale. At this stage, experiments have been realised at pilot plant scale.

This chapter discusses the origins of chocolate and the different ingredients and processes involved for its production and manufacture. Then reviews based on the general colloid science background of the project concerned with the rheology and stability of dispersed systems, and the relationship of colloidal interactions to dispersion properties will be developed. Secondly, we will investigate the chemical interactions of a model designed with the major ingredients of chocolate: sugar, oil and emulsifier. Thirdly, we will infer the comparison of physical interactions between sugar particles in oil systems by means of different sugar concentrations. Then, as the process has been further developed, we will check the veracity of the results obtained with the first mixing method with the results obtained with the improved dispersing method and thus will be based on the strength of sugar particles interactions. Finally, we will discuss the importance of ingredients chemistry as well as their addition order for controlling the dispersion of sugar particles within chocolate.

## *I.2. Brief history of chocolate*

Chocolate is a well-known marketed food product of the 20th century, but throughout its history, it has undergone to various changes in processing and flavour, in order to enhance its popularity amongst the different civilisations. Coe (1996) explained that chocolate originated in approximately 600 AD from the Maya civilisation in Southern and Central America, and it was first produced mainly in Mexico and Peru. Cocoa beans were taken from Theobrama cocoa trees, collected, and roasted. Once dried, these beans were reduced into powder. Chocolate was mainly consumed as a drink when mixed with cold water.

Coe (1996) emphasized that the European arrivals in the New World, including Christopher Columbus and Herman Cortès were not too impressed with the Mayan beverage, and they accommodated it to their own taste by adding some honey to it. Nevertheless, these conquistadors brought back some cocoa beans to Spain, and familiarity with chocolate drinks was gradually extended throughout Europe over the centuries.

Ayral (2001) said that cocoa beans generally contain up to 53 % fat, and the major problem encountered with drinking chocolate was the fact that, once cold, phase separation between fat and water would occur, leading to fat crystallisation of cocoa butter at the top of the pot and particles of chocolate powder dropping to the bottom of the pot. In order to overcome this problem, Van Houten (1828) developed a hydraulic press based on the process of boiling and skimming the beans which would remove most of the cocoa butter. With this technique, Van Houten managed to reduce the fat content down to 27 – 28 %. Moreover, by treating the powder with alkaline salts (e.g. sodium carbonate), chocolate mix was more dispersable in water and taste was improved (Dutching process).

As cocoa butter was expelled from the hydraulic press, Fry (1847) had the idea of blending cocoa powder and sugar with melted cocoa butter instead of warm water. To these crumbs was added some additional fat to form the first chocolate bar. Meanwhile in Switzerland, Sanders (1827) combined the hot chocolate liquor with hot milk to create the first drinkable milk chocolate. By using his process and the blending process of Fry, Nestlé and Peter (1879) created the first milk chocolate bar. Their process involved drying out the moisture in the mixture and replacing it with chocolate. The chocolate bar was then poured into a mould.

Further improvements were made to this chocolate bar in order to extend its shelf life (longer than 3 months) and its quality. Lindt, in 1879, invented the conching process which pushed the warm chocolate backwards and forwards for a certain length of time. This process led to a glossy appearance on the material surface and a tastier chocolate. Along the centuries, three main and different types of chocolates involving different processes and ingredients were created: dried chocolate also known as cocoa powder, dark chocolate and milk chocolate.

## *I.3. Chocolate ingredients*

The basic composition of chocolate has not greatly changed over recent centuries. Three main ingredients are required: firstly, cocoa beans, the seeds of the cocoa tree, are essential for providing cocoa butter and cocoa liquor, requested ingredients for chocolate making; secondly, sugar and its derivatives are required for the solid content and the sweetness; finally, milk and its components are required for the making of milk chocolate.

### *I.3.1. Cocoa*

The production of cocoa beans is mainly carried out in tropical and sub-tropical areas. Minifie (1989) has defined cocoa beans as the seed of the cocoa tree (*Theobroma cacao*, Linnaeus). He explains that cocoa beans refer to the whole seed, which has been fermented and dried. The proper climate is necessary for full maturation of the cocoa pods, nuts of the cocoa trees. These cocoa pods contain the cocoa beans required for the chocolate preparation. The relatively high humidity and high temperatures (> 25 °C) throughout the year required for a good chocolate are found in Western Africa, South East Asia and Latin America. These continents provide the sole production of cocoa beans to the world.

The Centre for International Cooperational and Agricultural Research (CIRAD) has estimated cocoa bean turnover to be £1.65 billion in 1997. The total annual production of cocoa beans has been estimated at 3 million tonnes. Figure 1 represents the global production of cocoa beans for 1997-98.

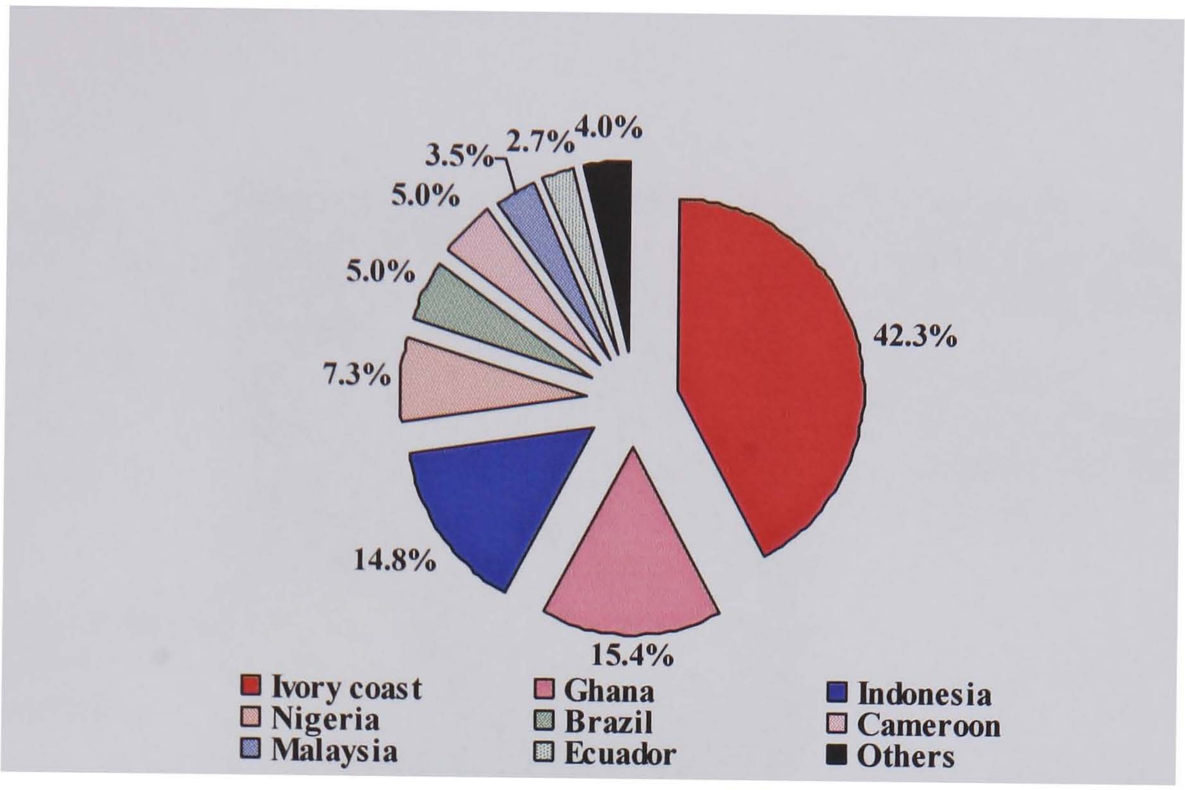


**Figure 1.1:** Share of market of cocoa beans according to country in 1997–1998. (Source: CIRAD, Centre For International Cooperational and Agricultural Research).

Figure 1.1 shows that Western Africa represent the main producers of cocoa beans (70 %) followed by the South East Asian countries (18.3 %) and the Latin American countries (7.7 %). Differences in quality of the crops are observed between the continents. The South East Asian countries, which started their production in the 1980's, have a relatively low quality of beans; on the other hand, Brazil which has reduced its production over the last 30 years, accounts for beans of the finest quality. West Africa aims for mass production, and it remains the primary supplier of cocoa beans to Europe.

The production site of the cocoa beans is fairly important, as it affects the flavour of the chocolate and its fat composition. Three characteristic cocoa bean varieties exist (Criollo, Forastero and Trinitario) and they grow in different parts of the world with their own morphological properties.

Approximately 40 cocoa beans are enclosed in pod (see Figure 1.2) and it requires up to 6 months to obtain a mature pod. Cocoa pods are generally 20 cm long and 15 cm wide. Once the pods are opened and the beans removed from their mucilage (white cover), they contain about 55 % fat, and 45% moisture. In order to use these beans for the manufacturing of chocolate, several process treatments are required.

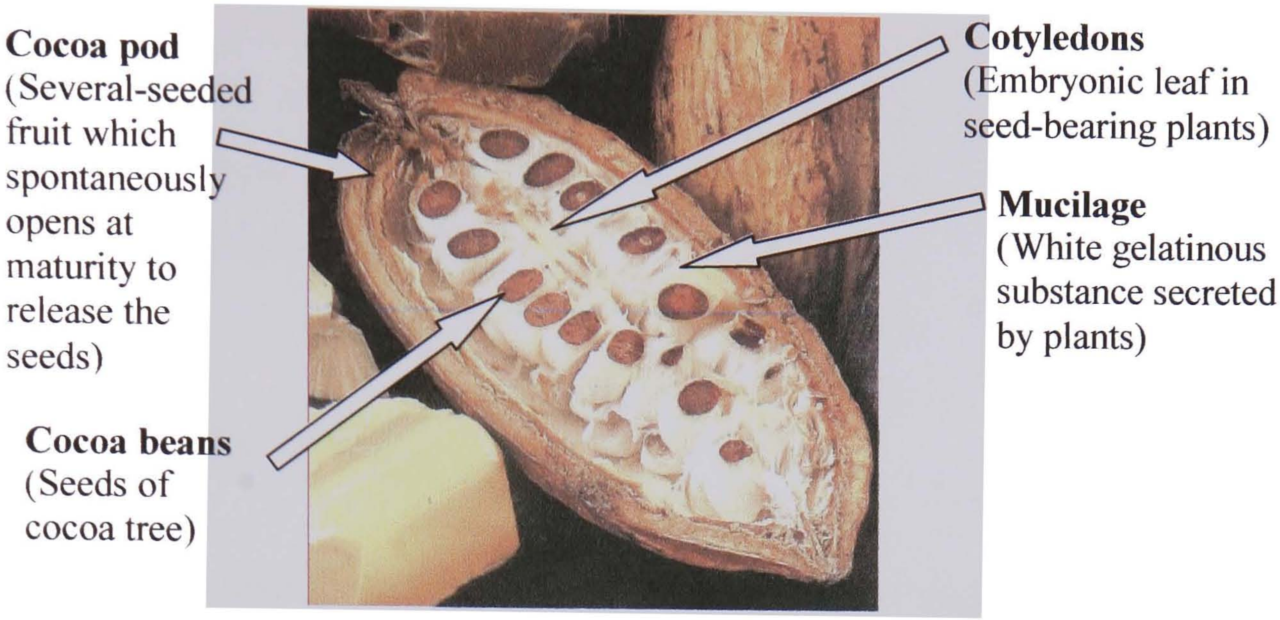


**Figure 1.2:** A pod with cocoa beans recovered by mucilage (from Ayral, 2001).

The most important factor which differentiates a raw cocoa bean from a bean ready for chocolate manufacturing is the level of moisture. In order to reduce the moisture content, two techniques need to be carried out before the transport of the cocoa beans: fermentation and drying. The complex process of fermentation determines the final flavour of the chocolate. Fermentation is carried out via heap or box fermentation techniques. For the heap method, fresh beans (approximately 2500 kg) are piled up and are recovered by banana leaves. On the other hand, box fermentation technique uses perforated wooden boxes which can hold up to 2 tonnes of coca beans. Each operating system has the same objective of ventilating cocoa beans over a week. Beckett (2000) has explained that the smaller the heaps or boxes fermentation are, the better the beans are ventilated and the better are the flavours are produced. More recently, fermentation still remains complex, as different reactions (chemical and enzymatic ones) occur within the bean. On the other hand, the principle of the drying stage is more well-known. Drying avoids the growth of moulds and micro-organisms. Cocoa beans are generally sun-dried for a week, which will allow a reduction of their moisture content to 7–8 %. At this stage, beans will be ready for further processing ( e.g. grinding; milling; roasting) by the chocolate manufacturer leading to the production of cocoa butter, the vegetable fat from the cocoa, and the cocoa liquor/cocoa mass which is the paste produced when cocoa beans are roasted and ground.

After discussing of the most characteristic ingredient for the conception of chocolate, we will bring our attention to sugar. Sugar is the second most important ingredient which has been added to enhance the consumption of the chocolate products.

### *I.3.2. Sugar*

Beckett (2000) has explained that sugar is an important component of the chocolate bar recipe which accounts for more than 50 % in a normal chocolate bar. Several molecular varieties of sugar exist, all of them are designed for different types of chocolate. Amongst these varieties, sucrose and lactose are the predominantly used. Sucrose is a disaccharide and is made of a molecule of glucose linked to a molecule of fructose. Its chemical formula is presented in figure 1.3.

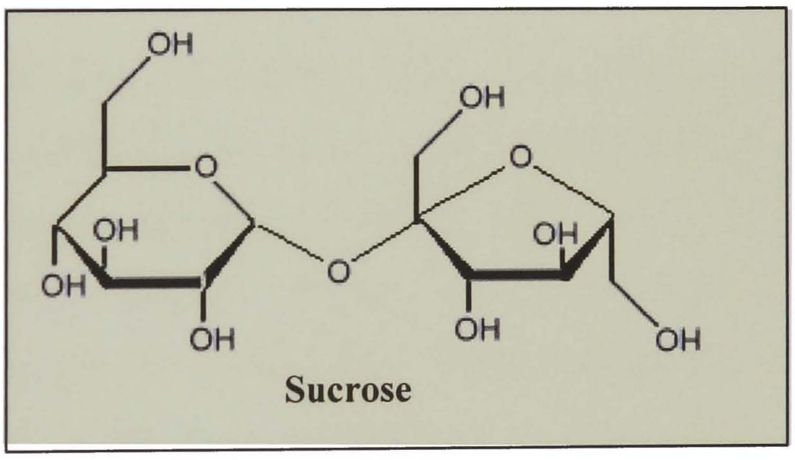


**Figure 1.3:** Haworth's representation of the chemical formula of sucrose.

Beckett (1999) has explained that the particle sizing of the sugar in chocolate confectionery is extremely important as it substantially alters the sensorial qualities of the product. Sucrose which is used in the chocolate confectionery has a particle size dispersion of 0.6 up to 1.0 mm diameter. Within this range of particles, the product is not gritty and feels smoother. In the present study, the sugar particles have been specifically milled to a size of 0.3 mm, allowing us to have a specific surface area of 0.8417 $m^2$ $g^{-1}$. Most of its sugars are in crystalline state rather than glassy state. It has been shown that the physical state of the sucrose interferes with the flavour and the flow properties of liquid chocolate (Beckett, 2000). The surface properties of the sugar particles have shown strong affinity with water molecules. As an example, Dedinaite and Campbell (2000) have observed that water molecules were creating bridges at the surface of sugar particles perturbing the contact between the emulsifier and the sugar. Moreover, in the presence of water, sugar particles tend to stick to each other, creating strong aggregates. Consequently, in this project the sugar has been specifically coated with cocoa butter in order to prevent these sugar aggregations.

Another ingredient which is commonly used in the chocolate confectionery is milk and its derivatives: milk fat and milk proteins. These ingredients have been further developed in the following section.

### *I.3.3. Milk and derivatives*

Beckett (2000) showed that several parts of the milk, milk fat and milk proteins are used at different stages of the production of milk chocolate and milk chocolate bars. Milk fat is essentially made of triglycerides, diglycerides sterols and a small amount of surfactant (lecithin). Table 1.1 compares the amounts of triglycerides and three free fatty acids (stearic, oleic and palmitic) present in three different fats involved in the chocolate production: cocoa butter, palm kernel oil and milk fat.

**Table 1.1:** Percentage of triglycerides and determination of the free fatty acids (stearic (16:0), oleic (18:1) and palmitic (18:0)) in the different fats (from Gunstone & Paddley, 1997; and O'brien, 2004).

| Fat components | Cocoa butter | Milk fat | Palm kernel oil |
|---|---|---|---|
| Triacylglycerols | > 98 % | > 98 % | > 98 % |
| Free fatty acids (16:0) | 25 % | 27 % | 56 % |
| Free fatty acids (18:1) | 36 % | 28.5 % | 32 % |
| Free fatty acids (18:0) | 34 % | 12 % | 2.5 % |

In this table, the three fats are mainly compared through their percentage content of free fatty acids. Cocoa butter contains principally oleic and palmitic acids, whereas milk fat contains mostly stearic and oleic acids. Palm kernel oil contains essentially stearic acid. Beckett (2000) explained that milk fat can be used for all chocolates recipes as it plays an important role as a texture and flavour enhancer in chocolate. It gives a better flow ability to the liquid chocolate as it is liquid at room temperature and gives a sweet flavour to the chocolate bar. Unfortunately, this ingredient is expensive and its usage is highly controlled by manufacturers. To overcome this situation, the ingredients which replace milk fat have generally butter fat origin and may be fractions of it (e.g. stearin and/or olein).

Milk proteins are mainly used in the making of milk chocolate, improving the texture (creamy), the flavour (acidic) and the adding some nutritional values (minerals).

### *I.3.4. Other ingredients in the chocolate recipe*

Over the centuries, the production of chocolate has been developed in terms of the process (e.g., conching, tempering) and also in terms of the recipe (e.g., addition of sugar or milk). As the definition of 'chocolate' was not clearly established at the beginning of its commercialisation, different ingredients have been added to the chocolate, resulting in many variations and adulterations of the product over the centuries. The first two adulterations in 1815, as reported by Coe (1996), involved mixing flour made with potato, lentils, peas or rice to the chocolate (1815). By 1847 the traceability of these unwanted ingredients could be detected in the product with the staining treatment of iodine. Later the same century, cocoa butter was also subjected to adulteration, as this ingredient was by then highly expensive. Coe (1996) and Ayral (2001) explained that cocoa butter, once extracted, was replaced to varying degrees by oils of different origins, such as olive oil, sweet almond oil, egg yolk or suet of veal. Their presence could be detected by the short shelf life of the resulting chocolate and its flavour degradation ('cheesy').

At the beginning of the 21st century, the development of adulteration based on cocoa butter replacement is still important, and several pieces of legislation are being considered to define more precisely chocolate product recipes. Several definitions of cocoa butter replacement such as CBA (Cocoa Butter Alternative), CBE (Cocoa Butter Equivalent), CBS (Cocoa Butter Substitute) have been introduced to the public domain. Lipp and Anklam (1998) gave a full list of cocoa butter alternatives. In this list they noted that most of the cocoa butter alternatives had a lauric acid origin. The required compositional values of cocoa butter (sterols, triglycerides and fatty acids contents) have been stated, and they observed that the best way to control adulteration remains by analytical method such as Gas Chromatography or High Pressure Liquid Chromatography. The latest European legislation produced in 2003, allows confectionery manufacturers to use limited amounts of vegetable fats considered as Cocoa Butter Equivalents (CBE's). Only a maximum of 5 % of these fats, such as palm oil fractions, shea, sal, illipé and kokum can be added to all the different types of chocolate (Yates, 2003). The addition of such ingredients to the chocolate may enhance the product properties by improving the flow properties, the texture and the shelf life of the new material. Economically, he explains that the presence of Cocoa Butter Equivalent reduces the overall cost of the chocolate, as the cocoa bean price is high and it fluctuates greatly. Moreover the quality of the beans has declined over the past years and the supply of such cocoa beans is more expensive than other tropical beans (e.g. varieties of palm oil and coconut oil).

The evolution of chocolate has also been effective with the addition of different ingredients and the constant change of the process. Beckett (1999) explained that in order to design three main types of chocolate (cocoa powder, plain and milk chocolates) the mixing of these ingredients were not sufficient and process greatly contributed to the development and creation of these chocolate products. In the following section, we describe further the process of chocolate making from its original ingredient (cocoa beans) to its final products (cocoa butter and cocoa powder).

## *I.4. From beans to cocoa butter and cocoa powder*

The process of chocolate manufacture has been improved over the centuries, and different types of chocolate can now be reproducibly obtained by following several well-defined stages. The process is mainly concerned with optimising the texture and flavour of the final product. The processing stages involve the use of cocoa beans under various forms (beans, cocoa mass, and cocoa butter) throughout the whole process. Figure 1.4 summarises the different steps under which cocoa beans are processed up to the formation of cocoa butter and cocoa powder.

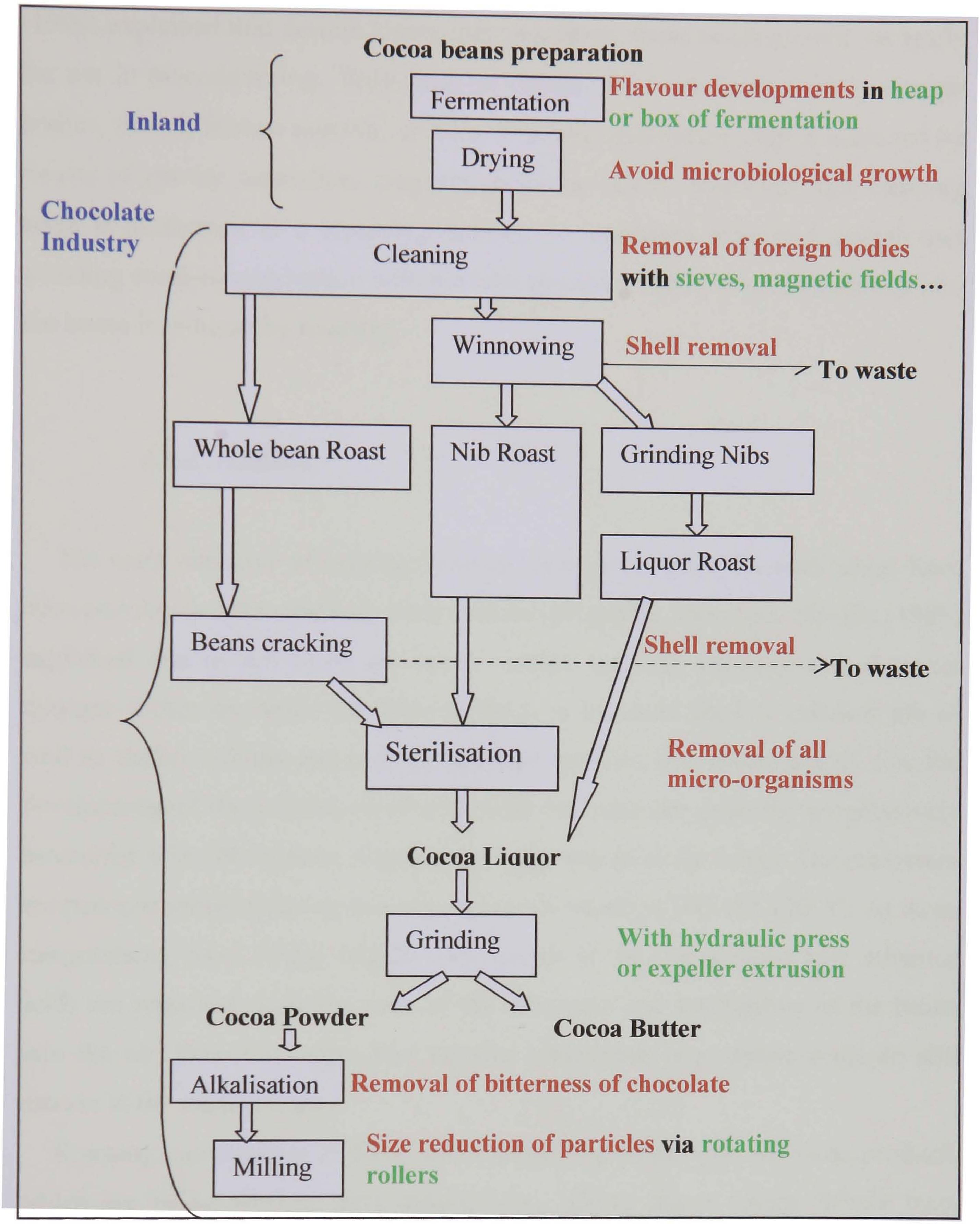


**Figure 1.4:** Diagram representing the manufacture of cocoa powder and cocoa butter

### *1.4.1. Fermentation and Drying*

Firstly, the beans which generally arrive at the chocolate company have been submitted to fermentation and drying processes in their original countries. Beckett

(1999) explained that despite losing their mucilage, these beans are not yet ready for use in manufacturing. They must be cleaned from contamination by foreign bodies, stones, ferrous material or dust. The decontamination step is achieved by means of gravity separation, magnetic fields or sieves. Moreover, this cleaning stage is performed in a separate chamber for restricting microbial growth and avoiding cross-contamination with the later products. Micro-organism growth into the beans is reduced by roasting.

### *1.4.2. Roasting*

The main objective of roasting however, is to develop the flavours which have been previously enhanced with fermentation and drying processes. Minifie (1989) explained that as the beans are being roasted, textural, physical and chemical changes within the beans are observed (e.g., a loosened shell, a softened nib as well as darker colours and a degradation of proteins and amino acids). For the development of the full flavour of chocolate, the beans are generally progressively heated for 45 to 70 minutes, depending on the origins of the beans. The maximum temperature reached during this process varies between 100 and 120 °C. At these temperatures, most of the volatile components of the cocoa beans (e.g. ethanoic acid) are removed releasing most of the bitterness and astringency of the beans into the air. However, some less volatile compounds (e.g. oxalic acid) do still remain in the roasted beans.

Roasting can be done in these ways depending of the type of cocoa products which are being worked with, whole bean, nib or liquor roasts. Whole bean roasting is no longer frequently used due to the migration of fat from the nib into the shell. This fat migration represents a loss of fat out of the nib of approximately 0.5 % fat. This method also requires more energy than the other methods, as in order to roast the nib, energy must be spent to roast the shell first. In the case of nib and liquor roasts, the shell is first removed before roasting. This process, known as **winnowing**, removes the shell from the cotyledons via infra red or saturated steam technique (Beckett (1999)). The shell is separated from the nibs on vibrating sieves and generally disposed to animal feed or used as fertiliser. Beckett (2000) explained that the difference between nib and liquor roasts occurs at the stage of roasting. For nib roast, the nibs are individually roasted whereas as for liquor roast, nibs are finely ground to give cocoa liquor (also known as cocoa mass) prior to being roasted.

Minifie (1989) has stated that after roasting, the level of micro-organisms, even if substantially reduced, still remains potentially harmful, and a final sterilisation step is generally necessary. This is frequently done at the end of the roasting operation by spraying with a solution of an anti-microbial agent. Then nibs are then cooled with sterile air.

### *1.4.3. Grinding*

The next stage is the grinding operation, which is important for the production of cocoa butter. The principal aim is to remove as much fat as possible from the nib, and also to reduce the size of cocoa particles. The nib's structure is made of cellular particles which contains various amount of fat. Figure 1.5 represents the cellular structure of a cocoa nib.

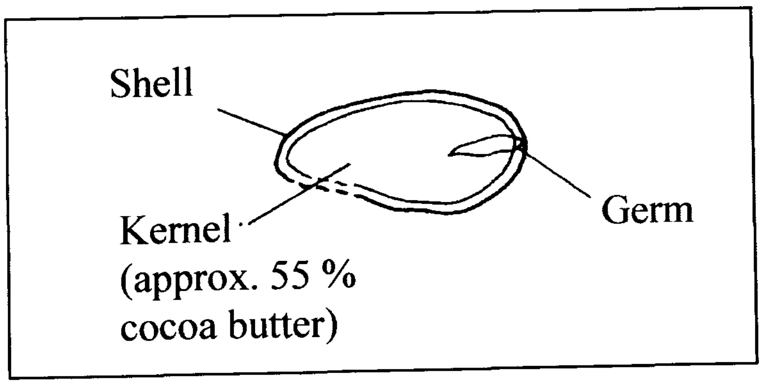


**Figure 1.5:** Cellular structure of a cocoa nib (representation taken from Beckett, 1999).

The process of friction disrupts the cell walls, allowing the release of the solid fat. Grinding should be done under warm conditions, as the fat will melt and the viscosity of the material will be reduced so that less force is required to mill the nibs. Beckett (1999) states that such an operation should be carried out with a low amount of moisture as the presence of water alters the texture of the cocoa mass by making a denser paste. Grinding is often done by impact or disc mills with adjustment of the gap size between the two discs. Figure 1.6 is the schematic representation of grinding mill.

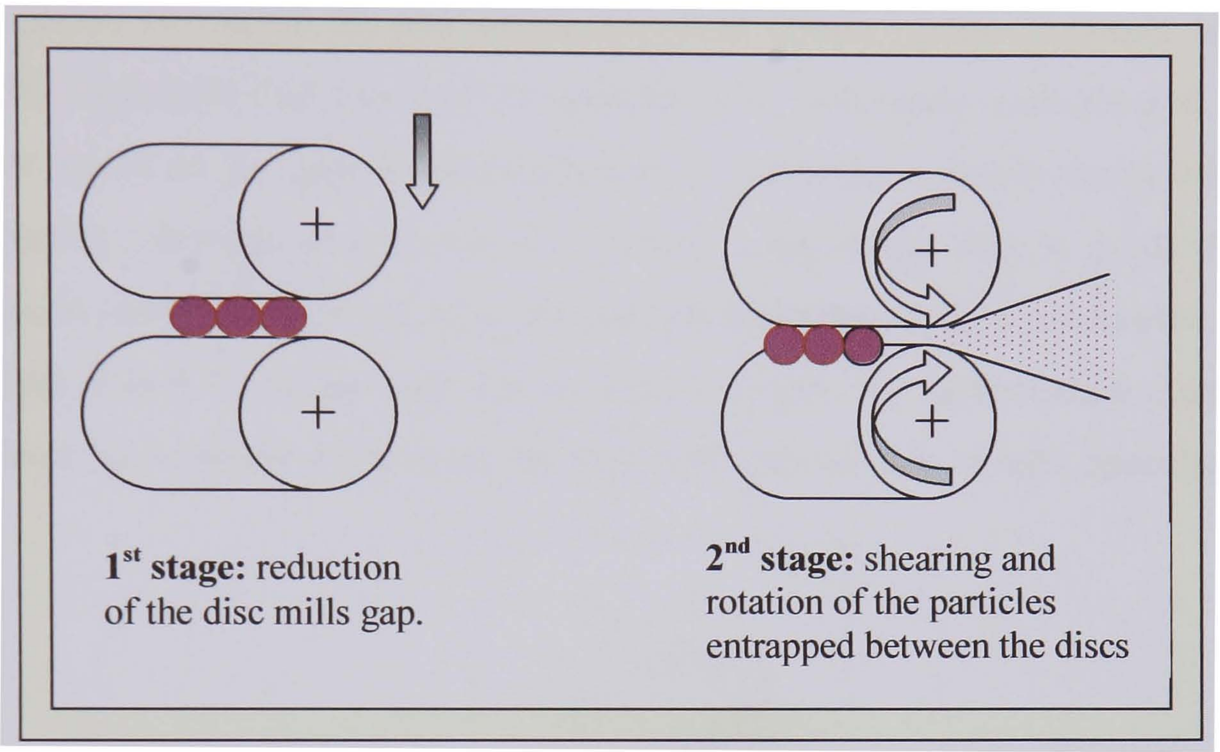


**Figure 1.6:** Grinding mill representation (representation taken from Beckett, 2000)

Grinding represents the last stage of the cocoa bean preparation process. After this operation, the two main constituents of cocoa beans, the cocoa butter and the cocoa powder, are separated for incorporation into different chocolate products.

### *1.4.4. Pressing*

In order to separate cocoa butter from cocoa liquor, a pressing system is further used for the extraction of fat. Beckett (2000) stated that the hydraulic press and expeller extrusion are the two most common ways to extract cocoa butter. In order to obtain the best quality of cocoa butter as well as cocoa powder, a hydraulic press is generally preferred. However, only 55 % of cocoa butter can be extracted with this method and the by-products (e.g. pressed cocoa powder cakes) may contain variable amounts of fat from 8 to 12 % up to 24 %.

Expellers or screw presses are also used for the extraction of cocoa butter, but the principle is no longer to aim for the high quality of the extracted compounds but mainly to aim for the quantity or the yields which can be produced. Minifie (1989) explained that this type of processing is commonly used for low grade cocoa beans or partially fermented beans. As a result, a cloudy cocoa butter is frequently obtained due to the fat mixture composition and it needs further filtration and centrifugation to be purified. On the other hand, pressed cocoa cakes contain 8 to 9 % of fat with this technique. Figure 1.7 represents a picture of purified cocoa butter ready to use for the production of dark or milk chocolate.

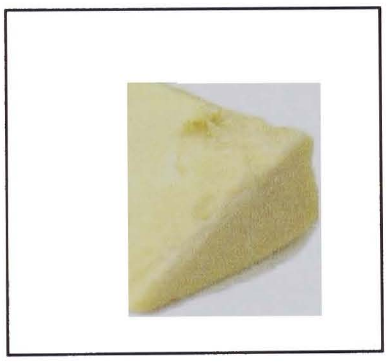


**Figure 1.7:** Picture of solidified and marketed cocoa butter taken from www.beautyofasite.com

The presence of impurities greatly affects the sensorial perception of chocolate, especially flavours. Minifie (1989) explained that if the level of impurity is still high following the two techniques of roasting and grinding, cocoa butter may be deodorised by steam distilling. Such processing will remove part of the strong chocolate flavours which are not appropriate for the production of white chocolate.

The cocoa butter fraction is then integrated at a later stage in the production of chocolate bars.

Pressed cocoa cakes cannot be directly used to make cocoa powder, due to the flavour of the powder which is bitter and astringent and its texture which is dense due to the force applied by the hydraulic presses or expellers (Beckett, 2000).

### *1.4.5. 'Dutching' process*

By 1828, Van Houten had created a process which allowed a better dispersibility of cocoa powder in water or in milk. This process is known as the 'Dutching' process or "alkalisation" due to the presence of alkaline solutions. Currently, this process is extensively used in the cocoa powder production but restricted in chocolate production. The principle is to treat each cocoa component separately: nibs, cocoa powder or the beans, with an alkaline solution of potassium carbonate ($K_2CO_3$) or sodium carbonate ($Na_2CO_3$). The texture of the cocoa powder is not the sole parameter to be affected, as changes in colour and flavour are also noticeable. The change in colour is due to a several chemical reactions during chocolate processing (oxidation, polymerisation and reactions with other chocolate components). It involves the formation of polyhydroxyphenols (tannins) from epicatechin molecules.

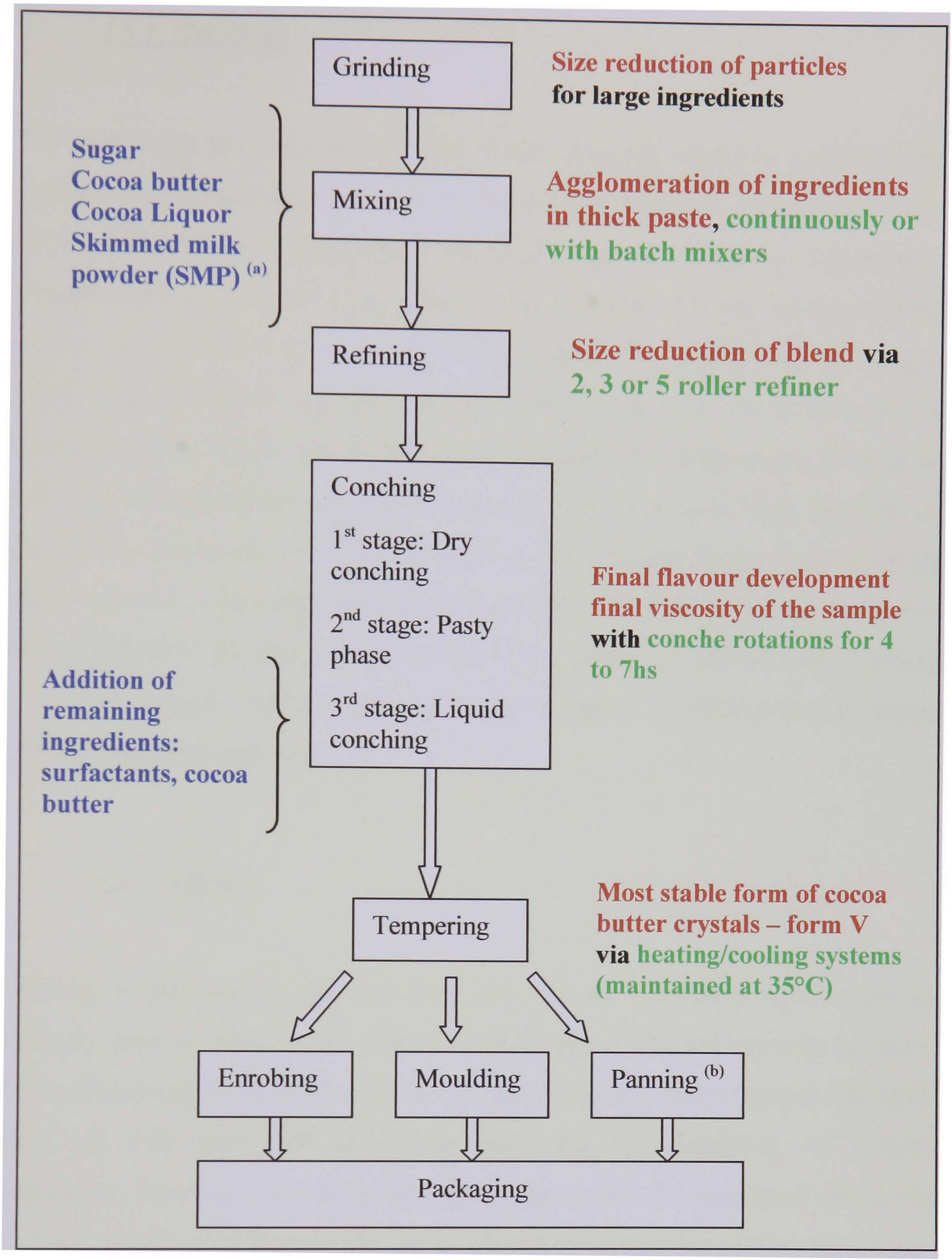
The addition of a small amount of alkaline solution will give a bitterness flavour to the product whereas a high quantity of alkaline solution will give a soapy flavour. As a consequence, the addition of these alkaline solutions is rigorously controlled. In order to regulate the action of the alkaline solutions, some food acids, e.g. tartaric and ethanoic acids, are generally sprayed over beans after the alkalisation process (Minifie, 1989).

### *1.4.6. Milling*

Pressed cocoa cakes need to be further milled to obtain a suitable cocoa powder. This is achieved by using two consecutive milling stages. The first phase breaks the cakes into large agglomerates of approximately 3 cm diameter when passed through rotating rollers. Then, these agglomerates are finely ground by hammer mills, and sieved. Optional ingredients such as sugar or milk may be added during the second milling phase. Finally, the powder is transported through systems of pipes and packaged immediately. This milling process is carried out at a cool temperature, as above 34 °C the cocoa butter will melt, leading to a sticky powder. Moreover, the transport should avoid all contact with moisture as the presence of water will tend to aggregate with sugar particles creating lumps or small aggregates of cocoa powder which may be undesirable for the customer. Two types of cocoa powder are commonly produced: the high fat cocoa powder (from 20 to 25 % fat content) which is mainly used for chocolate drinks and the low fat cocoa powder (from 10 to 12 % fat content) which is commonly used for flavourings and coverings of cakes, ice creams or biscuits.

## *I.5. Process design of chocolate making*

In the manufacture of chocolate on an industrial scale, the common ingredients requested are sugar, cocoa nibs, cocoa liquor, cocoa butter, butter fat, some emulsifiers, and milk powder for the production of milk chocolate. The addition of these ingredients must be rigorously performed and should follow several process stages including grinding, mixing, refining, conching and tempering for the manufacturing of either plain or milk chocolate. The following diagram in Figure 1.8 illustrates the general procedure of chocolate making.



*Notes:* (a) skimmed milk powder is only used in milk chocolate production; (b) chocolate panning means that the chocolate is used as coating for hard centres such as nuts.

**Figure 1.8:** Diagram representing the method of chocolate making.

### *I.5.1. Grinding*

The first stage of chocolate making is the grinding stage, to conserve the smoothness of the chocolate texture and reduce the sensation of grittiness from specific ingredients. Due to the broad size distributions of the various ingredients, the largest particles must have their sizes reduced below 30 µm. Beckett (1999) explained that at this size or below, the perception of grittiness is no longer reported by the consumer. On the other hand, there may be a benefit for specific formulations in the mean particle size of some ingredients being larger, in order to enhance the associated flavours. As an example, the presence of large particles of cocoa nibs in chocolate will tend to leave a strong cocoa flavour and a rough texture, whereas sugar particles confer sweetness to chocolate, and the gritty aspect will tend to disappear once melted in the mouth. A preliminary grinding step can be achieved while mixing, but the effectiveness of milling can be further improved at the refining stage.

### *I.5.2. Mixing*

Mixing is the second process achieved for the chocolate production as previously seen in Figure 1.8. Minifie (1989) stated that mixing can be done either continuously or in batch mixers, but there is a strong preference for batch mixers, as this procedure commonly increases the reliability of system composition. This issue of compositional accuracy plays an important role in the chocolate factory, as it permits the manufacturer to sustain long-term consistency in the texture of the product. The mixing time will be variable depending on the ingredient physical properties, but it must be carried out until a thick paste is obtained. Commonly up to 10–15 minutes of mixing is necessary in large-scale manufacturing. The production of a thick paste is crucial for the rest of the operating procedure. It should possess a rough texture and also have a plastic consistency (Beckett, 1999). The pictures in Figure 1.8 were taken during the trial at the Product Technology Centre of Nestlé in York in May 2004. In the present case, ingredients were mixed in the Crypto, Lodigë Mixer for five minutes twice. The mixture consisted of a granulated thick paste which needed to be further ground in order to obtain a smooth chocolate. The following stage allows a size reduction of particles 25 µm achieved by roller refiners.

**Figure 1.9a**

**Figure 1.9b**

**Figure 1.9a and 1.9b:** Ingredients in the Crypto Lodigë Mixer before and after 10 minutes mixing. Further information on the formula compositions and processing procedures will be given in Chapter VII.

### *I.5.3. Refining*

Refining is an important stage, as it induces the breaking of some aggregates not properly mixed, and the release of some fat for evenly coating the different kinds of particles. This process is done with a series of roller refiners, generally with two rollers and then five rollers. The preliminary stage allows the reduction of the particle size down to 100 µm, but this size is still larger than the one required for chocolate making. Therefore, a second refining stage is used for a reduction of the particles down to 30 µm, which is the optimum size of particles for chocolate making. The principle is to push the cocoa liquor through the rollers with a defined gap. The 5-roller refiner is set up horizontally whereas the 2-roller refiner is set vertically. The first roller is commonly used for feeding, whereas the fifth roller collects the flake by a knife blade.

Each roller is thermostatically controlled via inner cooling/heating systems. The temperature plays an important role; for instance, if the roller is too cold, the fat will crystallise on it. The local pressure and the shearing rate are the two main factors which control the extent of size reduction of the particles. The pressure in the gap is hydraulically controlled, and the shearing action depends on the relative roller speed. Shearing causes the particles to be pulled apart and pushed towards the faster moving roller. By adjusting the gap size, the operator can generate vary the amounts of shear and so different amounts of product pushed towards the faster roller. The average size of the particles obtained in the final stage depends greatly on the gap between rollers, the speed of the refining rollers and the original size of the particles (Minifie, 1989; Beckett, 1999; Beckett, 2000).

In the trial run carried out at the Product Technology Centre of Nestlé in York (May 2004), a 3-roller refiner was used due to the small size of the trial. This refiner was set up vertically, with the first two rollers in the feeding area and the third roller collecting the flakes. Figure 11 shows the product obtained after this 3-roller refining. The feeding was done through the grid above the first two rollers and a powder was obtained after the treatment. In this procedure, the mean particle size was 34 ± 1 μm. Figure 1.10 represents the 3-roller refiner used during the trial.

**Figure 1.10:** Flakes or powdery product obtained after 3-roller refining.

### *I.5.4. Conching*

From the powdery state to the chocolate making, the product needs to follow another stage which will develop the chocolate aroma and will confer the right texture on the final product. The process used in this case is conching which was developed by Lindt in 1878.

The principle of conching is to enhance the flavour and the texture of the powdery state by a series of periodic mixings. Mixing involves a series of forward and backwards movements for a long time. Even if the length of this application has been reduced (96 h originally) due to more powerful preliminary mixing stages, the conching process still remains long (up to 7 h). This extended mixing time is necessary for the development of the final chocolate flavour and the correct viscosity for the final product. During this process, the chocolate texture evolves in three stages: dry conching, the pasty phase, and liquid conching. The aim is to mix the powder for as long as possible in the dry state. Dry conching allows the removal of undesirable volatile compounds, e.g. ethanoic acid, through its powerful mixing. It also permits the disruption of aggregates and the release of fat to coat more of the particles. Moreover, it has been observed that, during the earlier stage, the chocolate paste loses part of its water content thereby contributing greatly to the fluidity of the system. This water release, under vaporised form, is enhanced by the temperature of the conche (70–80 °C). As the cocoa butter melts and water is lost, the chocolate is transformed from a powdery state (or flakes) into a thick paste. The slow motion of the rotary blades allows uniform mixing throughout the paste. Figure 1.11 is the schematic representation of a Frisse conche.

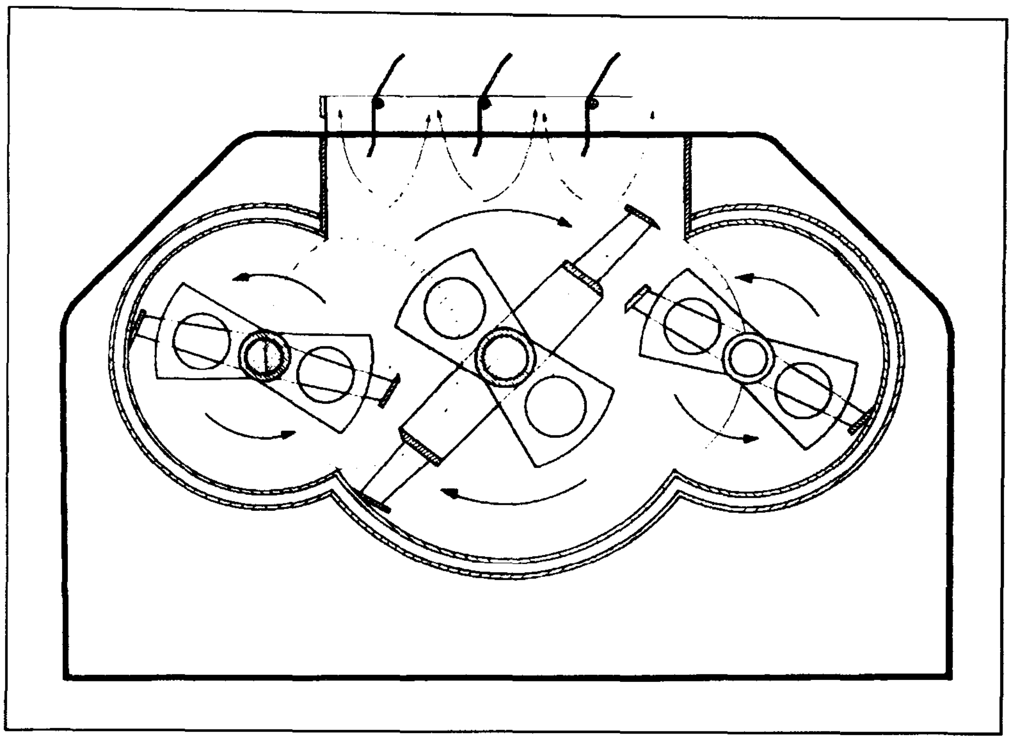


**Figure 1.11:** Schematic representation of a Frisse conche (taken from Beckett, 1999)

In the Figure 1.11, three blades of different sizes overlap each other and allow a continuous movement for mixing the powdery cocoa. Three vents at the top of the apparatus can be observed which will release water vapour and undesirable volatile compounds. The viscosity of the sample gets thinner as more cocoa butter is present in the liquefied state and more water is lost during the process. In the last stage of the conching, the mixing is increased and the remaining ingredients such as cocoa butter and emulsifier are added to the system. These ingredients bring more fluidity to the dispersed system and play an important role in determining the viscosity of the final product. In our pilot plant trial, conching was carried out for 4 h with a conche of capacity about 10 kg at 60 rpm. Figure 1.12 is a picture taken of the conche containing chocolate after a conching time of 4 h.

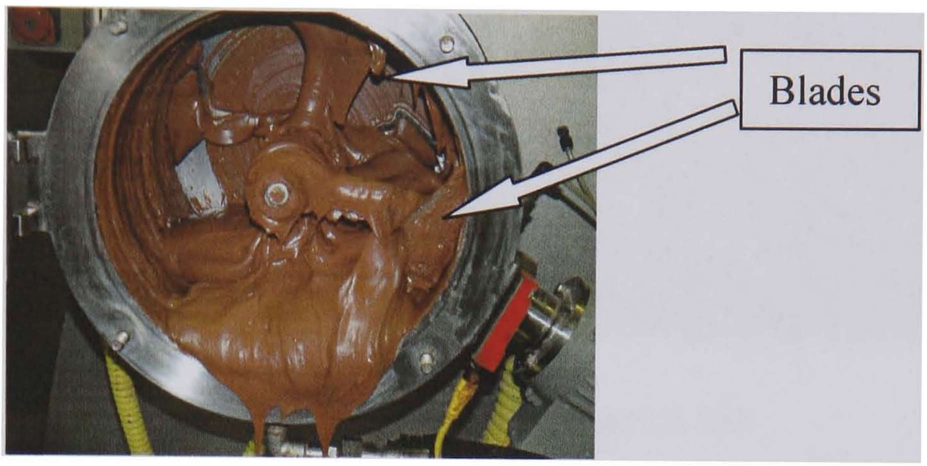


**Figure 1.12:** Picture of the inner view of the conche. Blades are fully coated by chocolate.

At this stage of the process, a few more processing procedures are required before the product can be packaged and sold.

### *I.5.5. Tempering*

Cocoa butter exists in six different crystals states, however only one state, state V, which has the most stable form of crystals, has the full requirements for making a good chocolate. The crystal states of cocoa butter are further explained in chapter section 1.15.3.3.

In order to obtain the right type of cocoa butter crystals, the chocolate needs to be tempered. This means that the chocolate should become crystallised at a temperature below 32 °C. Once seed crystals are formed, chocolate follows a heating and cooling process on tempered tables. This process favours the formation of the crystals in the V form rather than the VI form. In our trial, the chocolate was tempered using a thermostatically heated plate (~ 35 °C) and was further continuously mixed with a scraper to uniform the chocolate crystals. The following pictures in Figures 1.13a and 1.13b represent the movements of chocolate in a thermostatically controlled table while chocolate is tempered.

**Figure 1.13a**

**Figure 1.13b**

**Figure 1.13a & 1.13b:** Pictures of the movements brought to chocolate while tempering.

Chocolate is finally processed while tempered. While it is still warm, melted chocolate flows easily into moulds, or it can be used to enrobe different types of sweets or biscuits. Prior to packaging, the chocolate is cooled down online to approximately 15 °C. Finally the chocolate is stored in chilled area away from direct light exposure. The nutritional value and quality of chocolate may last up to a year, but it is generally recommended to be eaten within a month for best results.

### *I.5.6. Conclusion of Cocoa Historical Approach*

In this chapter, it has been shown that chocolate, which has been consumed for more than five centuries throughout Europe, originates from the South American countries. Throughout these 500 years, chocolate has been submitted to several changes in order to become one of the most popular food products. Consequently, we have seen the additions of sugar as well as milk to chocolate for the consumption of chocolate drinks. The addiction to this product has been enhanced by the constant process development to cocoa beans, including alkalisation and conching stages for the expansion of new chocolate products such as chocolate and milk chocolate bars.

For centuries, the price of cocoa beans has been fluctuating greatly and their quality has varied greatly. In order to overcome this problem, adulteration of the product has been observed since the 18th century. New European legislation allows the usage of Cocoa Butter Equivalent (CBE's) or surfactants (e.g., lecithin) for the partial replacement of cocoa butter.

The next section further develops the chocolate theory under a colloids approach with the interaction of the sugar particles in oil. This development includes the determination of a colloidal model and the different forces applied to this system.

## *I.6. Colloidal Background of Interactions between Particles*

Many authors have attempted to define colloidal systems. Hunter (2001) explains that colloidal systems represent materials in which particles are dispersed through a continuous solvent, such that the dispersed particles are larger than molecules. Walstra (1996) limits the size of colloidal particle to the range from 10 nm to 1 mm, but Hunter (2001) says that the upper limit is not so well-defined and the particle radius may extend beyond 1 mm.

The properties of colloidal systems are dependent on the characteristics on the dispersed phase (gas, liquid or solid) and also on the properties of the continuous phase (gas, liquid or solid). There exist two types of colloidal dispersions: lyophilic and lyophobic ones. This refers to the work of Freundlich (1926) with particles being either *'solvent loving'* or *'solvent fearing'* (Hunter, 2001; Hiemenz, 1986). The nature of the solvent, the continuous phase, mainly determines the characteristic properties of the dispersions. For the particular case of chocolate, Walstra (1996) says that this product is made of sugar and cocoa particle aggregates dispersed in a partly crystallised fat matrix.

The difference between lyophilic and lyophobic colloids lies in the fact that lyophilic dispersions are commonly formed spontaneously. Alternatively lyophobic dispersions are subdivided with two or more phases and format of these dispersions is not spontaneous (Hiemenz, 1986). Hunter (2001) states that lyophilic dispersions are reversible and thermodynamically stable with a reduction in the free energy. However, lyophobic dispersions are irreversible and thermodynamically unstable with an increase in the free energy. Walstra (1996) explains that such dispersions are commonly stabilised by the addition of amphipilic molecules. These molecules possess two defined regions: a hydrophobic part soluble in oil, and a hydrophilic part soluble in water (Hunter, 2001).

Nevertheless, the difference between lyophilic and lyophobic dispersions is not always clear. Walstra (1996) suggests that dispersions are better classified in terms of particle shape and volume fraction. The values of these two parameters influence greatly the physical properties of the dispersions (Goodwin, 2004; Walstra, 1996). There is a strong relationship, for instance, between the viscosity and the volume fraction of particles depending on the nature of the particles, as illustrated in Figure 1.14.

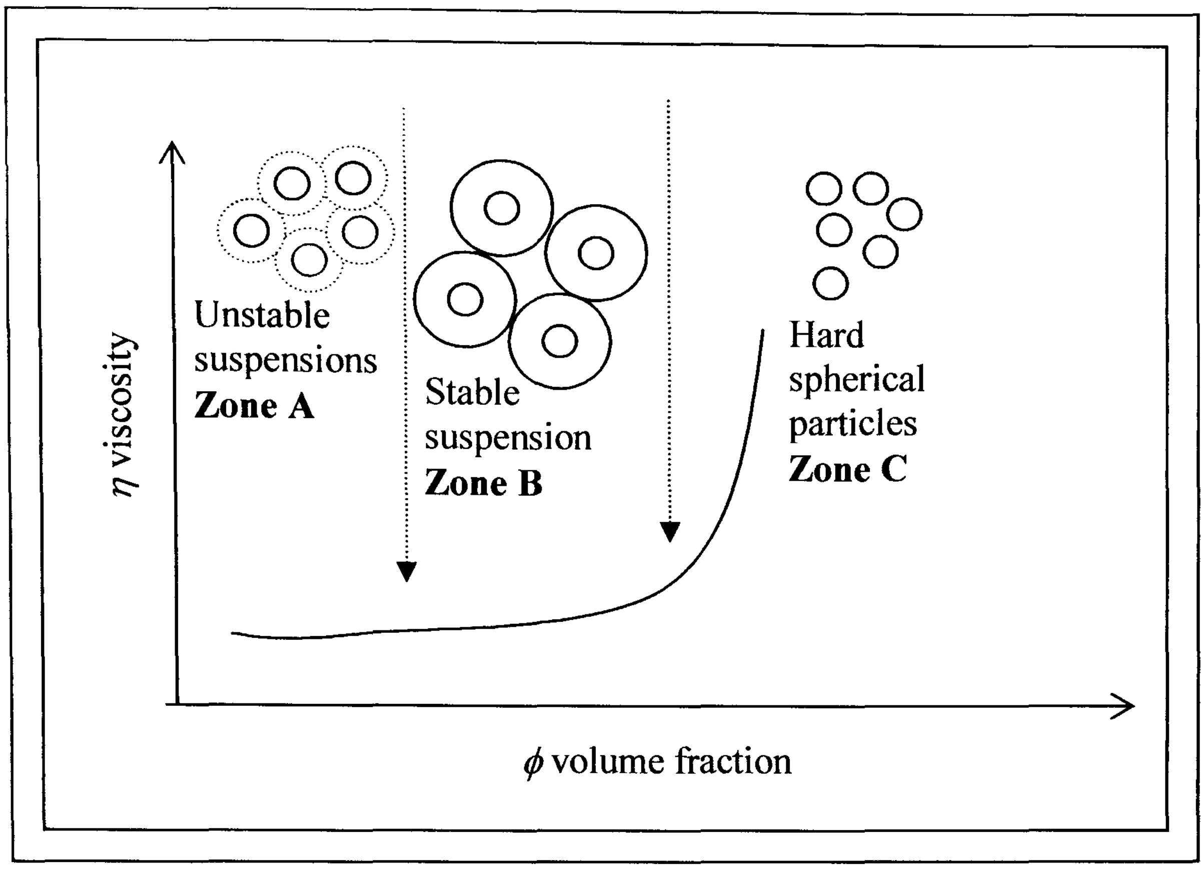


**Figure 1.14:** Correlation between the viscosity ($\eta$) and the volume fraction ($\phi$) of different particles (taken from Goodwin, (2004)).

As shown in Figure 1.14, it can be observed that the particle models used to describe colloidal systems are mainly spherical. Goodwin (2004) explains that in "**Zone A**", the dispersions behave like Newtonian fluids and have a low viscosity. This is generally observed with unstable suspensions such as oil/water mixtures. "**Zone B**" samples exhibit slight viscosities with a shear thinning behaviour. As the volume concentration increases, Goodwin (2004) explains that "**Zone C**" represents the region of complex fluid with the limitation of the Newtonian behaviour. In this region, he specifies by saying that dispersions have weak gel formation. Hunter (2001) explains that in order to understand the theoretical basis of colloidal dispersions, simple geometric shapes are commonly used: spheres or an infinite flat plate. In the particular case of this project, sugar particles will be mainly treated as hard spheres and the volume fraction of spheres will be the main parameter controlling system properties.

Colloidal stability depends on the interactions between the particles. The state dispersion of particles arises from a combination of repulsive and attractive forces (Hiemenz, 1986; Israelachvili, 1992; Hunter, 2001). The effects produced by the interaction of two colloidal particles are commonly defined by their potential energy. Figure 1.15 represents a schematic diagram of the potential energy curves plotted against the distance of separation of these two particles. In Figure 1.15, ***r*** represents the centre-to-centre separation distance for equal spherical particles, and ***s*** is the distance of surface separation for symmetrical spheres.

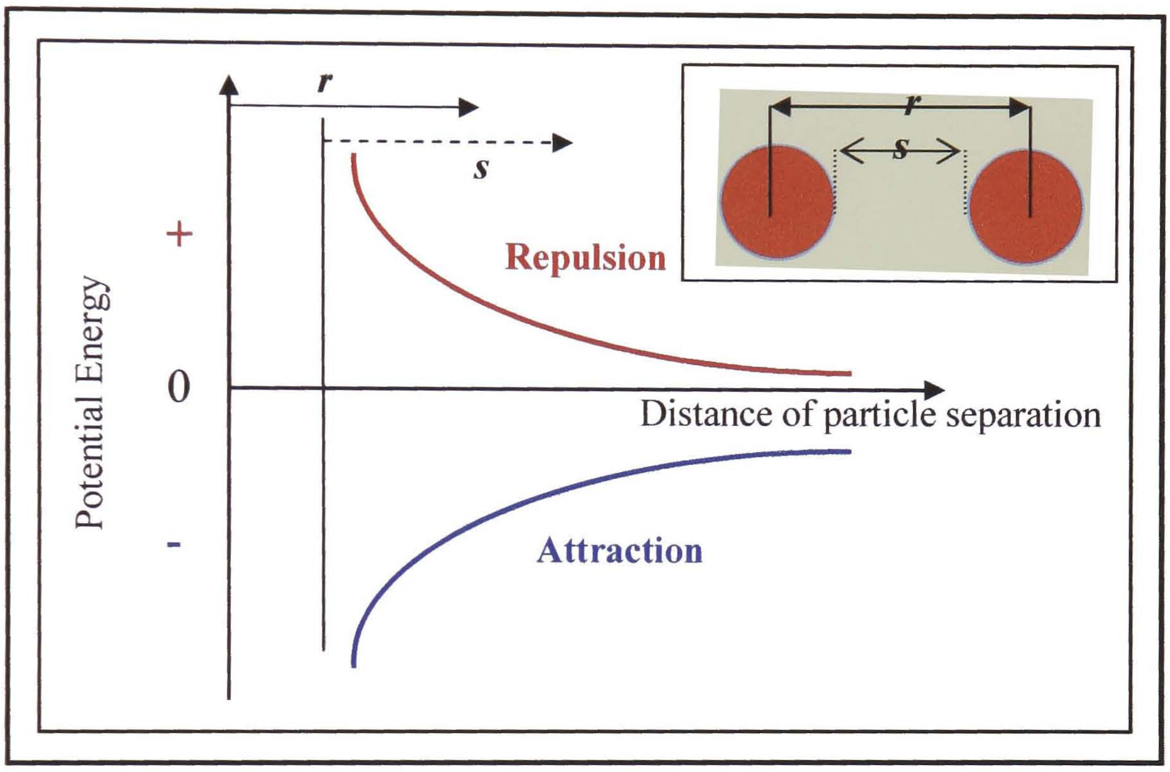


**Figure 1.15:** Potential energy curves for the interaction of two colloidal particles. By convention negative values correspond to attraction and positive values to repulsion. (Figure inspired by Hiemenz P. C., 1986, In *Principles of colloids and surface Chemistry, 2nd edition,* Marcel Dekker, Inc., N.Y).

The colloidal system considered in this project is a suspension system: solid particles dispersed in a continuous liquid oil phase. Based on the apolar solvent characteristics, this dispersion is mainly classified as lyophobic, and it requires amphiphilic molecules, emulsifiers, in order to stabilise the sugar particles in the oil phase. For simplifying the theoretical interpretation, the sugar particles are conveniently considered as hard spheres with a variable volume fraction.

The next section describes the forces involved acting between the sugar particles dispersed in the oil phase.

### *I.6.1. Van der Waals Attraction Forces*

Van der Waals forces were involved to explain the deviations from the ideal gas law. Van der Waals showed a direct link between the strength of the intermolecular attractive forces and equation of state of a condensed system. He defined the parameters by the following equation:

$$\left(P+\frac{n^2a}{V^2}\right)(V-nb)=nRT \qquad \textbf{(1.1)}$$

Here $P$ is the pressure (Pa); $V$ is the volume ($dm^{-3}$); $n$ is the number of moles (mol.); $R$ is the universal gas constant; and $T$ is the temperature (K). The quantity $a$ represents the strength of intermolecular attractive forces (long-range attractive forces), and the quantity $b$ is the finite volume occupied by the gas molecules (short-range repulsive forces).

Further work identified three major different kinds of intermolecular forces (Debye, 1920; Keesom, 1921; London, 1930). These intermolecular interactions are generally described by an inverse power law of the separation distance of particles (represented by $s$ in Figure 1.15). Based on the developments of Debye, Keesom and London on different kinds of intermolecular forces, Hiemenz (1986) describes some similarities between the various kinds of van der Waals forces. All of them possess a sixth power inverse power law dependence. Therefore the dependence of the attractive energy potential can be written as $1/r^6$ with $r$ being the fixed distance of separation between the centres of two identical spheres (Hunter, 2001; Israelachvili, 1992). As these equations have a negative power law factor and consequently a negative potential energy, and all these forces result in net particle attraction (see Figure 1.15).

However these equations report to specific interactions and Hiemenz (1986) explains that permanent dipole-induced dipole interaction is characterised by the Debye equation (induction force); whereas Keesom equation details the interactions of permanent dipole-permanent dipole (orientation); and induced dipole-induced dipole is defined by the London equation (dispersion). He explains that these three equations contribute preferentially to the net van der Waals force between molecules. Hiemenz (1986) specifies that induction and orientation forces, calculated from Debye and Keesom equations, have small percentage contributions on the van der Waals forces (~ 10 % and ~ 20 % respectively) in comparison with the dispersion force from London equation (~ 70 %) and thus depending of the molecule type. As a result, most of the literature relating on the attractive interactions at microscopic level deals only with the London theory (Israelachvili, 1992; Hunter, 2001).

Israelachvili (1992) describes some common features of van der Waals forces. He defined these forces as long-range forces which may have a variable distance from 0.2 nm up to 10 nm. He explained that van der Waals forces may be attractive. Israelachvili (1992) states that dispersions forces, represented by the London equation, may influence the alignment and the orientation of mutual particles as well as attracting these particles together.

Despite being quite useful, the London equation has some limitations such as the effects of the medium on the interaction of particles (Hiemenz, 1986; Israelachvili, 1992; Walstra, 1996; Hunter, 2001). In order to overcome the problem of medium interaction, Mc Lachlan (1963) developed a new equation involving all the van der Waals forces and the interactions in solvent (Israelachvili, 1992). Furthermore, Hunter (2001) discusses the Hamaker theory. In this theory, it is suggested that long range van der Waals forces could explain colloid coagulation by summing pairwise intermolecular forces. However, this theory still remains limited when particles are dispersed into a solvent. This is due to the difficulty to interpret the interactions between particles and with the solvent. Israelachvili (1992) defines the Hamaker constant by the following equation:

$$A = \pi^2 \times C \times \rho_1 \times \rho_2 \tag{1.2}$$

where C is the coefficient in atom-atom pair potential and $\rho_1$ and $\rho_2$ represent the number of atoms per unit volume in two particles.

The Hamaker constant of solid particles has been calculated in organic media (van der Hoeven and Lyklema, 1992; Bergström, 1997; Visser, 1972; Israelachvili, 1992; Hough and White, 1980). Van der Hoeven and Lyklema (1992) summarise the value of the Hamaker constant for salts dispersed in organic solvents of 0.04 × $10^{-20}$ J. They explain that their calculations on Hamaker constant may have significantly different values depending of the type of salt analysed. Walstra (1996) further describes that Hamaker constant was dependent on the material particle and the surrounded fluid. Moreover, this value can determine the force of adhesion and the interaction energy of two identical particles at a macroscopic scale (Israelachvili, 1992). These parameters can be calculated by the following equations.

Adhesion force:

$$F = \frac{A \times R}{12 \times D^2} \tag{1.3}$$

Van der Waals interaction free energy:

$$V_A \approx \frac{-A \times R}{12 \times D} \tag{1.4}$$

Here $F$ represents the adhesion force (N), $A$ is the Hamaker constant (J); $R$ represents the radius of the two particles (m); $D$ is the interparticle distance (m) and $V_A$ is the interaction energy (J) (Walstra, 1996; Israelachvili, 1992).

From these equations, the adhesion force and the van der Waals interaction attraction energy required to pull apart two particles can be estimated. The current sugar particles have a mean diameter size of 36 µm and by varying the interparticle distance of the two particles more knowledge on the surface energy and force can be gained. Table 1.2 shows the value of the adhesion force and the interaction energy, calculated from equations 1.3 and 1.4 and thus at variable interparticle distances.

**Table 1.2:** Calculated estimations of the adhesion force and interaction energy of two sugar particles in organic medium. Their mean radius is equivalent to 18 μm, the Hamaker constant equivalent to 0.04 × $10^{-20}$ J, $1k_BT = 4.114 \times 10^{-21}$ J (for 298 K).

| *D*: interparticle distance (nm) | *F*: adhesion force (N) | $V_A$: interaction energy (J) | Interaction energy ($k_BT$) |
|---|---|---|---|
| 0.25 | $9.6 \times 10^{-9}$ | $-2.4 \times 10^{-18}$ | ~ - 583.4 |
| 0.5 | $2.4 \times 10^{-9}$ | $-1.2 \times 10^{-18}$ | ~ - 291.7 |
| 1 | $6.0 \times 10^{-10}$ | $-6.0 \times 10^{-19}$ | ~ - 145.8 |
| 2.5 | $9.6 \times 10^{-11}$ | $-2.4 \times 10^{-19}$ | ~ - 58.3 |
| 5 | $2.4 \times 10^{-11}$ | $-1.2 \times 10^{-19}$ | ~ - 29.2 |
| 10 | $6.0 \times 10^{-12}$ | $-6.0 \times 10^{-20}$ | ~ - 14.6 |
| 20 | $1.5 \times 10^{-12}$ | $-3.0 \times 10^{-20}$ | ~ - 7.3 |

Table 1.2 shows that the adhesion force and the interaction energy become smaller as the interparticle distance increases, common observations to van der Waals interaction (Hiemenz, 1986; Israelachvili, 1992; Walstra, 1996; Hunter, 2001; Van der Hoeven and Lyklema, 1992).

In order to understand better the interaction of van der Waals forces under the influence of the particle sizing, Figure 1.16 has been designed. It represents the interaction energy versus the interparticle distance of two different particles size of 18 μm and 2.0 μm. The first radius dimension refers to the radius of the sugar particle, and the latter one has been selected from the published work of van der Hoevn and Lyklema (1992).

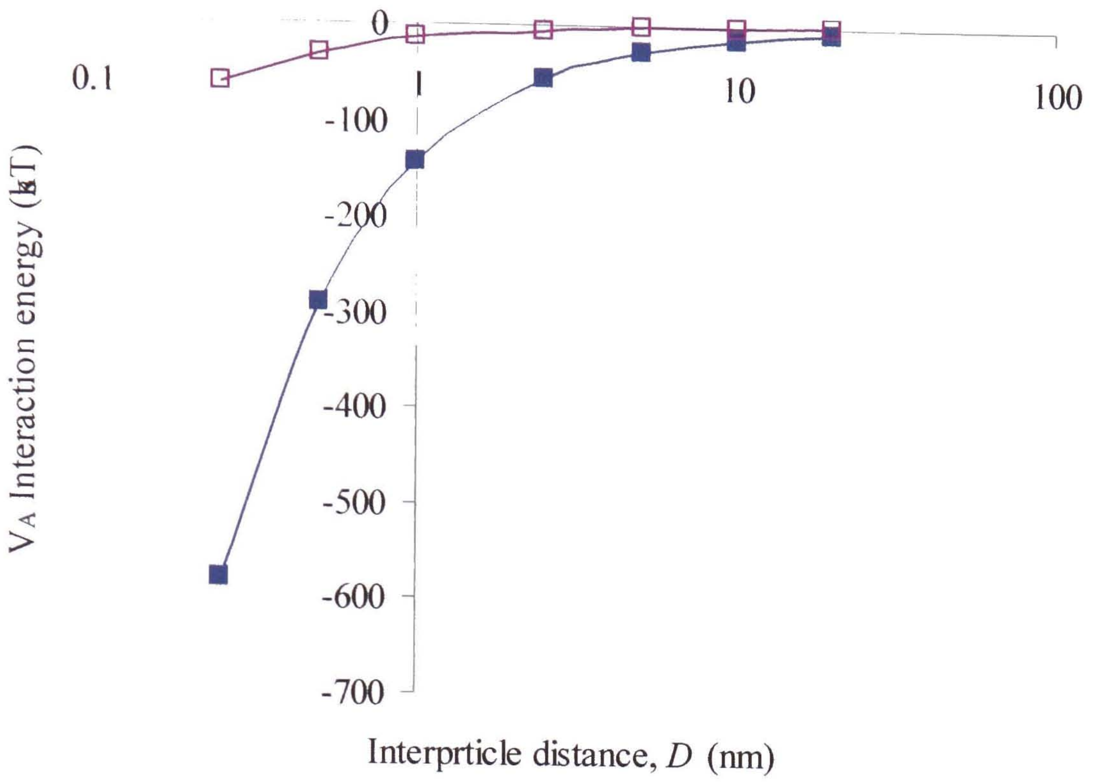


**Figure 1.16:** Interaction energy (VA) versus interparticle distance (D). ■ represents the interaction energy for particles of $r$ = 18μm; □ shows the interaction energy of particle of $\boldsymbol{r}$ = 2.0 μm.

In this figure, the interaction energy of the particles having a mean radius of 2.0 μm have been calculated in the same way as for the particle of 18 μm from the table 1.2. It can be noted that large particle size shows strong attractive interactions, ~ - 292 $k_BT$, at short distance (0.5 μm) in comparison with small particles which generate lower interactions: ~ -32 $k_BT$. However, as the interparticle distance increase up to 10 nm, interaction energy for both sizes of particles is substantially increased. Such features which show that large particle size generates strong attractive interactions (Walstra, 1996). Moreover, Hiemenz (1986) stated that the interaction energy becomes weaker as the interparticle distance becomes greater and thus regarding the shape of the particles.

However due to the limitations and rough estimations of Hamaker constant and its theory, further developments were suggested involving mechanical and quantum field theories like the DLP theory (Dzyaloshinskii, Lifshiftz and Pitaevskii) [Barrera & Duke, 1976]. This theory is based on the quantum field theory which consequently improves the van der Waals theory at a macroscopic level. Moreover, Hiemenz (1986) explains that this theory is applied to different shapes of particles.

At a macroscopic level, properties of van der Waals forces have been further advanced. For instance, Israelachvili (1992) shows that in presence of solvent medium, van der Waals forces have low interaction magnitude in comparison with particles suspended in vacuum. Wastra (1996) mentions that the magnitude of the interaction depends on the material of the particles and the specific interaction between the particles and the solvent. Hiemenz (1986) further explains this feature by saying that the particles' bonds may be broken in the presence of the solvent and new bonds may be formed between the solvent and the particles. Therefore, the aggregation state, the flocculation and the coagulation of particles may be retarded with the action of the solvent.

In conclusion, van der Waals forces originated mainly from the contribution of three forces: induction, orientation and dispersions forces, represented by the Debye, Keesom and London equations. The van der Waals forces depend greatly on the dispersion forces at microscopic scale. These forces are omnipresent in each molecule and atom which presents dipole moments. At a macroscopic scale, van der Waals properties altered as several parameters need to be taken into account: solvent medium, particles size and particles material which may rapidly increase the attractive forces between particles and thus facilitate the separation between particles within the fluid; or, may slowly increase if the attractive forces involve solvent interaction and thus restrict the sedimentation of particles in a fluid.

Nevertheless, if van der Waals forces are omnipresent in common molecules and atoms, several forces may impart on the interaction of particles in different media, either attractive interactions, like van der Waals or repulsive interactions, like steric effect which is important in our system of hard spheres.

LIBRARY LEEDS

### *I.6.2. Steric forces*

Steric repulsion is generally encountered when two flexible molecules or particles tend to overlap each other due to different parameters such as heavy concentrations. Israelachvili (1992) specifies that repulsive force arises from the overlap of the electrons surrounding the atoms. This force determines the distance at which atoms or molecules are getting close to each other. On the other hand, Walstra (1996) explained that steric repulsion occurred when molecules were flexible and extend part of the molecule in the continuous phase. At a macroscopic scale, Hunter (2001) described that adsorbed chains contained in molecules cause repulsion between them. Hiemenz (1986) reported that general polymers, homopolymers or copolymers, are the precursors of such forces. Looking at these definitions, steric repulsion involves different types of particles surface overlap, from the electrons to polymers and to the molecule shape. However all these particles induce a specific colloidal stability (Hiemenz, 1986; Israelachvili, 1992; Hunter, 2001).

Israelachvili (1992) explains that repulsive forces have a very short range and the magnitude of their potential increases rapidly as the molecules are approaching each other. Figure 1.17 describes the potential energy of a repulsive force.

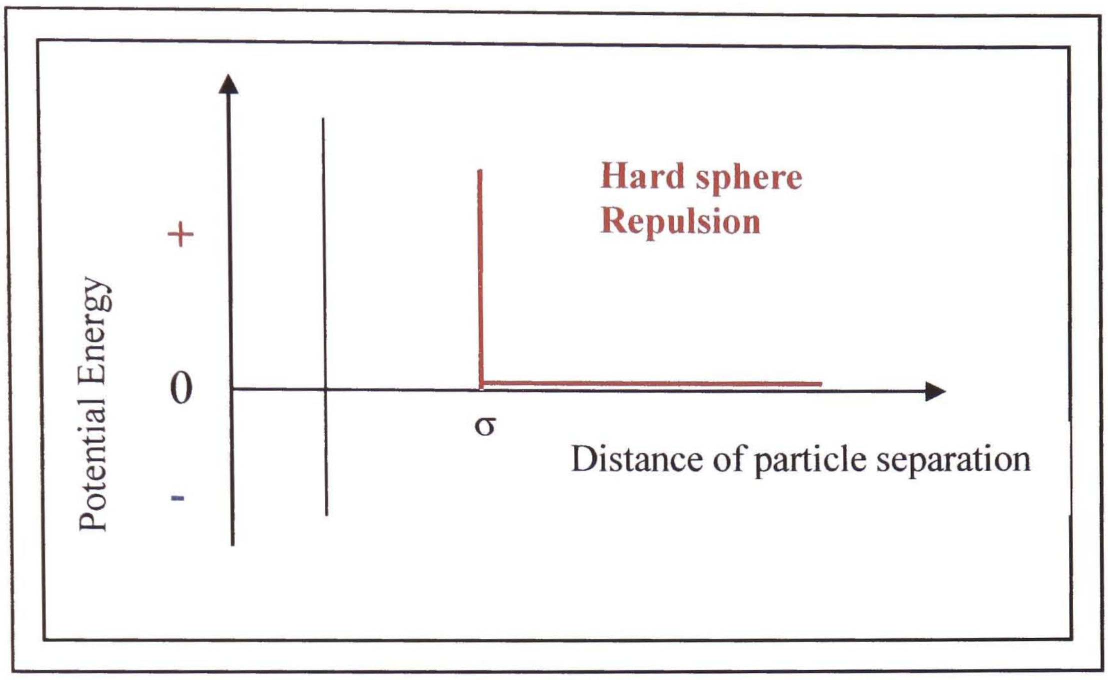


**Figure 1.17:** Potential energy curve representing the repulsion force of two identical and spherical colloidal particles. σ represents the hard sphere diameter of the particle. (Figure inspired by. Israelachvili, 1992).

This graph may be applied to atoms, molecules or particles depending of the considered representation of σ as a hard sphere diameter of an atom, a molecule or a particle (Israelachvili, 1992; Hunter 2001).

Despite the complexity of such forces in food systems, repulsive interactions can be simplified with the help of a hard sphere model in presence of polymers and dispersed in medium. Hunter (2001), Hiemenz (1986) and Walstra (1996) refer to the free energy which can be calculated when two 'hairy' particles approaching. The free energy equation has been commonly defined by the Gibbs law.

$$\Delta G_R = \Delta H_R - T \times \Delta S_R \qquad \textbf{(1.5)}$$

Here $\Delta G_R$ is the free energy of repulsive interaction (J); $\Delta H_R$ is the enthalpy of repulsion (J); T is the temperature (K) and $\Delta S_R$ is the entropy of the repulsive interaction (J). By convention, the positive value of $\Delta G_R$ refers to a repulsion interaction. Hiemenz (1986) further details this aspect by saying that this positive energy protects the particles from flocculation and coagulation. Hunter (2001) adds that the stability of the colloidal system generates a positive free energy as the particles are approaching each other. He defines three domains of approach: non-interpenetration (stage 1), interpenetration (stage 2) and interpenetration with compression (stage 3). Each of these domains are summarised in the Figure 1.18.

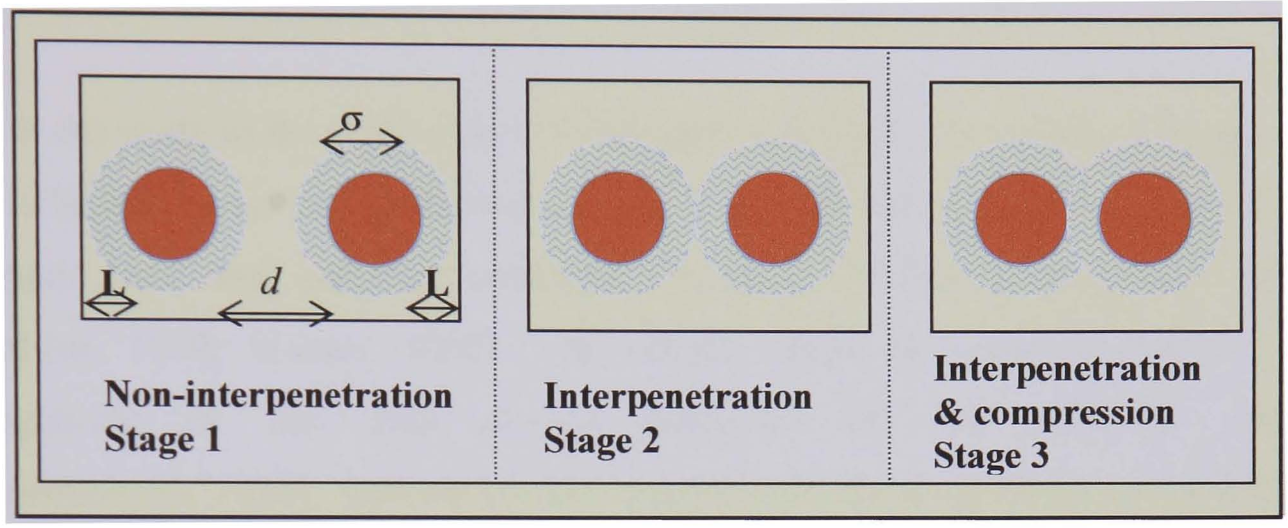


**Figure 1.18:** Schematic representation of the steric repulsion of two polymerised particles. The following colour ■ is the particle; whereas, ■ represents the polymer. Part of the polymer is attached to the surface of the particle and its other part known as 'tails' or 'hairs' are dispersed into the medium (in yellow). *d* is the impenetrable distance of the two particles; **L** is the steric layers from the polymer tails and $\sigma$ is the hard sphere diameter of the particles.

In stage 1, Hunter (2001) explains that the polymer chains do not interact with each other. He states that at this stage the distance of the two particles follows the relation: $d > 2\,\mathbf{L}$. Hiemenz (1986) says that in this stage $\Delta G_R$ is nil as no polymer overlapping is present. Israelachvili (1992) specifies that each polymer tails contained in a particle interact independently from the other tails of the other particle.

In stage 2, Hunter (2001) explains the mechanism of overlapping by stating the relation: $\mathbf{L} \le d \le 2\mathbf{L}$. Both Hiemenz (1986) and Walstra (1996) describe this stage by an increase in the density of polymer tails. Walstra (1996) further explains that overlap is caused by the effect of restriction volume generated by the tails. As the polymer tails overlap, these can no longer move freely and their original conformation is restricted. Walstra (1996) specifies that at this stage, a loss of entropy is observed ($\Delta S_R$) which will result in a rapid increase of the free energy.

Finally in stage 3, Hunter (2001) reports that the distance between the two particles can be written as the following relation $d \leq \mathbf{L}$. He explains that polymer overlapping and elastic contribution from tail mechanisms leading to slow rearrangement of the particles occur in this domain.

In the study of the steric effect, it should be noted that the repulsive forces may be affected by several parameters, such as the solvent quality, the size of the particle and the polymer structure (Hiemenz, 1986; Israelachvili, 1992; Walstra, 1996; Hunter, 2001). The solvent plays an important role in the magnitude of the free energy generated by the repulsive forces (Israelachvili, 1992). Walstra (1996) explains that the good quality of a solvent will generate high repulsive force. Hiemenz (1986) shows that the stability of a colloid system occurs in presence of a large quantity of polymer present in the system. However Walstra (1996) explains that the molecular structure of the polymer should also be considered. As thin adsorbed layers will stabilise less large particles than long adsorbed layer of polymers. At this stage, Walstra (1996) mentions that van der Waals attractive interactions may be more powerful and the system will be coagulated.

This section has shown that repulsive forces may be multiple depending of the size of the considered particles. In this section we mainly focus our work on steric effect and thus use a hard sphere model. The presence of the polymer has helped us to understand better the mechanism of interpenetration referred by Hunter (2001). Finally we looked at the different parameters which influence such stability. However, another type of stabilising effect may be considered in the study of our model. Israelachvili (1992) explains that steric forces may be further promoted by the action of molecule bridges.

### I.6.3. Water bridges, Hydrogen bonding

Hydrogen bonding commonly arises from the polarity of the water molecule and its electrostatic interaction. The intramolecular bond between the oxygen and one hydrogen atom is approximately 0.10 nm; whereas intermolecular distance between one oxygen and hydrogen atom is approximately 0.176 nm.

The strength of the hydrogen bonding varies from 10 to 40 $kJ.mol^{-1}$. Israelachvili (1992) observed that this specific bond remains stronger than a van der Waals bond (~ 1 $kJ.mol^{-1}$) but weaker than a covalent bond (~ 500 $kJ.mol^{-1}$). Heimenz (1986) explains that hydrogen bonding allows the linkage of two highly electronegative atoms. Furthermore, Fennema (1996) also reports that hydrogen bonding depends on the electronegativity and the specific orientation, resulting from the Coulombic interactions of the two associated molecules (Israelachvili, 1992). Figure 1.19 represents hydrogen bonding of water molecules with the formation of a tetrahedral network.

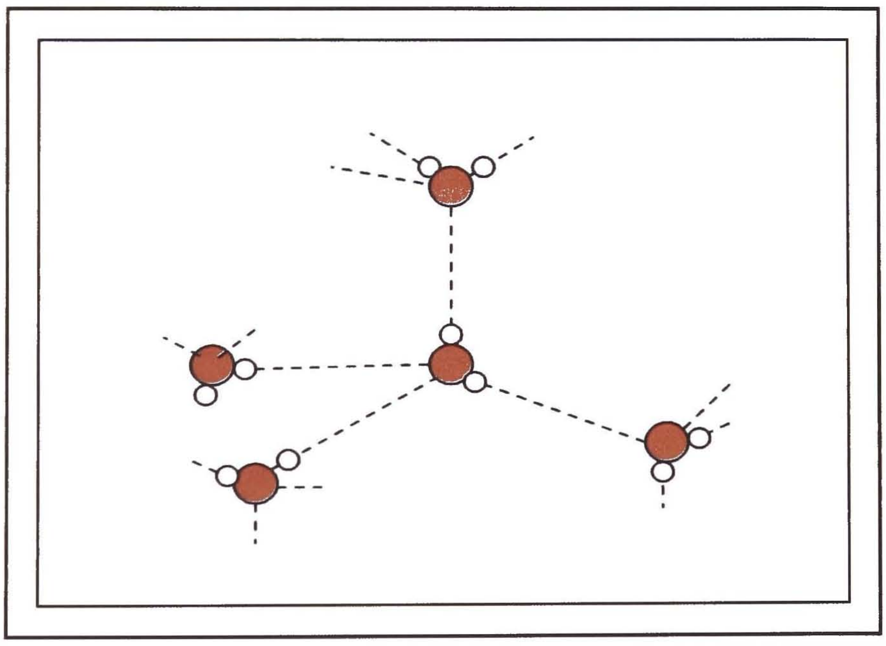


**Figure 1.19:** Hydrogen bonding of water molecules. ● represents the oxygen atoms and ○ represents the hydrogen atoms. Hydrogen bondings are represented in dashed lines

(~ 0176 nm). Covalent bonds distance is approximately 0.10 nm (Figure inspired by Fennema, 1996).

Due to the presence of hydrogen bonding in water molecules, water is formed of multiple dipoles which interact between each other (Fennema, 1996). Dickinson and Stainsby (1982) have reported that the presence of water leads the formation of a continuous and distorted network. The presence of a hydrogen bond can form a three-dimensional 'structures' network. Heimenz (1986) explains that this network has a loose structure which can be easily disrupted and rebuilt depending of the medium properties. Moreover, he enumerates several factors (e.g. salt concentration, pH and temperature) which can affect the hydrogen bonding network.

In relation with our project, Dedinaite & Campbell (2000) publication relates on the effect of water, hydrogen bonding, on the adsorption of oil at mica surface and thus at different water activities. They note that at a specific water activity, $a_w > 0.9$, water capillaries were formed between the surface of the two mica plates. This was mainly dependent on the strength attractive interactions between water molecules. They explain that water and hydrogen bonding in colloidal systems are important and compulsory, unless scientific experiments are performed under specified atmosphere. As a result the presence of water is inevitable in common food and its effects should not be ignored.

The previous sections have shown that van der Waals, steric interaction and hydrogen bonding play important roles in the stability of our original model. However, the stability of our colloidal system depends also on other forces, hydrophobic and electrostatic, to a less extent than the previous detailed forces. Considering our non-aqueous model, the studies of hydrophilic or depletion interactions seem irrelevant for our project.

### *I.6.4. Hydrophobic Effects and Electrostatic Interactions*

Hydrophobic interactions are strong bonds and are commonly formed between hydrophobic molecules and the surfaces in contact with water. The interactions produced between hydrophobic and water molecules are attractive in the same range as van der Waals interactions. Attempts have been made to calculate the strength of the hydrophobic effects, however, due to the complexity of the system which involves many molecules no satisfactory theory has yet been discovered (Israelachvili, 1992). Hiemenz (1986) explains that the hydrophobic effects are mainly based on the association of molecules, involving entropy changes, and, are very important on the stability of surfactant structure mixed in water.

Walstra (1996) states that particles in aqueous media present electrical charges and he observes that it is generally negatively charged in food. Israelachvili (1992) shows that such charged particles can be obtained via two mechanisms: ions adsorption and ionic surfactants dissociation. Adsorption of ions can be arise from a solution onto a preliminary uncharged surface (Hiemenz, 1986); whereas the gain of ionic surfactant is done by ionising or dissociating groups of protons from the surface and thus leaving negative charges surrounding the surface of the particle.

Israelachvili (1992) explains that the charge at the surface is balanced by the presence of counter ions, ions of the opposite surface charges. Walstra (1996) details that counterions are present in excess and commonly formed the *electric double layer*. This layer is also known as *Stern* or *Helmholtz layer* (Israelachvili, 1992).

Walstra (1996) explains that such layer keeps fluctuating by the on/off movement of medium molecules. He stated that this layer is approximately 1nm or less. For particles dispersed in non aqueous systems, the thickness of the Stern layer is even smaller than in aqueous systems (Walstra, 1996). Walstra (1996) explains that despite charged surfaces the ionic strength is negligible and not relevant if considering electrical interaction.

### *I.6.5. Conclusion of the Interactions between particles*

In this chapter we have used a colloidal model in which we can be applied for understanding the forces encountered between sugar particles dispersed in oils. By analogy, this model has been considering that sugar particles were hard spheres dispersed in non aqueous solvent.

We then looked at the main forces contributing to the stability of these particles in the media. Attractive interactions, van der Waals forces, and repulsive interactions, steric effects accounted for the primary sources of interactions in the stability of the system. Hydrogen bonding has been also considered, as water molecules may be present in non-purified solvents. We also have mentioned the presence of other interaction forces such as the hydrophobic effects and the electrostatic forces. However, for our colloidal model the most important forces which contribute to the stability of our system are the van der Waals, the steric and the hydrogen bonding forces.

## *I.7. Introduction to Sugar in Oil Suspensions*

Chocolate is a complex system, composed of various kinds of particles suspended in a highly concentrated liquid mixture. In order to understand better how the behaviour of the system is independent on the properties of these particles, we can firstly reduce the number of compositional elements involved, with work here being based on a simple model suspension (including sugar particles, surfactants and liquid oil phase); and secondly we can vary the concentration of each component in order to probe ingredient effects on specific interactions between the dispersed particles.

This section will describe the general properties of suspensions observed a wide range of colloidal including chocolate. The main interactions encountered in aggregating systems will be characterised as well as the different parameters which influence the strength of these interactions. We encounter parameters relating to the physical and chemical structures of the particles and surrounded fluid, as well as to the presence of surface-active impurities or additives (emulsifiers). We consider how much their effects impart on the structure, rheology and chemistry of the fats or fat blends used to make up the continuous phase in chocolate.

## *I.7.1. Types of Particles in Suspension*

The field of particles in suspension is important industrially. A wide range of industrial products depends on, or is based on, particle technology. For example, catalysts, inks, paper, dyes and pigments, adsorbents, thickening agents, medicinal products, aerosols, and some adhesive products all rely directly on the properties of small particles in bulk dispersion.

### I.7.1.1. Multiphase Systems

Coulson and Richardson (1991) mentioned that there exist various kinds of systems where particles are in suspension; these are generally called multiphase mixtures. Such systems include:

- a mixture of liquid with gas or vapour,
- solid particles mixed into liquids (commonly known as hydraulic transport),
- wholly or partially suspended solid particles in gases (pneumatic transport),
- systems containing both solids, liquids and gases.

The behaviour of these systems is generally dependant on diverse physical, process engineering and chemical factors. The properties of the components, the flow rates and the geometry of the system should be taken into account. As an example, flow rates can depend on the density difference between the phases. In case of a mixture of the solid/ liquid type, the liquid is generally less dense than the solid.

Colloidal systems have a great range of applications and the factors relevant to each particular system are numerous. In this research, we consider chocolate as a complex concentrated suspension made of solid particles dispersed in liquid fat, once melted. The rheology of the melted chocolate can therefore be understood by considering the factors affecting the rheology of suspensions of solid particles in general.

### I.7.1.2. Solid-Liquid Mixtures

Hydraulic transport characterises the flow of a system of solid particles dispersed in a liquid. On the industrial scale, the transport of particles is greatly dependent on the handling system, such as the diameter and length of the pipeline. In order to ensure a reliable system, the system is mainly dependent on the controlled physical properties of the liquid and the solids. The physical properties of the liquid include the density, the viscosity (more generally, the rheology) and the reactivity with surfaces. In terms of solid, the relevant physical properties are the mean particle size and the size distribution, and the shape and density of the particles.

Coulson and Richardson (1991) report that two types of suspensions exist: a *fine suspension* which is characterised by a uniform distribution of colloidal particles in the liquid and a *coarse suspension* of large particles, which tend to fall in the bottom of a pipe. They define the size of fine particles from 0.1 to 10 µm and the size of coarse particles above 100 µm. However, they explain that the boundaries between these particles are small and are generally, influenced by their flow rate and their particle concentrations. The separation of these two groups is somewhat arbitrary, however greatly depending on the flow rate and the concentration of solids dispersed in the fluid.

### *Fine Dispersions*

Coulson and Richardson (1991) explain that particles in this type of system are mainly individually dispersed in liquid or they might also be present as flocs. Flocs contain a large number of particles, which are bound loosely together. At low solid concentrations, the fluid behaves like a Newtonian liquid. The rheology of fine suspensions will be further detailed in Chapter II. In the Newtonian flow of a liquid like water or a vegetable oil, the shear stress is proportional to the rate of shear strain. This behaviour is mainly due to the fact that such fine particles do not take part in the viscosity of the fluid. In the case of highly concentrated suspensions, however, the flow is no longer Newtonian and shear thinning behaviour is generally observed. As a result of this system there can be observed a build up of structure between the particles. One feature of a concentrated suspension is the fact that it exhibits a yield stress, which is the pressure that a substance is capable of supporting without fracturing.

### *Coarse Suspensions*

Coulson and Richardson (1991) report that depending of the equipment and the way particles are suspended, horizontally or vertically, coarse particles behave differently. In a case of a horizontal pipeline, the fluid will generally flow within two different velocities and particles will tend to settle at the bottom of the pipe. The major factor, which determines the settling of particles, is the terminal falling velocity in the liquid. For a vertical pipe, the suspension will tend to be uniformly distributed along the pipe. The predominant factor in this situation will be the relative velocity between liquid and solid.

In order to understand further the mechanism of interactions between sugar particles in oil, we detailed the characteristics of both solid particles and liquid.

### *I.7.2. Solid Characteristics*

Solid systems is the result of complex interactions between the particles which explains the difficulty to completely determine the physical state of the material. Individual particles are generally characterised by their *composition*, *size* and *shape*. Otterstedt and Brandreth (1998) explained that single particles might also be classified in terms of their conductivity and their electrical and optical properties. They extended the particles' characteristics to chemical and physical properties and showed that, on a small scale, the spatial distribution of the particles has an effect on the packing of small particles.

#### **I.7.2.1. Single Particles**

Single particles are generally characterised by their simplest shapes: the sphere. In the case of uniformed particles and with the particles composition, it is possible to determine the density, the conductivity, the turbidity and the viscosity of the system. Properties of particles include porous and crystalline state, their amorphous structure, and also their ability to form a matrix. Single particles are mainly defined following the *surface*, the *shape* and the *distribution size* of the particles.

The Sauter mean, D (3, 2), diameter together with the volume fractions determines the surface area. The surface area has a great importance for the material especially for small particles. It generally affects the settling rate of solids and is defined as surface area. It has been observed that, as the particle size decreases the surface to volume ratio increases.

The **"shape"** of the particles may be regular or irregular. Rhodes (1998) states that the shape of particles affects the drag coefficient ($C_D$). The general equation of the drag coefficient is:

$$C_D = \frac{R'}{\left(\frac{1}{2}\rho_f U^2\right)} \qquad \textbf{(3.1)}$$

where R′ represents the force per unit projected area of the particle (N $m^{-2}$), $\rho_f$ is the density of the fluid or filtrate (kg $m^{-3}$), and U is the superficial velocity (m $s^{-1}$).

This coefficient is a function of the relative velocity of the particle in a fluid. Particles are classified as regular if they are spherical or cubical. In this case, mathematical equations permit to define precisely their shape. In terms of irregular particles, these are generally expressed in terms of particular characteristics of regular shaped particles.

Finally, **"particle size distribution"** remains important in order to quantify the spread of sizes and to determine different sizes of particles present. This method is used for sedimentation, as it permits to understand the flow behaviour of particles when settling out.

Chocolate is based on the dispersion of several particles in the continuous fat phase. Many particles, including sugar ones, interfere between each other to give chocolate. However, their chemical formula still remains important as well as their physical properties. Krüger (1999) reports that sucrose is mainly used in the chocolate confectionery. This sugar is present in its crystalline form. He explains that sugar particles behave differently in the presence of the surrounding fat fluid as compared with the individual particles. In fact, the properties of such solids dispersed into liquid depend on the physical properties of individual particles, i.e. size, shape and size distribution, as well as on the inner properties, molecular and physical structures, of the continuous fluid.

But single particles generate different behaviours whether in the presence or not of a medium. And other parameters such as the liquid medium, need to be taken into account for the determination of rheological properties of dense slurries. This refers to the nature of the surrounding fluid and the addition of surface-active components.

### *I.7.3. Liquid Characteristics*

There exist different types of dispersing media, which may have different properties in terms of their chemical and physical structures. Media are subdivided in two groups: the aqueous solutions and the organic phases. Aqueous solution refers mainly to water based samples whereas organic phase refers mainly to oils. Oils' characteristics are numerous in terms of their inherent chemical components and their physical parameters. Their origins (animal, vegetable or mineral) impart greatly on these parameters (Coulson and Richardson, 1991). Prior to study the organic phases, we present an example of the possible interactions encountered in a solid/ water suspension.

For solid/ water dispersions, Otterstedt and Brandreth (1998) explain that the most important characteristic of particles in suspension in a solution is their insolubility in this liquid. The most common dispersing fluid is referred to as water. They stated that particles, which are insoluble in water, do not react with the medium and do not tend to vaporise easily. They also mentioned that the particles must have great "wettability" with the medium or create a surface film between the medium and the dispersed particles. Most of the particles, which can be suspended in water, contain the following elements: Se, Pt, C, as well as oxides of heavy metals such as $Cr_2O_3$, $Fe_2O_3$ and $Mn_2O_3$. Aluminium oxide ($Al_2O_3$) and silicon oxide ($SiO_2$) are also partly insoluble in water. In the case of $SiO_2$, this oxide presents a particularity and could be soluble in water due to its amorphous cell. It should be noted that the use of water is very common due to its simple structure. But aqueous phases are not the only fluids used which can disperse particles and other examples include organic fluids. Different types of particles may be dispersed in organic phases. In this case different solvents and oils are used: carbon black particles in silicone oil (Kawaguchi *et al.*, 2001) or sugar particles in cocoa butter (Johansson and Bergenståhl, 1992b).

### I.7.3.1. Organic Phases

Several dispersed systems exist which all remain different to each other (Coulson and Richardson, 1991). Many of them involve multiphase mixtures. In this section, our aim is to concentrate on the inherent properties of the elements specially involved in the chocolate process. As a consequence, oils, which may have different origins such as vegetable, animal or mineral, and mainly edible fats will be discussed.

Nawar (1996) explains that the characteristics of a food oil are variable depending on its composition, its crystalline structure, its melting properties, and on its ability to associate with $H_2O$ and other non-lipid molecules. O'Brien (1998) states that fats and oils contain a combination of glycerides and free fatty acids. Lipp (1998) shows that fats and oils can be differentiated from each other by their triglycerides, fatty acids and sterols content. Such compositional analyses are mainly performed these days with gas chromatography or high pressure liquid chromatography due to the sensitivity of these techniques (Christie, 1982).

### I.7.3.2. Chemical Properties of the Fat Phase

The individual glyceride molecule has a tri-ester structure. Food oils and fats are mixtures of different triacylglycerides (TAG), which are represented by the general formula shown in Figure 1.20.

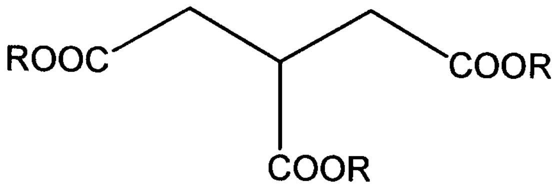
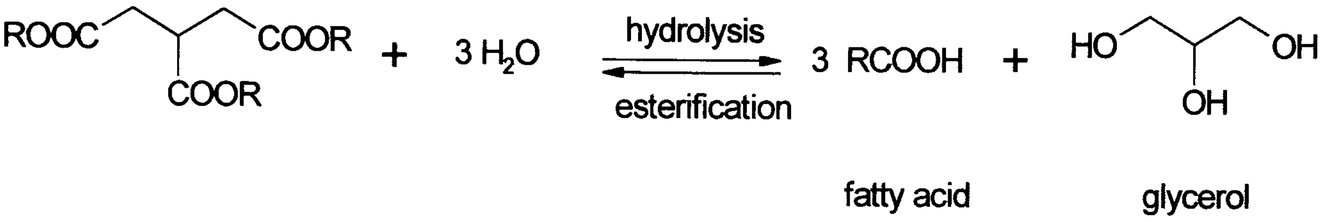


**Figure 1.20:** Ingold schematic representation of a general triacylglyride (TAG). R represents a hydrogen atom or fatty acyl groups derived from condensed acids.

Glycerides can be hydrolysed to give fatty acids and glycerol (Taylor, 1965):



By convention we recognise that the physical difference between fats and oils is that at room temperature fats are solid whereas oils are liquid. On the molecular scale, this difference in behaviour between the two can be understood mainly by the fact that saturated fatty acids are predominantly present in fats whereas oils are mainly constituted of unsaturated fatty acids. Saturated fatty acids include palmitic (C-16:0), stearic (C-18:0) or arachidic (C-20:0) acids; on the other hand, unsaturated fatty acids include acids such as palmitoleic (C-16:1), oleic (C-18:1) or linoleic (C-18:2).

Another factor, mentioned by O'Brien (2004), is that the length of the chain also affects the physical properties of the glycerides. The range of fatty acids for lipids varies from $C_4$ to $C_{24}$. In this range, different levels of unsaturation are possible. As an example, for $C_{18}$, three different fatty acids exist with this carbon chain: unsaturated, stearic acid C18:0; mono-saturated, oleic acid C18:1, di-saturated, linoleic acid C18:2 or tri-saturated C18:3. But other parameters, which are discussed in the following section, might influence the physical properties of these components.

## I.7.3.3. Crystallisation Behaviour of Triglycerides

The physical changes of a common triglyceride may be influenced by several factors (Koyano *et al.*, 2002): the *polymorphism*, the *phase behaviours of fat mixtures*, and the *fat crystal network*.

Koyano *et al.* (2002) define **“polymorphism”** as the ability for a lipid to form different crystalline structures. O’Brien (2004) explains that polymorphism is in fact a succession of different crystalline forms without changes in the chemical structure. Four main different triglyceride forms exist. These are: γ, α, **β’ and β.** These crystals are generally classified by their order of stability: β > β’ > α > γ, where the β form is the most stable. The presence of specific triglyceride crystals impart on the physical properties of the fat. Due to the formation of different physical stages, Weiss (1983) enumerates six different melting points, which may be characterised by different polymorphs and may be defined by several techniques, such as X-ray diffraction. Nawar (1996) explains that the transitions of fat polymorph, from one form to the other, are the result of several factors such as, purity, temperature, rate of cooling and the presence of solvent. Figure 1.21 represents the different polymorphic structures of fats in terms of (a) unit cell and (b) the chain length.

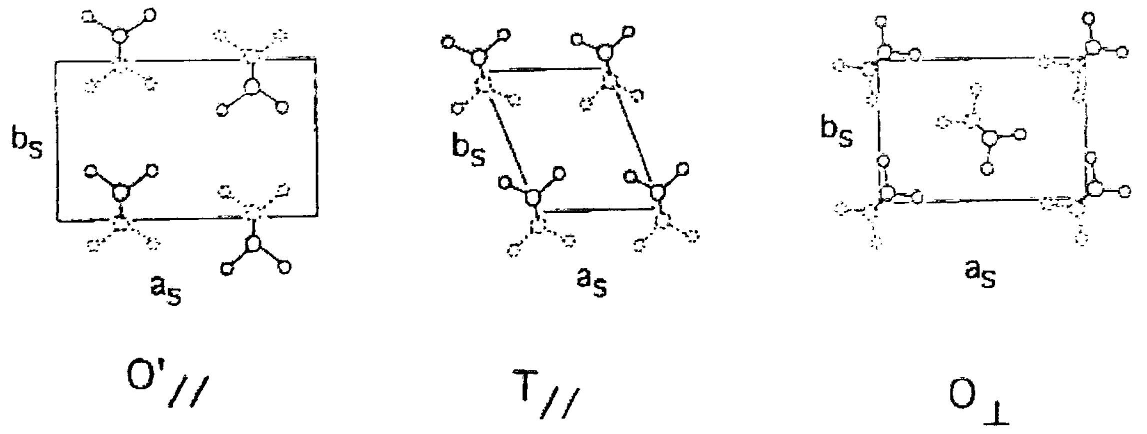


**Figure 1.21a:** Three major unit cells composing different polymorphs: coplanar (O’$_{//}$), triclinic parallel (T$_{//}$), orthorhombic perpendicular (O$_{\perp}$) subcells. b$_s$ and a$_s$ represent the axes of the each unit cell. Their dimensions for a common triclinic parallel polymorph are about $a_s = 5.54$ Å and $b_s = 7.38$ Å (taken from Kaneko, 2001).

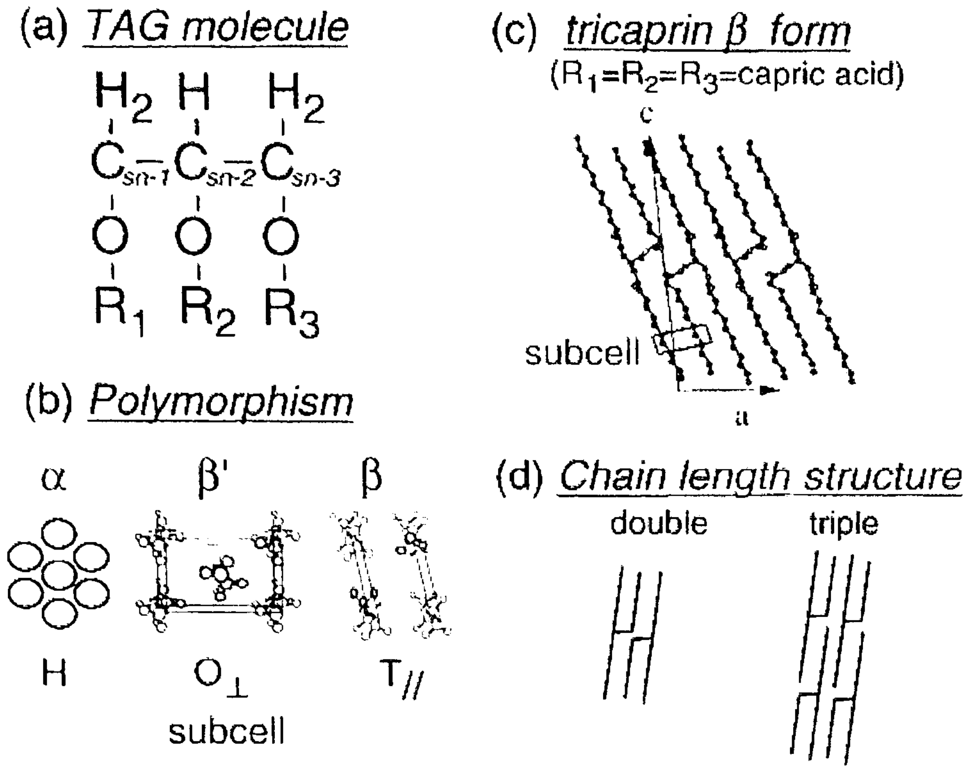


**Figure 1.21b:** Scaling representation of the fat structure from the molecular approach to the general structure of the fat crystals. (a) the molecular triacylglycerides; (b) the unit cell also known as subcell. α polymorphs have hexagonal subcell (H); β' polymorphs have orthorhombic-perpendicular subcell ($O_{\perp}$); and β have triclinic-parallel subcell ($T_{//}$); (c) one crystal structure of β polymorph for tricaprin; and (d) the chain length of the fat structure. Double chain length obtained when chemical properties of the three acids are almost similar. Triple chain length occurs due to chain sorting of the molecule. It is obtained when one of the acid is extremely different from the other two acids (taken from Sato and Ueno, 2001).

Characteristic physical properties of the different triglyceride crystalline forms, are listed in Table 1.3.

**Table 1.3:** Physical properties of the three main fat crystal forms: α, β' and β.

| Characteristic | α form | β' form | β form |
|---|---|---|---|
| Chain packing | Hexagonal (H) | Orthorhombic perpendicular (O⊥) (a) | Triclinic (T//) (b) |
| Short spacing ( Å ) | 4.15 | 4.2 to 3.8 | 4.6, 3.9, 3.7 |
| Stability | Least stable | Meta-stable | Most stable |
| Density | Lowest | Intermediate | Highest |
| Melting point | Lowest | Intermediate | Highest |
| Morphology | Amorphous-like | Rectangular | Needle shaped |

*Note:* (a) Acyl chains perpendicular to the nearest neighbours; (b) Planes are parallel to each other

It was shown that acylglycerides present a complex structure due to the presence of carbon chain segregation. Nawar (1996) notices that highly randomised fats such as palm oil, cottonseed oil milk fat and modified lard from β' crystals first and slowly transform to β forms. In comparison, soybean, peanut, and coconut oils, as well as cocoa butter, tend to crystallise in the β form.

Koyano and Sato (2002) explain that three mixture **"phases"** can exist involving triacylglycerides. These phases are the *solid solution phase* for less stable polymorphs, the *eutectic phase* for stable polymorphs, and *compound formation* for mixtures of saturated/unsaturated acids. Each of these mixtures depends on the polymorphism of an original subcell and the interactions of atoms in the chain length. The interactions produced are mainly from acid chain to other acid chain which depends on the molecular structure of the triacylglyceride. The types of reactions encountered in triacylglycerides occur generally in the chain length. The reactions are mainly isomeric configuration transformations and hydrogen or carbonyl group addition from unsaturated to saturated molecule (Koyano and Sato, 2002).

Koyano and Sato (2002) state that the nature of the **"fat crystal"** remains one of the most important factors influencing the rheological behaviour of lipids. Fat crystals are characterised in terms of morphology, size, size distribution and network formation. The formation of specific types of crystals depends on the processing conditions applied to the product. In the case of cocoa butter, this fat exhibits four polymorphic forms which are represented in Figure 1.22 (Sato *et al.*, 2001).

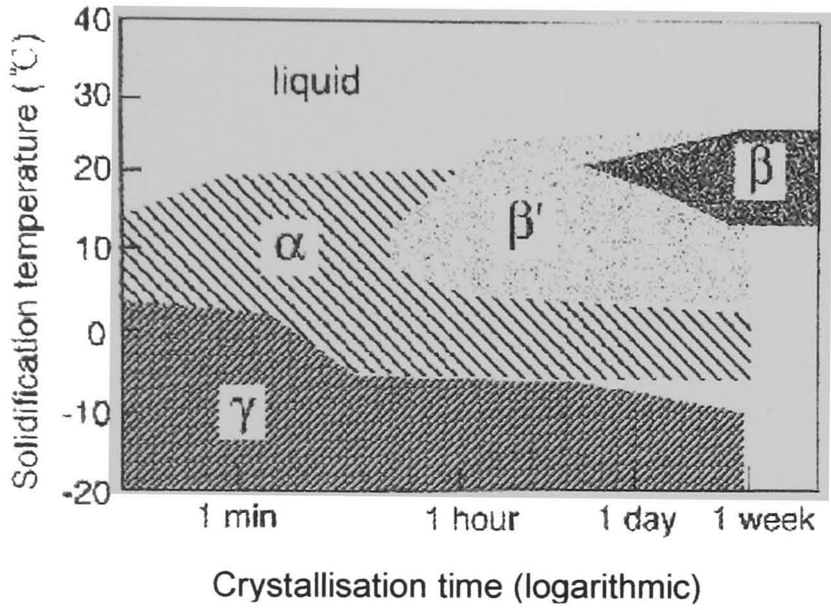


**Figure 1.22:** Representation of the cocoa butter polymorphs as analysed by dynamic crystallisation (from Sato and Koyano, 2001).

Fat crystallisation is an important factor as it affects the viscosity of the product. Melting of fat is a difficult process to understand mechanistically as the molecules are oriented in many different ways and several different kinds of interactions *via* nuclei may form disordered structures. Povey (2001) emphasises this by saying that the crystallisation process is complex and it results from the interactions of foreign molecules at their surfaces. This phenomenon is known as *heterogeneous nucleation.* Povey (2001) explains that rheological behaviour arises from factors such as the particle shape and distribution, the heat transfer between the particles and the continuous phase, the viscosity of the continuous phase, and the surface energies between the contacting surfaces, as well as the surface nucleation.

Aquilano *et al.* (2001) confirm that the crystallisation process is not in equilibrium, and it results in crystal nucleation in the bulk phase with the presence of small quantities of surfactants. Walstra *et al.* (2001) indicate that the state of aggregations of the crystals is the dominant factor determining the firmness of the bulk fat system. The build-up of rigidity is due to the formation of a continuous network. They explained that this mechanism involves a sequence of steps illustrated in Figure 1.23.

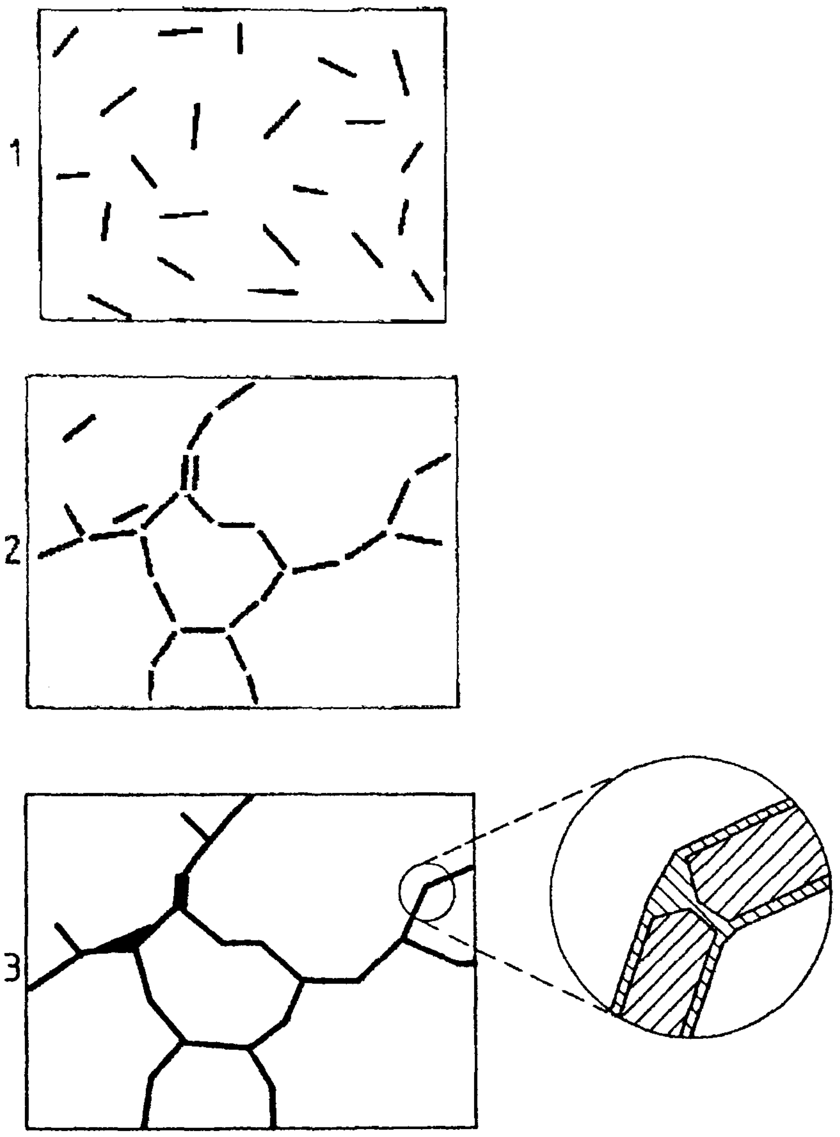


**Figure 1.23:** Schematic representation of the growth of fat crystals. (1) represents the 1st step: formation of the nuclei; (2) represents the 2nd step: growth of the nuclei in order to form crystal, (3) represents the 3rd and 4th steps: formation of crystal aggregates and formation and development of continuous network respectively. In the circle, the sintering effect is shown, e.g. growing together, of adjacent crystals (from Wastra *et al.*, 2001).

The formation of the network is permanently changing. In order to explain the importance of the fat crystallisation in food, Aronhime *et al.* (1988a), have shown that the process of fat crystallisation is also dependent on the rate of cooling of the fat product. They showed that the cooling rate affects the enthalpies of crystallisation ($\Delta H_c$) and fusion ($\Delta H_f$) of the transitions between the different phases of cocoa butter. As an example, for a cooling rate of 0.3 °C / min, $\Delta H_c$ = 88.7 J/g and $\Delta H_f$ = 94.5 J/g; on the other hand when the cooling rate is 0.1 °C / min, $\Delta H_c$ = 100.7 J / g and $\Delta H_f$ = 103.7 J / g. They showed that the different polymorphic forms of this fat result in a different chemical composition.

Johansson (1995) studies the particle network properties of a specific fat in relation to temperature. She explained that, as the temperature increased, the fat network became stronger in terms of inter particle interactions. She stated that this was the result of a partial but strong adhesion between melted crystals. She was able to identify the type of crystals dispersed in the continuous liquid oil phase. In fact, these crystals were flocculated and were producing a semi-solid fat layer made by the aggregations fat crystals in surrounding oil. This semi-solid fat layer remained thicker than the oil's viscosity itself.

### I.7.3.4. Properties of Specific Oils

In this project, eight different edible fats and oils have been studied. The originally selected oils were soybean oil, sunflower oil, palm kernel oil and cocoa butter. Work was later extended to include milk fat, a fat mixture (blend of 83.5 % cocoa butter and 16.5 % milk fat) and two milk fat fractions ('stearin' and 'olein').

#### *Soybean oil*

Soybean oil once partially hydrogenated and fractionated is mainly formed of large and easily filtered crystals (O'Brien, 1998). These crystals are generally created in the β form. Soybean oil has a great capacity for hydrogenation, as confirmed by its high iodine value. Gunstone (1997) states that the presence of a high content of essential fatty acids (6-10% linolenic acid) makes this oil undesirable for food products. Due to the presence of linolenic acid, O'Brien (2004) recommends handling this oil carefully in order to prevent oxidation. Table 1.4 lists the typical characteristics and fatty acid composition of soybean oil.

**Table 1.4:** Physical characteristics of soybean oil and its fatty acid composition

| ***Characteristic*** | ***Typical value*** | ***Typical Range of values*** |
|---|---|---|
| *Specific gravity, (25°C) (a)* | *0.9175* | *0.917 to 0.921* |
| *Refractive index, 25°C* | *1.4728* | *1.470 to 1.476* |
| *Iodine value* | *131* | *123.0 to 139.0* |
| *Saponification number (b)* | *192* | *189 to 195* |
| *Unsaponifiable number (c)* | *0.6* | *0.6 to 1.6* |
| *Titer (°C) (d)* | *24.0* | — |
| *Melting point (°C)* | *-22* | *-20.0 to -23.0* |
| *Solidification point (°C)* | — | *-16.0 to -10.0* |
| *Cloud point (°C)(e)* | *-9* | — |
| *Cold test (hours) (f)* | *25* | — |
| *AOM stability (hours) (g)* | — | *12.0 to 15.0* |
| *Tocopherol content (ppm):* | | |
| *α -Tocopherol* | *100* | *56 to 165* |
| *β -Tocopherol* | *23* | *16 to 33* |

*Note:* (a): Specific gravity of liquid at 25/25 °C (ASTM D – 4052); (b) Saponification number is the measure of alkali-reactive groups in fats and oils. It can also predict the types of glycerides present in a sample; (c) Unsaponifiable number is the measure of the proportion of lipid material other than fatty acids of the original sample. It is calculated as a percentage. (d) Titer is the measure of the amount of concentration of a substance in a solution. It refers to the method AOCS Cc12-59; (e) Cloud point is the temperature at which haziness is first observed at the bottom of a jar when the oil is cooled and examined under specific conditions (ASTM D2500); (f) Cold test refers to the experiments set up to measure the cloud point and the pour point; (g) Active Oxygen Method (AOM) stability estimates the oxidative stability of fats and oils.

**Table 1.4**: Physical characteristics of soybean oil and its fatty acid composition (continued)

| *Characteristic* | *Typical value* | *Range of values* |
|---|---|---|
| *γ -Tocopherol* | *842* | *593 to 983* |
| *δ -Tocopherol* | *363* | *328 to 411* |
| *Fatty acid composition (%):* | | |
| *Myristic (C-14:0)* | *0.1* | *<0.2* |
| *Palmitic (C-16:0)* | *10.6* | *8.0 to 13.3* |
| *Palmitoleic (C-16:1)* | *0.1* | *<0.2* |
| *Margaric (C-17:0)* | *0.1* | — |
| *Stearic (C-18:0)* | *4.0* | *2.4 to 5.4* |
| *Oleic (C-18:1)* | *23.3* | *17.7 to 26.1* |
| *Linoleic (C-18:2)* | *53.7* | *49.8 to 57.1* |
| *Linolenic (C-18:3)* | *7.6* | *5.5 to 9.5* |
| *Arachidic (C-20:0)* | *0.3* | *0.1 to 0.6* |
| *Gadoleic (C-20:1)* | — | *<0.3* |
| *Behenic (C-22:0)* | *0.3* | *0.3 to 0.7* |
| *Erucic (C-22:1)* | — | *<0.3* |
| *Lignoceric (C-24:0)* | — | *<0.4* |
| *Triglyceride composition (%):* | | |
| *Trisaturated (GS3)* | *0.1* | — |
| *Disaturated (GS2U)* | *5.6* | — |
| *Monosaturated (GSU2)* | *35.7* | — |
| *Triunsaturated (GU3)* | *58.4* | — |
| *Hydrogenated crystal habit* | *β* | — |

*Note:* G = glyceride; S = saturated; U = unsaturated.

## Sunflower oil

Sunflower oil is one of the most commonly used triglyceride oils and its composition varies due to the origin of the crop and the temperature. Gunstone (1997) observes that the general composition of sunflower oil is 60-65% linoleic acid and > 90% oleic + linolenic acids. O'Brien (2004) states that the linoleic acid range in the sample varies from 48.3 to 74.0 %, whereas linolenic acid is almost absent with less than 1%. Table 1.5 lists the characteristics and fatty acid composition of sunflower oil. Sunflower oil is used in a wide range of salad oils and cooking oils, as well as in the production of shortenings and margarines (O'Brien, 1998; Gunstone, 1997).

**Table 1.5:** Physical characteristics of sunflower oil and its fatty acid composition

| ***Characteristic*** | ***Typical value*** | ***Range of values*** |
|---|---|---|
| *Specific gravity, (25°C)* | — | *0.915 to 0.919* |
| *Refractive index, 25°C* | — | *1.472 to 1.475* |
| *Iodine value* | *133.0* | *125.0 to 136.0* |
| *Saponification number* | — | *188 to 194* |
| *Unsaponifiable number* | — | *0.3 to 1.3* |
| *Titer (°C)* | — | *16.0 to 20.0* |
| *Melting point (°C)* | — | *–18 to –20* |
| *Solidification point (°C)* | *–17.0* | — |
| *Cloud point (°C)* | *–9.5* | — |
| *Cold test (hours)* | *24 dewaxed* | — |
| *Wax (%)* | — | *0.02 to 0.35* |
| *AOM stability (hours)* | *11* | *10 to 12* |
| *Tocopherol content (ppm):* | | |
| *α -Tocopherol* | *690* | *690 to 778* |
| *β -Tocopherol* | *26* | *21 to 33* |
| *γ -Tocopherol* | *5* | *5 to 9* |
| *Fatty acid composition (%)* | | |
| *Myristic (C-14:0)* | *0.1* | *<0.2* |
| *Palmitic (C-16:0)* | *7.0* | *5.6 to 7.6* |
| *Palmitoleic (C-16:1)* | *0.1* | *<0.3* |
| *Stearic (C-18:0)* | *4.5* | *2.7 to 6.5* |
| *Oleic (C-18:1)—* | *18.7* | *14.0 to 39.4* |
| *Linoleic (C-18:2)* | *67.5* | *48.3 to 74.0* |
| *Linolenic (C-18:3)* | *0.8* | *<0.2* |
| *Arachidic (C-20:0)* | *0.4* | *0.2 to 0.4* |

**Table 1.5**: Physical characteristics of sunflower oil and its fatty acid composition (continued)

| ***Characteristic*** | ***Typical value*** | ***Range of values*** |
|---|---|---|
| *Gadoleic (C-20:1)* | *0.1* | *0 to 0.2* |
| *Behenic (C-22:0)* | *0.7* | *0.5 to 1.3* |
| *Erucic (C-22:1)* | — | *0 to 0.2* |
| *Lignoceric (C-24:0)* | — | *0.2 to 0.3* |
| *Triglyceride composition (%):* | | |
| *Trisaturated (GS3)* | *0.3* | — |
| *Disaturated (GS2U)* | *3.1* | — |
| *Monosaturated (GSU2)* | *26.6* | — |
| *Triunsaturated (GU3)* | *70.2* | — |
| *Hydrogenated crystal habit* | *β* | |

## *Palm kernel oil*

Gunstone (1997) stated that palm kernel oil is a well-known lauric oil obtained from fractionation and hydrogenation of palm oil. Its composition is not similar to that of palm oil, despite it being one of its fractions, but it is comparable to that of coconut oil. O'Brien (2004) states that palm oil is essentially composed of palmitic and stearic acids whereas palm kernel oil is mainly formed by lauric acids. Talbot (1999b) explains that the use of palm oil has been expanded due to its composition of certain glycerides being similar to cocoa butter. Table 1.6 describes the characteristics and fatty acid composition of palm kernel oil.

**Table 1.6**: Physical characteristics of palm kernel oil and its fatty acid composition

| ***Characteristic*** | ***Typical value*** | ***Range of values*** |
|---|---|---|
| *Specific gravity, 40/20°C* | — | *0.860 to 0.873* |
| *Refractive index, 40°C* | *1.451* | *1.448 to 1.452* |
| *Iodine value* | *17.8* | *16.2 to 19.2* |
| *Saponification number* | *245* | *243 to 249* |
| *Unsaponifiable number* | *0.3* | *0.3 to 0.5* |

**Table1.6:** Physical characteristics of palm kernel oil and its fatty acid composition (continued)

| ***Characteristic*** | ***Typical value*** | ***Range of values*** |
|---|---|---|
| *Titer (°C)* | — | *20.0 to 29.0* |
| *Melting point (°C) (MDP)* | *28.3* | *26.8 to 29.8* |
| *Solidification point (°C)* | — | *20.0 to 24.0* |
| *AOM stability (hours)* | *100+* | *15 to 100+* |
| *Tocopherol content (ppm)* | *3* | *3 to 10* |
| *Fatty acid composition (%):* | | |
| *Caproic (C-6.0)* | *0.2* | *0.1 to 0.5* |
| *Caprylic (C-8:0)* | *3.3* | *3.4 to 5.9* |
| *Capric (C-10:0)* | *3.4* | *3.3 to 4.4* |
| *Lauric (C-12:0)* | *48.2* | *46.3 to 51.1* |
| *Myristic (C-14:0)* | *16.2* | *14.3 to 16.8* |
| *Palmitic (C-16:0)* | *8.4* | *6.5 to 8.9* |
| *Stearic (C-18:0)* | *2.5* | *1.6 to 2.6* |
| *Oleic (C-18:1)* | *15.3* | *13.2 to 16.4* |
| *Linoleic (C-18:2)* | *2.3* | *2.2 to 3.4* |
| *Arachidic (C-20:0)* | *0.1* | *Trace to 0.9* |
| *Gadoleic (C-20:1)* | *0.1* | *Trace to 0.9* |
| *Crystal habit* | *$\beta$.* | — |
| *Solids fat index (%) at:* | | |
| *10.0°C* | *48.0* | — |
| *21.1°C* | *31.0* | — |
| *26.7°C* | *11.0* | — |
| *33.3°C* | *0* | — |

It can be observed in Table 1.6 that palm kernel oil contains a low content of medium-chain fatty acids and a large quantity of oleic acids (O'Brien, 2004).

Palm kernel oils have the physical characteristic of melting rapidly when the temperature reaches 30 °C. Due to this property, palm kernel oils can be used as a Cocoa Butter Equivalent (CBE) or a Cocoa Butter Substitute (CBS) for the partial or full replacement of cocoa butter in the confectionery industry (O'Brien, 2004; Gunstone, 1997). Table 1.7 shows the percentage composition of triglycerides in palm oil and cocoa butter, using the data of Talbot (1999b).

**Table 1.7** Percentage composition of triglycerides in palm oil and cocoa butter

| Triglyceride | Palm Oil | Cocoa Butter |
|---|---|---|
| No double bond | 8.5 | 2.2 |
| MOP(a) | 1.2 | 0.9 |
| MOSt(b) | Nil | 1.3 |
| POP(c) | 24.1 | 12.0 |
| POSt(d) | 7.0 | 34.8 |
| StOSt(e) | 0.5 | 25.2 |
| Total SOS (f) | 32.8 | 78.2 |
| Total SSO (g) | 5.3 | 4.1 |
| Two double bonds | 35.0 | 16.0 |
| Three double bonds | 19.8 | 3.5 |
| More than three double bonds | 0.5 | Nil |

*Notes*: (a): M = maleic acid, O = oleic acid, P = Palmitic acid; (b) Maleic acid – Oleic acid - St = Stearic acid; (c) Palmitic – Oleic – Palmitic triacid; (d) Palmitic – Oleic – Stearic triacid; (e) Stearic – Oleic – Stearic triacid; (f) and (g) S = saturated fatty acids (mainly palmitic and stearic).

Palm oil contains 45.3 %, 41.6 % and 8.3 % of saturated, mono-saturated and poly-saturated fatty acids, respectively (Paul & Southgate, 1993). The oil contains a high amount of vitamin E: 33.12 mg per 100 g of oil. Gunstone (1997) explains that palm oil is different from the others fats, like cocoa butter, due to its almost equal amount of saturated (palmitic, stearic) and unsaturated (oleic and linoleic) fatty acids. He reported the fatty acid composition of palm kernel oil, which is listed in the Table 1.8a and Table 1.8b, and represents the major triacylglycerols (mol %).

**Table 1.8**: Fatty acid composition of palm kernel oil. (a) Percentage composition of fatty acids in palm kernel oil; (b) predominant triacylglycerols contained in palm kernel oil.

**Table 1.8a**

Fatty acids composition

| Fatty acids | Palm kernel (wt %) |
|---|---|
| 6:0 | 0.5 |
| 8:0 | 2.5 |
| 10:0 | 4 |
| 12:0 | 49 |
| 14:0 | 16 |
| 16:0 | 9 |
| 18:0 | 2 |
| 18.1: | 14 |
| 18:2 | 2 |

**Table 1.8b**

Major triacylglycerols in palm kernel oil

| Saturated acyl groups | Isomers Nb | Palm kernel |
|---|---|---|
| 12, 12, 8 (LLCaprylic)* | 3 | 6.4 |
| 12.,12,10 (LLCapric)* | 3 | 4.7 |
| 12,12,12 (LLL)* | 1 | 19.8 |
| 12,12,14 (LLMyr)* | 3 | 14.1 |
| 14,12,8 (MyrLCaprylic)* | 6 | 3.6 |
| Others | - | 51.4 |

*Notes*: (*): L = Lauric (12:0); Caprylic (8:0), Capric (10:0); Myr = Myristic (14:0).

The main applications of palm kernel oil listed by Gunstone (1997) are in the production of margarine, shortenings and Cocoa Butter Substitutes (CBS) or Cocoa Butter Replacements (CBR).

### *Cocoa butter*

Cocoa butter composition consists of just three main fatty acids (Talbot, 1999a). These are palmitic, stearic and oleic acids, which represent over 95% of the cocoa butter composition. The typical fatty acid composition listed by Talbot (1999a) is reproduced in Table 1.9.

**Table 1.9:** Fatty acid composition of cocoa butter (from Talbot, 1999a).

| **Fatty acid** | **Composition (%)** |
|---|---|
| Myristic acid (C-14:0) | 0.1 |
| Palmitic acid (C-16:0) | 26.0 |
| Palmitoleic acid (C-16:1) | 0.3 |
| Stearic acid (C-18:0) | 34.4 |
| Oleic acid (C-18:1) | 34.8 |
| Linoleic acid (C-18:2) | 3.0 |
| Linolenic acid (C-18:3) | 0.2 |
| Arachidic acid (C-20:0) | 1.0 |
| Behenic acid (C-22:0) | 0.2 |

The structure of cocoa butter remains complex as it is composed of different triacylglyceride groups, which each have their own characteristics. Due to the presence of the variable compositions of the triglyceride groups, cocoa butter exhibits six polymorphic forms. Nawar (1996) explains that such forms vary from the least stable (Form I) to the most stable (Form V). Table 1.10 lists the different crystal forms of cocoa butter.

**Table 1.10:** Characteristics of cocoa butter polymorphs

| Wille & Lutton[a] nomenclature | Larsson[b] nomenclature | Melting point (°C) | Chain Length Structure (c) |
|---|---|---|---|
| Form I | $\beta'_2$ | 16-18 | Double |
| Form II | $\alpha$ | 21-22 | Double |
| Form III | Mixed | 25.5 | Double |
| Form IV | $\beta'_1$ | 27-29 | Double |
| Form V | $\beta_2$ | 34-35 | Triple |
| Form VI | $\beta_1$ | 36 | Triple |

*Notes*: (a) Reference: Wille R. L. and Lutton E. S., (1966), *J. Am. Oil Chem. Soc.*, **43**, 491; (b) Reference: Larsson K., (1976), In: *Food Emulsions* (S. Friberg, ed.), Marcel Dekker, New York, 39; (c): chain length represents the structure of the fat network.

The Form V is generally suitable for chocolate coating as it is made of the most stable fat crystal β form. Form VI is a transformation of Form V, the rate of formation of which depends mainly on the experimental time and the storage conditions. Such a transformation is also known as "chocolate bloom".

### *Milk fat*

Milk fat is mainly made up of triglycerides, 98 %, and a small quantity of polar lipids such as di- and monoglycerides and phospholipids. O'Brien (2004) reported that milk fat has the highest amount of fatty acids in comparison with all the vegetable or animal fats. These fatty acids have a low molecular weight similar to enriched lauric fats. Depending on the purposes of usage of the milk fat, it can be fractionated into different butters to give soft or hard fractions (Gunstone 1997). Table 1.11 lists the characteristics and fatty acid composition of milk fat.

**Table 1.11:** Physical characteristics of bovine milk fat and its fatty acid composition

| ***Characteristic*** | ***Typical value*** | ***Range of values*** |
|---|---|---|
| *Specific gravity, 40/20°C* | — | *0.907 to 0.912* |
| *Refractive index, 60°C* | *1.4465* | — |
| *Iodine value* | *34.0* | *25.0 to 42.0* |
| *Saponification number* | — | *210 to 250* |
| *Unsaponifiable number* | — | *0.4 max.* |
| *Titer (°C)* | *34.0* | — |
| *Melting point (°C) (MDP)* | *35.0* | *28.0 to 36.0* |
| *Solidification point (°C)* | — | *19.0 to 24.5* |
| *AOM stability (hours)* | *42.0* | — |
| *Fatty acid composition (%):* | | |
| *Butyric (C-4:0)* | *3.6* | *2.8 to 4.0* |
| *Caproic (C-6.0)* | *2.2* | *1.4 to 3.0* |
| *Caprylic (C-8:0)* | *1.2* | *0.5 to 1.7* |

**Table 1.11:** Physical characteristics of bovine milk fat and its fatty acid composition (continued)

| Characteristic | Typical value | Range of values |
|---|---|---|
| Capric (C-10:0) | 2.5 | 1.7 to 3.2 |
| Decenoic (C-10:1) | — | 0.1 to 0.3 |
| Lauric (C-12:0) | 2.9 | 2.2 to 4.5 |
| Dodecenoic (C-12:1) | — | 0.1 to 0.6 |
| Myristic (C-14:0) | 10.8 | 5.4 to 14.6 |
| Myristoleic (C-14:1) | 0.8 | 0.6 to 1.6 |
| Pentadecanoic (C-15:0) | 2.1 | — |
| Palmitic (C-16:0) | 26.9 | 26.0 to 41.0 |
| Palmitoleic (C-16:1) | 2.0 | 2.8 to 5.7 |
| Margaric (C-17:0) | 0.7 | — |
| Stearic (C-18:0) | 12.1 | 6.1 to 12.5 |
| Oleic (C-18:1) | 28.5 | 18.7 to 33.4 |
| Linoleic (C-18:2) | 3.2 | 0.9 to 3.7 |
| Linolenic (C-18:3) | 0.4 | — |
| Gadoleic (C-20:1/C:22:1) | 0.1 | 0.8 to 3.0 |
| Crystal habit | β | — |
| Solids fat index (%) at: | | |
| 10.0°C | 33.0 | — |
| 21.1°C | 14.0 | — |
| 26.7°C | 10.0 | — |
| 33.3°C | 3.0 | — |
| 40.0°C | 0 | — |

The above table shows the variety of fatty acids, with no individual fatty acid exceeding 30 %. Milk fat generally contains 28.5 % of oleic acid (O'Brien, 2004).

### *Fat mixture*

The so-called 'fat mixture' used here is made of a mixture of 83.5 % cocoa butter and 16.5 % milk fat. This fat blend is currently used at variable ratios in chocolate confectionery for economical and technological reasons. This particular ratio was specifically formulated by Nestlé UK Ltd (York) for this project.

Consequently, all the physical properties and the fatty acid composition of the pure fats present in this blend can be found in the two sections on cocoa butter in Table 1.9 and milk fat in Table 1.11.

### *Stearin and Olein*

These two fractionated fats can be extracted from various origins such as cottonseed oil or milk fat. The present material had milk fat origin. The purpose of studying these two specific fractions was based on the difference between their physical and chemical properties. Olein is essentially made of oleic fatty acids. Due to the presence of these unsaturated fatty acids olein fraction can be easily melted at room temperature. Its melting point is about 20 °C. In the case of stearin, this fat fraction is mainly formed of stearic fatty acid. These fatty acids are saturated and possess a carbon chain length of 14 carbon atoms. Stearin's molecular structure greatly influences its melting point which is variable between 35 - 45 °C. O'Brien (2004) explains that new methods such as dry fractionation have been used to create these fractions with the conservation of part of the physical and chemical properties of the original product. By using dry fractionation to obtain stearin and olein, this technique allows the preservation of the fatty acid profile from milk fat. The principle of the dry fractionation is based on a slow cooling of melted fat and thus under controlled conditions. This technique is based on the removal of small quantities of waxes (winterisation) and liquid oil (hydraulic press) and also the crystal fractionation without solvent addition. Moreover, this method preserves the crystal structure, β' in the two different fractions. Specific composition and properties are given in Chapter II.

### *I.7.4. Surface-active Components in Suspended Systems*

The presence of surface-active components is another parameter which directly influences the physical properties of the ingredients and their blends. Dickinson (1992) states that surfactants are particles which lower the surface tension between two immiscible products at low concentrations. Numerous examples of our environment refer to the use of these surfactants. It can be found in colloidal systems such as particles dispersed in solid, liquid or air, or liquid droplets suspended into another liquid. Surfactants are generally constituted of two different parts: one part which has strong affinity with the aqueous media (polar head) and the other part which has a strong affinity with non-aqueous media (organic tail). Figure 1.24 gives a schematic representation of a singular emulsifier.

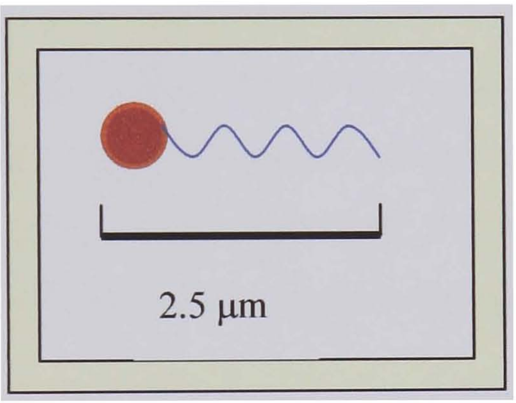


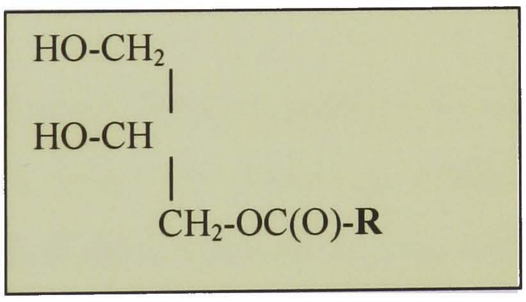
**Figure 1.24:** Schematic representation of an emulsifier. The polar head is defined as the red filled circle and the organic tail is represented as the blue oscillating line. Bar indicates approximate scale of the structure.

The effects of such particles onto colloidal systems can be observed by measuring the surface activity of a solution at either its surface (liquid/air interface) or at the interface of immiscible liquids. Moreover surfactants improve the process of emulsifications as well as the emulsion's stability. These components are specifically known as emulsifiers.

In the food area, common food emulsifiers are classified into four categories: non-ionic, anionic, cationic or zwitterionic. Dickinson (1992) explains that some of these emulsifiers may be naturally present in the food itself. On the other hand, some products may require the addition of emulsifier to be better stabilised. The following section will investigate on the compositions and properties of the natural and added emulsifiers to the systems.

### I.7.4.1. Natural emulsifiers already present in Oils

Food oils are constituted by a mixture of triglycerides. The hydrolysis of these substances releases some fatty acids as well as monoglycerides and/or diglycerides and glycerol molecules. All of these substances are surface-active components. Depending of their concentration, their chemical structure, all these substances will impart on the texture of the fat itself or in the blend of particles with fat. As an example, the different types of butter textures (e.g. hard and soft butters) which are marketed are differentiated by their amounts of surface-active components and the fact that they can bind with more water molecules. Inherent surface active ingredients which have been extensively used in literature (Johansson and Bergenstahl, 1992a,b,c) are commonly monoglycerides and diglycerides. These molecules are well used for their emulsifying properties. Figure 1.25 represents the formula of a typical monoglyceride molecule.



$$\begin{array}{l} HO\text{-}CH_2 \\ \qquad | \\ HO\text{-}CH \\ \qquad | \\ \qquad CH_2\text{-}OC(O)\text{-}\mathbf{R} \end{array}$$

**Figure 1.25:** Representation of a monolglyceride molecule. (**R** represents a fatty acid chain).

Monoglycerides and specifically monolooein has been selected in our project to compare its surface-activity with others emulsifiers such as phosphatidylcholine or polyglycerol of polyricinoleate, also known as PGPR.

As a conclusion, the presence of monoglycerides, diglycerides and triglycerides, which are already present in fats, may impart on the texture properties of these fats and oils. However, the control of the fat texture may be arbitrary depending of the presence of these surfactants into the systems. In order to improve and control efficiently the texture of the fat additions of some surface active components or specific emulsifiers may be required.

### I.7.4.2. Addition of specific emulsifier

In the case of cocoa butter and the specificity of chocolate making, emulsifiers such as lecithin, are commonly added to improve the flowing texture of the chocolate. Lecithin is a blend of four different phosphatides: phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidyl inositol (PI) and phosphatidylcholine (PC). As shown in Figure 1.26.

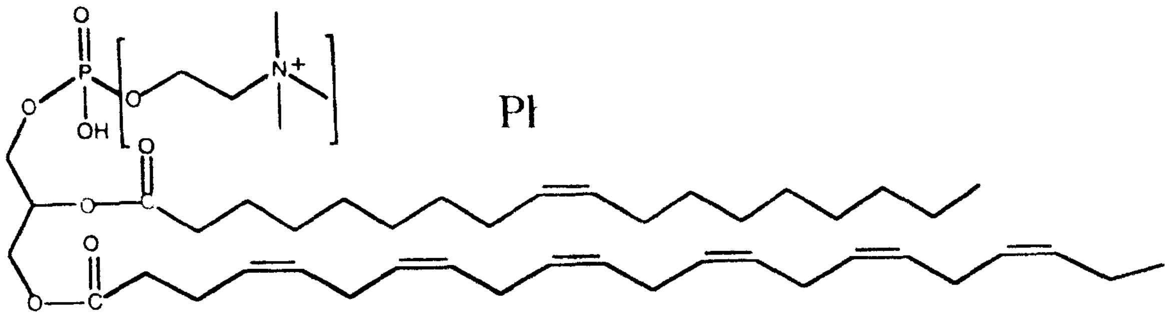


**Figure 1.26**: Chemical representation of phosphatidylcholine molecule (Kyle, 2002).

Lecithin is generally extracted from vegetable oils with variable amounts of phosphatides between each other. In order to obtain defined fractions of phosphatides, lecithin can be further purified by enzymatic hydrolysis, de-oiling or fractionation (O'Brien, 2004).

Another type of emulsifier which is commonly used in the chocolate confectionery is the PGPR, as shown in Figure 1.27.

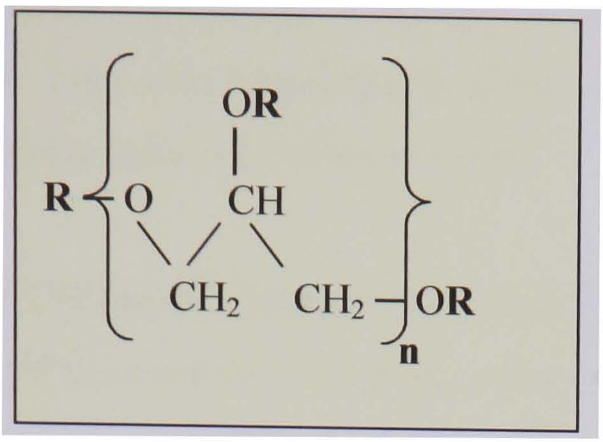


**Figure 1.27:** Chemical representation of PGPR molecule. **R** represents a hydrogen atom or a fatty acyl group derived from polycondensed ricinoleic acid. **n** represents the degree of polymerisation of glycerol (average > 1).

PGPR is generally prepared by the esterification of condensed castor oil fatty acids with polyglycerol molecules (Rajah, 2002). This surfactant is principally used for its viscosity modifier and its ability to maintain a good emulsification (Chevalley, 1999). In the example of chocolate, PGPR is commonly used as a lecithin substitute. It has a substantial effect on the yield value, the force required to make it flow any type of material, and reduces further the plastic viscosity of the heavily concentrated suspension such as chocolate. In this case, the plastic viscosity represents the measure of the maximum viscosity at which the chocolate sample does not flow. Therefore it has a similar behaviour as a *plastic* material.

The presence of surfactants in suspended systems is important for controlling stability, rheology and texture. The addition of a specific emulsifier prior to another one will contribute differently to the physical effect of the original sample or of the blend (Dickinson, 1992). The following paragraphs show the precise effect of different emulsifiers onto cocoa butter or chocolate blend.

The main surfactants, which are used to suspended particles in oil, are generally oil-soluble of low HLB (*hydrophile - lipophile balance*) number. An example of a surfactant is the sorbitan monostearate. Aronhime *et al.* (1988a) explain that the presence of 5% sorbitan monostearate in the system influences its physical properties. The solidification point was found to remain higher than without emulsifier, and the crystallisation and fusion enthalpies were lower than for pure cocoa butter. They also noted that sorbitan monostearate has a direct impact on the polymorphic behaviour of cocoa butter.

Schantz and Rohm (2005) have shown that the multiple addition of emulsifiers such as lecithin and PGPR contribute to a substantial effect on the yield stress and the plastic viscosity of dark and milk chocolates. They noted that a mixture of these emulsifiers could tailor the flow properties of melted chocolate and consequently they could create several products adaptable to different processing systems. They observed that the specific blend, lecithin/PGPR (30:70) had a great impact on the reduction of the yield stress value. However, blends of lecithin/PGPR, 50:50 and 75:25, reduce respectively the viscosity of dark and milk chocolates.

Johansson (1995) studied the influence of non-ionic and ethoxylated emulsifiers on the interactions and crystal structures in semi-solid fats. The emulsifiers investigated were mainly pure monoolein, sorbitan tristearate (HLB value: 2.1) and monostearate (HLB value: 4.7), and technical lecithin. She studied their behaviour via sedimentation, rheology and microscopy. Using the sedimentation technique, Johansson discovered that the presence of emulsifiers was able to suppress the flocculation and sintering processes. This tended to decrease the firmness of the fat at elevated temperatures. This effect was particularly noticeable with sorbitan tristearate, but the other emulsifiers also had a minor effect on the system. It was also observed that the technical lecithin did not bind to the fat crystals, but had weak interactions with them. Johansson noted that lecithin did not suppress the flocculated crystals. It was suggested that lecithin leads to desorption at high temperatures.

Aronhime *et al.* (1988 b) observed the effect of a wide range of sorbitan and ethoxylated sobitan stearates on the heat capacity and the NMR relaxation time of the triglycerides. They also showed that the addition of such substances influences the transformations' conditions. In fact their presence does not lead to the formation of any preferred polymorph but it generally controls the mobility of the molecules, especially the hydrocarbon chain methyl ends. Emulsifiers facilitate the configurational changes undergone by the molecules. In the case of tristearin fatty acid, the emulsifier is able to control both the structural and the chemical polymorphic transformation.

Johansson and Bergenståhl (1992a) investigated the influence of food emulsifiers on fat and sugar dispersions in oils. They analysed the interactions of these molecules within the system using adsorption, sedimentation and rheological techniques. They discovered that the emulsifiers had less adsorption of the fat crystals compared to the adsorption of sugar crystals. In the presence of fat crystals and at low emulsifiers concentrations, the adhesion between the molecules and crystals was reduced. All the emulsifiers interacted in the same way with the fat crystals, but monoglycerides interacted specifically with the sugar crystals for all the studied concentrations. In the case of sugar crystals, the general tendency was for the emulsifiers to form a tightly packed monolayer. The addition of phospholipids was found to result in denser sediments than with the addition of monoglycerides.

Mackie, Hey and Mitchell (1989) studied the behaviour of lecithin on silica particles in non-aqueous media. They discovered that in alcohol the emulsifier enhances the interactions between the silica particles. Conversely, in a dodecane medium, lecithin addition tends to reduce the adhesion between the particles. This was attributed to steric effects of an enthalpic rather than entropic nature. They also confirmed that the dispersion of particles coated by lecithin is dependent on the quality of the solvent that is used.

Garti and Yano (2001) described the role of such emulsifiers in fat crystallisation. It was stated that the presence of such molecules drastically influences the physical and functional properties of the products in terms of solid-liquid separation and the flow behaviour of the medium as well as the particles. Due to the amphiphilic character of the emulsifiers these can simultaneously interact with fat molecules and other types of polar molecule (e.g. water). They explained that interactions reduce the interfacial energy of oil/water systems.

Garti and Yano (2001) emphasized the fact that due to their particular amphiphilic structure, the strength of the emulsifier interactions is constantly changing within the two media. As a result, a migration of emulsifiers between the two phases occurs all the time. They also explained that the stable physical state of emulsifiers, such as solid for saturated emulsifiers, has a great effect on the crystallisation process. They extended their research to common emulsifiers like lecithin from different glycerides and from different esters (sorbitan and sugar ones). They discovered that the interactions of the lecithin with fat are complex and this results in aggregated networks involving fat and emulsifiers.

Garti and Yano (2001) explained that the effects of emulsifiers are involved in the preliminary stages of the fat network mechanism previously stated by Walstra *et al.* in 2001. In the formation of nuclei, the tendency of emulsifiers is to accelerate the rate of nucleation at the interface. In terms of crystal growth, Walstra *et al.* (2001) stated that emulsifiers will modify the rate of polymorphic transitions in order to enhance the suitable adsorptions of fat crystals with the emulsifiers. Garti and Yano (2001) referred to the work carried out by Johansson and Bergenståhl (1992a) in terms of emulsifiers affecting the dispersion of particles in oil via different methods. They noted that the addition of emulsifiers to food products brings wide ranges of reactions. This varies from the adhesion of fat crystals at the interface of different emulsions to the sintering of fat crystals *via* stability of food emulsions compensated by the addition of emulsifiers.

All these literature reviews show the importance of emulsifiers in food product formulation and indicate the range of reactions, which can be encountered while dispersing particles in a liquid media such as oil. The flow of such dispersions may also be dependent on residual surface-active compounds already present in the dispersion medium.

### I.7.4.3. Presence of residual compounds

Residual components in liquids are present in very small quantities. In order to work with purified samples and to remove all the undesirable compounds, several types of processes can be used. Coulson and Richardson (1991) stated that the most important separation process in the industry involves adsorption. This process involves the separation of molecules in the presence of two phases: solid/liquid and liquid/liquid (or liquid/gas). This separation depends on one component in the binary solution being more readily adsorbed than the other. They divided the mechanism into two stages:

1) The first step is the formation of a monolayer over the surface of the solid. This layer may be chemisorbed or associated with a change in free energy.

2) The second step is the successive formation of layers as the concentration in the fluid increases. The size of the adsorbent's pores determines the number of layers that can be formed.

In order to remove traces of residue, adsorbents are required to have particular characteristics. They are commonly used in the form of granules bigger than 6 mm. Coulson and Richardson (1991) have stated that good adsorbents have a large internal surface area which is accessible via large pores in order to admit adsorption of large molecules. Adsorbents should also be able to regenerate easily and should not loose their adsorptive properties while recycled. The most widely used adsorbents are molecular sieves and activated carbon. Table 1.12 shows the different properties of these two kinds of adsorbents.

**Table 1.12:** Physical properties of two major adsorbents

| Molecular Sieves | Activated Carbon |
|---|---|
| Lattice structure.<br>Tetrahedral Si or Al arranged in different ways<br>Dimension: from 0.3 nm to 1 nm (precise dimensions) | Require additional treatment (activation)<br>Treatment via ZnCl or $H_3PO_4$.<br>Surface area: 106 $m^2/kg$<br>Set pores diameter: 2 nm<br>Can be used as powder or granules. |

Coulson and Richardson (1991) discussed the fact that activated carbon has a low affinity for water molecules. Due to this characteristic, activated carbon will be mainly used in aqueous solutions or moist gases. However the presence of water in mixtures can alter on experimental results. Research conducted on activated carbon in order to understand its activity and behaviour in several systems, have been summarised as follows.

Johansson and Bergenståhl (1992c) investigated the influence of water in systems of fat and sugar crystals dispersed in oil. In order to purify their oils, they used activated carbon as the adsorbent. They discovered that the presence of water had a great impact on the adhesion of the fat and sugar crystals in oils whether emulsifiers were present or not. Water molecules also increased the surface activity of the oil. They also investigated the effects of the addition of minor oil crystals such as non-triglycerides into the system. These oil compounds produced a small increase in the adhesion of fat and sugar crystals in the oils. But they did not influence the adsorption of food emulsifiers in any of the cases.

Claesson *et al.* (1997) showed that the presence of surfactants has great influence on the adsorption of mica surfaces in a triolein media. They observed that at different water activities, water may have a dramatic effect on the structure and the re-orientation of triolein molecules at the mica surface. They also noted the formation of water capillary condensation. This phenomenon was observed for near water saturation at specific contact position. They explained that this phenomena, which will destabilise the colloidal dispersions, is induced by the formation of hydrogen bonds between water molecules and the ester group of the triolein molecules.

In addition to this work, Dedinaite & Campbell (2000) compared the surface interactions of polyglycerol polyricinolate (PGPR) and phosphotidylethanolamine (PE) and the combination of PGPR/PE in presence of water at the surfaces of mica plates. Their results showed that: PE was acting much more like a dispersing agent whereas PGPR was behaving like an additive with the formation of capillaries at the surface. The combination of these two surfactants at near water saturation led to a formation of a viscous, sticky adsorbed layer.

Gaonkar (1989) studied the effect of purification methods for different oils. He used three types of adsorbent and compared them in terms of interfacial tension (IT). He observed that silica gel and charcoal adsorbents are less effective at removing impurities, since their interfacial tension was observed to be decreasing with time. On the other hand, by using a column of florisil (40 g / 1 L), he noted that this adsorbent had a great efficiency in removing impurities. Florisil is made of activated magnesium silicate. He also tested different adsorbents for soybean, corn, canola and olive oils as well as for monopalmitin, olein, linolein and dilinolein. He explained that surface-active impurities interfere with the molecular adsorption resulting in erroneous data. The use of florisil is effective for purification of oil contaminated with different kinds of impurities. Gaonkar's work (1989) suggests that the interactions of colloids in dispersing media may be studied in different fields of rheology, such as viscosity and surface-active rheology.

Dedinaite *et al.* (1998) used a different approach to purify oils from water residues. In order to remove the water from all the constituents (melted oils, phospholipid solution, mica layer and sucrose crystals) they were kept in a hermitically closed box at 45 °C in presence of phosophorous pentoxide. Under these conditions, adsorptions of surfactants and minor entities to the mica surface plate were occurred.

## *I.8. Conclusion*

This chapter has briefly discussed on the different origins of the actual chocolate involving the ingredients and processes. Our main emphasis was to perform some research on the main components of chocolate which would alter the physical properties of the product: sugar, oil and emulsifier. In order to understand further the implication of such ingredients in our mixture, we attempted to define a colloidal model.

The dispersion of spherical particles in a liquid medium has been used to identify our colloidal model and the different interactive forces. We identified three main interactions present in the suspensions made of particles dispersed into a liquid. These interactions are the van der Waals, the steric interactions and the hydrogen bondings. We investigated how these interactions influence the suspension of spherical particles in the system.

Furthermore we looked precisely at properties of the sugar particles, the liquid oil and the emulsifier. These properties were investigated by several kinds of parameters: the particle itself with its nature, its particle's distribution and its shape; the surrounded liquid with its specific chemical and physical characteristics; and finally, the presence of surfactant which may be naturally present in the medium or may be added to the system. We showed through a provided literature review that all these parameters affect to a different extent the stability, the rheology, and the texture of a designed suspended blend.

Our research which was based on the interactions present in chocolate involved observation of these interactions in different liquid media and at different particle concentrations in the suspensions. We specifically investigated parameters such as the chemical and the physical properties of the surrounded liquid by studying eight oils; we looked at the effect of the sugar concentration in these systems at three different concentrations; and we observed the effect of the presence of emulsifier onto the stability of the interactions by adding several kinds of emulsifier; finally we looked at the competition of inherent and added surface active components of different oils.

This thesis contributes to gaining a specific knowledge towards the addition of surfactants in blends of sugar dispersed in eight different oils from molecular to pilot plant scales. It studies the stability of sugar suspended systems with the addition of different origins of surface-active components. It also investigates the specific relationship between added emulsifier and the presence of naturally present surface-active components contained in the surrounded fluid.

The following chapter relates on the different techniques of analysis which are currently used for determining the strength of the interactions of particles dispersed in liquid media. Four different techniques were selected to characterise the interactions of our systems: adsorption, sedimentation, confocal microscopy and rheology.

# *Chapter II Background to Experimental Methodology and Materials*

## *II.1. Introduction to Methodology*

In this chapter is discussed previous relevant literature related to the analysis the colloidal systems. The chapter is subdivided into four sections: rheology, sedimentation, emulsifier adsorption, and confocal microscopy.

Rheology is the main experimental technique which has been used in this project. The study of the different kinds of flow behaviour is reported and some theoretical models are mentioned. Sedimentation is a gravity settling method which has also been used extensively in this project. The use of this technique is based on the work of Johansson and Bergenståhl (1992a). Adsorption of emulsifier via chromatography has also been used in this project in order to define the level of phosphatidylcholine adsorbed at the surface of sugar particles. Finally attempts have been made with confocal microscopy in order to observe the effect of the interactions of the sugar particles in the different oils.

## *II.2. Rheology Technique*

The present section gives a brief general overview of the physical properties which are defined with the rheology technique. Rheology is the study of the flow and deformation of materials. Two main properties are exhibited in rheology: solid-like character and flow behaviour (Sherman, 1983). In principle everything flows at some particular velocity, even apparently solid-like materials. Dickinson (1992) explained that even hard materials can flow like liquids as long as they are subjected to large forces.

Considine (1983) discussed the behaviour of different fluids. He explained that flow properties vary between two extremes: the Hookean solid and the Newtonian liquid. Sherman (1983) subdivided rheological theory into three types: Newtonian flow, non-Newtonian flow, and viscoelasticity. Most of these definitions are based on the relationship between two physical entities: *stress* and *strain* (Ross-Murphy, 1988; Dickinson, 1992).

Rheology involves the understanding of the flow of systems under small or large deformations. In the case of chocolate confectionery, Chevalley (1999) explained that interest in chocolate flow properties is mainly limited to that of the melted state of this product. The flow behaviour of molten chocolate has been described in terms of the models of Bingham and Casson (Nelson and Beckett, 1999; Aguilar and Ziegler, 1995). The Casson equation will be further detailed in this chapter. However, more general theoretical models also seem adequate for the evaluation of chocolate flow behaviour (Servais *et al.*, 2004). The following section will detail principles underlying the different flow properties, both Newtonian and non-Newtonian.

### *II.2.1. Newtonian flow*

Hiemenz (1986) explained that, in the case of a liquid confined between two plates that are parallel and separated by a known distance, the liquid will tend to move in a similar direction as the force which is applied to it. In other words, Newton's law implies that the shear stress remains proportional to the shear strain-rate. Dickinson (1992) defined the shear stress as the force divided by the area parallel to the force direction, and the shear strain rate is defined as the velocity gradient as shown in Figure 2.1.

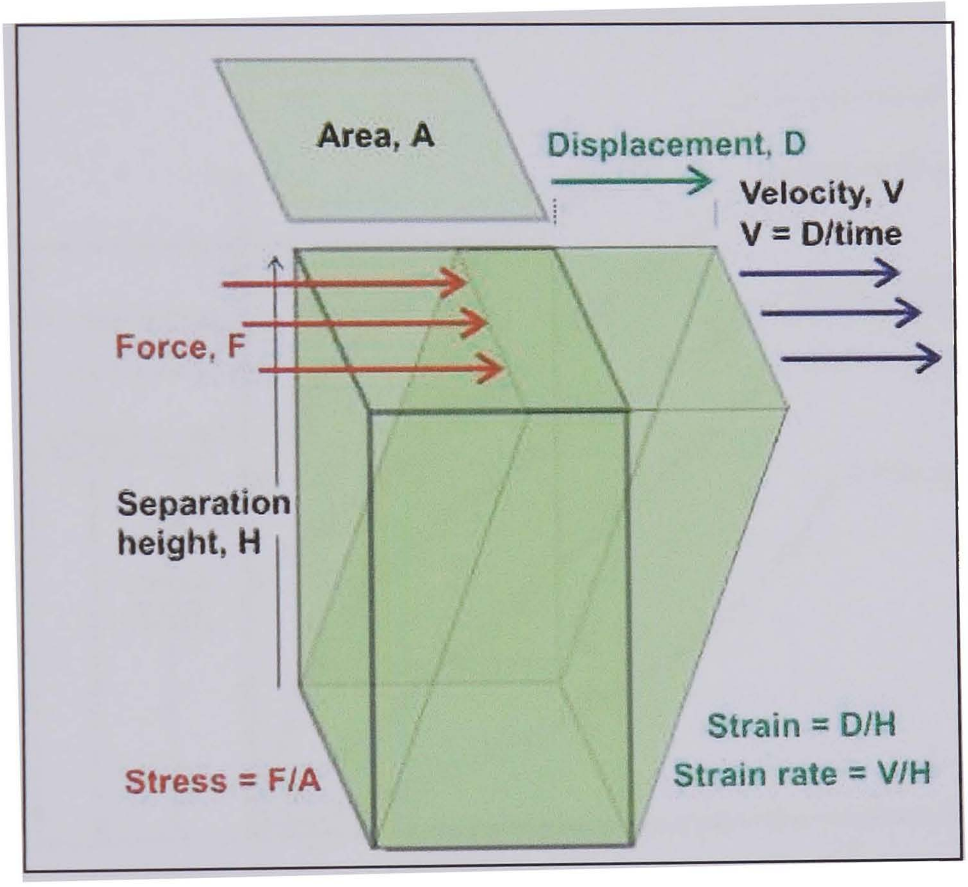


**Figure 2.1:** Representation of shear deformational properties (from Chaplin in www.sbu.ac.uk/water/, 2002). The red arrows show the force applied to the system; the green arrow shows the displacement of the system; and the blue arrows indicate the velocity.

Sherman (1983) stated that the constant linking stress and strain is known as the *viscosity,* and is commonly expressed as:

$$\eta = \frac{\tau}{\dot{\gamma}^{\alpha}} \qquad \textbf{(2.1)}$$

Here $\eta$ represents the viscosity (Pa $s^{-1}$), $\tau$ and $\dot{\gamma}$ are described as the shear stress and shear rate, respectively, and $\alpha$ is a constant of any positive value. Sherman (1983) explained that for Newtonian flow the value of $\alpha = 1$. True liquids exhibiting Newtonian flow have precise viscosity coefficients and are mainly very dilute solutions (e.g. milk at 35°C) or aqueous or organic systems (Dickinson, 1992).

Several representations of the relationship between shear stress and shear strain exist. Figure 2.2 shows only the curves associated with the Newtonian and the non-Newtonian behaviours as illustrated by Sherman (1983).

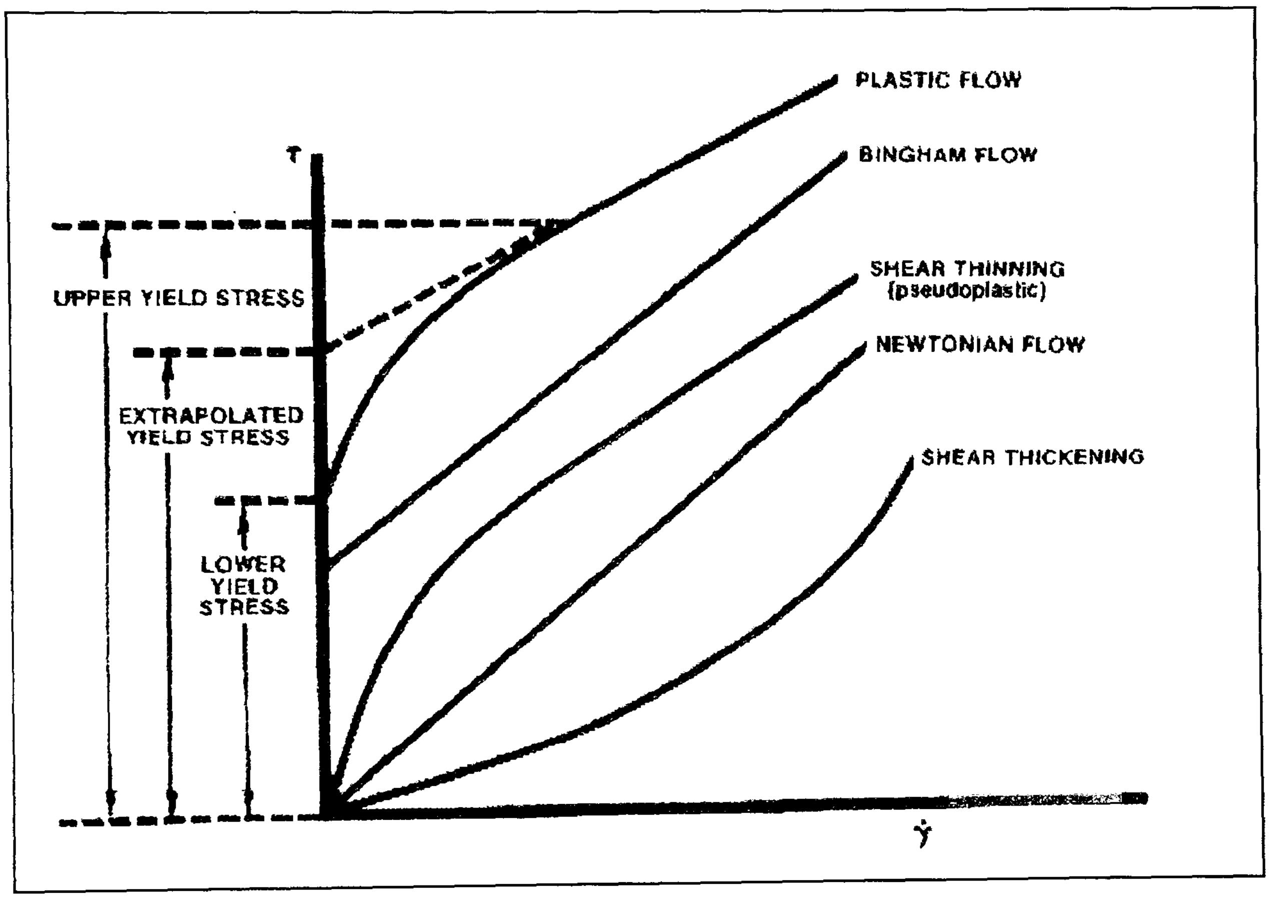


**Figure 2.2:** Types of Newtonian and non-Newtonian rheological behaviour commonly exhibited by fluids (from Sherman, 1983).

### *II.2.2. Non-Newtonian flow*

For many complex food systems, the shear stress and the shear-rate are not linearly related (Dickinson, 1992). Four different types of non-Newtonian behaviour exist: pseudoplastic (or shear-thinning), Bingham flow, plastic flow, and dilatancy (or shear-thickening) (Sherman, 1983; Dickinson, 1992).

**Shear thinning** is the most common type of non-Newtonian flow in food systems and is also commonly observed in the ink and paper industries (Dickinson, 1992). In reference to the Figure 2.2, the viscosity of the shear thinning flow exhibits a curvilinear shape and reaches a constant minimum value of shear rate. As the shear rate increases, the relationship between shear stress and rate becomes more linear. This type of flow behaviour may be expressed using the equation 2.1. However, Sherman (1983) explained that in the case of shear thinning behaviour, the constant $\alpha$ is less than 1, which is a lower value compared to that of the Newtonian flow ($\alpha = 1$).

**Bingham flow** shows linearity in the relationship between stress and rate over a wide range of applied rates. The only difference from Newtonian flow is that Bingham behaviour exhibits a yield stress value ($\tau_0$) whereas Newtonian flow has no yield stress (Sherman, 1983). In 1997, IUPAC gave a general definition of the yield stress, stipulating that the "*yield stress is the shear stress which yielding start abruptly*". The yield stress value is of the product itself at which yielding occurs (IUPAC, 1997). However both flows have the same value for the constant $\alpha$ which is equal to 1. Both curves are represented in Figure 2.2. At low stresses the Bingham model behaves like a Hookean solid, indicating that zero or little movement occurs below the yield stress (Considine, 1983; Dickinson, 1992). At a stress value equal to the stress yield value ($\tau_0$) the Bingham model will exhibit Newtonian flow. The rheology follows the equation:

$$\eta = \frac{(\tau - \tau_0)}{\dot{\gamma}^{\alpha}} \qquad \textbf{(2.2)}$$

Bingham-type flow behaviour is important in some systems, and it is a useful model in the chewing gum or chocolate confectioneries. It allows the determination of the plastic viscosity of various kinds of slurries (Considine, 1983; Chevalley, 1999; Nelson and Beckett, 1999)

In Figure 2.2, it can be noted that **plastic flow** behaves in the same way as a shear-thinning flow: its viscosity has a curvilinear shape at low shear rates, and as the shear-rate increases, a linear response is obtained between shear stress and shear rate. The difference between these two curves is that the plastic flow curve has a yield stress value. Sherman (1983) stated that the flow will not exhibit shear thinning properties unless its applied stress equals the yield stress value, and this is only the case when $\alpha$ is greater than 1. In Figure 2.2, three different definitions relate on the yield stress value. The "*lower yield stress*" represents the value at which shear thinning behaviour is observed. The "*upper yield stress*" represents the yield stress value at which the Newtonian flow behaviour is applicable. The "*extrapolated yield stress*" is obtained by extrapolating the flow behaviour to the Bingham model.

**Shear thickening** or dilatancy is a less common type of flow, which is totally differently to the others (Considine, 1983; Dickinson, 1992). In fact, it describes a flow curve that displays stress increasing with the shear rate (Sherman, 1983). The flow follows equation 2.1 with the constant $\alpha$ greater than 1. Considine (1983) gave some food examples, i.e. peanut butter and some candy compounds, which follow this type of flow behaviour.

Other forces referring to the mechanical spectroscopy may be encountered. These forces allow the differentiation of the plastic behaviour from the liquid behaviour in a sample. These forces are commonly defined by the storage (elasticity) modulus, G', and the loss (viscosity) modulus, G''. However, these forces are mainly used for determining the small deformations of the samples. As our study is based on large deformations of the sample, spectroscopy measurements is not appropriate in our study.

### *II.2.3. Theoretical models*

Chocolate can be generally considered as a concentrated suspension made of mainly sugar particles dispersed in an oil medium (Walstra, 1996). Dense slurries are complex fluids exhibiting characteristic rheological behaviour (Nelson and Beckett, 1999). These kinds of systems have been theoretically studied, and they are described with reference to models such as those of Einstein, Roscoe, and Krieger and Dougherty. Table 2.1 gives the common semi empirical equations used for characterising the viscosity of concentrated suspensions.

**Table 2.1:** Different models applicable to the rheological behaviour of dense suspensions of particles as a function of volume fraction $\phi$

| Equations name | Equation |
|---|---|
| Einstein | $\eta = \eta_0(1+[\eta]\phi)$ |
| Roscoe | $\eta = \eta_0(1-1.35\phi)^{-K}$ |
| Krieger-Dougherty | $\frac{\eta}{\eta_0} = (1-\frac{\phi}{\phi_{\max}})^{-[\eta]\phi_{\max}}$ |
| Casson | $\sqrt{\tau} = \sqrt{\tau_{CA}} + \sqrt{\mu_{CA}} \times \sqrt{\dot{\gamma}}$ |

Variable definitions: $\eta$: viscosity of the suspension; $\eta_0$: viscosity of the liquid/medium; $[\eta]$: intrinsic viscosity of the suspension; $\phi$: volume fraction of solid; $\phi_{\max}$: maximum packing fraction; $\tau$: yield stress; $\tau_{CA}$: Casson yield stress; $\mu_{CA}$: Casson viscosity; and $\dot{\gamma}$ : shear rate

Einstein (1906) stated that the viscosity of any dilute dispersion is directly linked to the volume fraction of solid present in the blend. As the dispersed phase concentration increases, the related viscosity also increases. This theoretical model is based on the assumption that the particles are not interacting between each other and that they are considered as unsolvated and rigid spheres (Prentice, 1894; Dickinson, 1992; Hiemenz, 1986; Pal, 1999). Ferraris (1999) and Pal (1999) have explained that this model remains mostly applicable only for dilute suspensions where the level of interactions is weak.

Based on Einstein's original model, Roscoe (1952) extended the relation between the suspended concentration and the viscosity to higher solid volume fraction. This refined model takes into account the fact that particles are interacting between each other (Ferraris, 1999). This leads to the assumption that newly concentrated systems are treated like dilute suspensions in a continuous medium viscosity. However, this model uses the volume fraction ($\phi$) which represents the volume of particles over the total volume of the mixture. This model is limited to the volume fraction at which the particles form a 3-D network (Chen and Fan, 2002). In the study of semisolid metal slurries, with a low interaction state, the maximum effective volume fraction obtainable with this model is $\phi = 0.6$. This value is still quite small, since in many systems of interest the volume fraction exceeds 0.6.

Krieger and Dougherty (1959) suggested that an equilibrium exists between individual spherical particles, which are constantly associated and then dissociated. From their work, they developed a rheological model based on the determination of the intrinsic viscosity via the particle-liquid interactions (Ferraris, 1999; Pal, 1999; Quemada, 1998). Moreover this equation takes into account the maximum packing factor, $(\phi)_{max}$, which is mainly used a fitting parameter for data for concentrated suspensions (Quemada *et al*, 1985; Quemada, 1998; Fang *et al.*, 1994). However, Quemada and Berli (2002) explain that Krieger and Dougherty's semi-empirical equation reflects only one area of the rheological modelling of suspensions. They further explain that Krieger and Dougherty's model uses only one parameter (the maximum packing factor) and omit different other parameters. Quemada and Berli (2002) state that phase transitions occur at different volume fraction for some particles, as influenced by the following parameters: polydispersity in size, shape or surface charge. Moreover, Quemada and Berli characterised the rheology of suspensions by using three distinctive theories: classical theory referring to the rate of processes for molecular fluids (Eyring's theory); balance of colloidal forces (Blachford's theory); and the structural concepts in which forces perturbing the microstructure. According to this last approach, they develop a viscosity model which has been subjected to testing in different applications. This equation takes into account the balance of hydrodynamic forces, and the Brownian motion for determining the viscosity of concentrated material. The interaction energy may also be represented by the electrostatic repulsion forces in the case that hydrodynamic interactions are greater than the Brownian motion.

Unfortunately, as Ferraris (1999) explained it, such defined equation models are not commonly used for specific suspensions. Systems such as concrete present extremely complex interactions that make application of the previous rheological models described above impossible (Ferraris, 1999). Despite the complexity of the system, chocolate has been analysed rheologically (Walstra, 1996; Nelson and Beckett, 1999). Chevalley (1999) refers to two kinds of models used for the interpretation of chocolate viscosity: the Casson equation and the Bingham flow.

The confectionery industry has commonly used the Casson equation to assess the quality of melted chocolate and to interpret its rheology (Nelson and Beckett, 1999; Chevalley, 1975, 1999; Aguilar and Ziegler, 1995). In most cases, the chocolate flow behaviour matches the equation for high shear rates and it crosses the shear stress axis to give the Casson yield stress value. The Casson equation is represented by

$$\sqrt{\tau} = \sqrt{\tau_{CA}} + \sqrt{\mu_{CA}} \times \sqrt{\dot{\gamma}} \qquad \textbf{(2.3)}$$

where $\tau_{CA}$ is the Casson yield stress value, and $\mu_{CA}$ is the viscosity exhibited at a yield stress equivalent to or bigger than the Casson yield stress value. The Casson yield stress is obtained by extrapolating the flow curve at a shear rate value of zero.

At lower shear rates, the rheology data generally do not fit the Casson equation so well. Prentice (1983) has explained that the Casson equation gives a precise specification of the stress required to initiate the breakdown of the chocolate structure. On the other hand, Servais *et al.* (2004) showed that, out of 46 chocolate samples, the Casson equation was not accurate enough for defining the rheological behaviour of their samples. Therefore they developed a method which measures the viscosity at two distinctive shear rates (5 and 40 $s^{-1}$). Servais *et al* (2004) explained that this technique has the advantage of being simple, accurate and readily applicable to different systems if the rheological history of the network is known.

In this thesis, we have promoted the use of the Casson equation for theoretically analysing our samples. However, the viscosity of trials performed in the industry have been analysed with the recently proposed Servais *et al.* (2004) technique. These latter results will be presented in chapter VII.

After describing some of the different theoretical models used to describe suspension rheology in the following section, a few relevant publications have been selected which describe the measured viscosity of dense slurries, explaining the network fragility of concentrated suspensions in terms of the rheology, and discussing the influence of emulsifiers on the rheology of sugar dispersions.

The first chosen publication shows the importance of particle size in coal samples. Wildemuth and Williams (1985) prepared three different powder stocks (stock I: 400 sieve size; Stock II: 400 – 200 sieves size; Stock III: blend of stock I & II) made with coal. They suspended each of these powders into three different media. The dispersing media had different chemical structures and different viscosities, but they all had the same density as the coal particles. They measured the relative viscosity and correlated it with the volume fraction under the maximal packing fraction. In this work, they observed the role of the particle-size distribution and that of the suspending fluid character. They developed a new model, which correlated the relative viscosity with the stress dependence for non-Newtonian fluids. Their investigation concluded that the stress and not the shear strain rate governs the microstructure and the rheology of such dense slurries. They also observed some anomalies, which were due to the suspension behaviour. Furthermore, Bouzas and Brown (1995) explained that the particle size distribution affects the flow properties of molten chocolate. They observed that the wider the particle size distribution is, the lower the viscosity is for the same solid content.

The second publication has been chosen in reference to the suspension of the particles (carbon black) in oil media. Kawaguchi *et al.* (2001) investigated the properties of a carbon black suspension in silicone oil. They observed that shear stress and steady state viscosities, as well as the linear dynamic moduli, were a function of the particle concentration. They noted that, at lower shear-rate, the suspension exhibited some structural breakdown, whereas at higher shear-rate, structural build-up was observed. These suspensions exhibited a solid-like viscoelastic behaviour under linear response and the behaviour of such slurries was interpreted as shear-thickening flow. The rheological behaviour of these particles suspended in oil can be compared with the rheological properties of our sugar particles dispersed in oils.

The third study is selected to indicate the use of specific theoretical models. Fang *et al.* (1995) studied the rheological behaviour of cocoa butter and cocoa mass in order to define the best rheological model for cocoa suspensions. They determined that the cocoa butter viscosity behaved like a Newtonian fluid at temperatures above 40 °C. On the other hand, the viscosity of cocoa mass followed the Casson model, with its yield stress decreasing with increasing temperature. They also fitted the rheological behaviour of concentrated cocoa suspensions ($\phi > 0.20$) to the Krieger and Dougherty model. This results is presented in Figure 2.3.

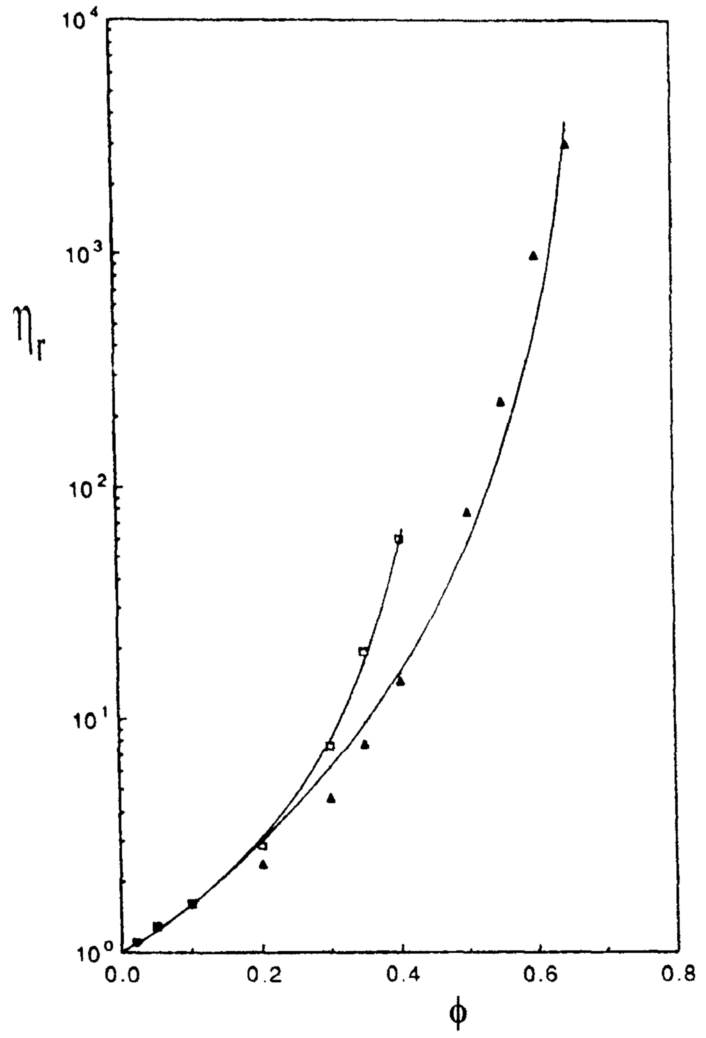


**Figure 2.3:** Relative viscosity ($\eta_r$) of cocoa suspension against the volume fraction ($\phi$) of powder at two shear stress levels: (□) $\sigma = 0.5$ Pa and (▲) $\sigma = 1028$ Pa (from Fang *et al.*, 1995). The solid lines represent the line of the best fit using the Krieger and Dougherty equation model.

Fang *et al.* (1997) showed that three different Cocoa Butter Replacers (CBR) exhibited Newtonian behaviour. They stated that the CBR dispersions could be fitted to the Casson or Quemada rheological models. Condensation of this study will permit us to compare our experimental results with the application of rheological models such as the Einstein model and the Casson equation.

Finally, reference is made to the publication of Johansson and Bergenståhl (1992b) which was a motivation behind our project in determining the importance of emulsifier on the dispersion of sugar particles in specific oils. Johansson and Bergenståhl (1992b) investigated the rheology of sugar dispersions in oils and particularly the influence of emulsifiers on the storage modulus and yield value of the fat. They concluded their work by saying that almost all the emulsifiers influenced the rheological properties of such dispersions. There was also a variation in the magnitude and direction of the rheological change depending on the concentration of the emulsifiers. Johansson and Bergenståhl (1992b) explained that the interactions between the particles were not only influenced by the van der Waals forces but also by the formation of water bridges. They used different models to correlate their results, but stated that the validity of their results properly to fit the different models was questionable. Inspired by their work, we will investigate further the interactions of sugar particles in a different set of oils under the influence or not of emulsifier using several experimental methods: rheology, sedimentation, emulsifier adsorption, and confocal microscopy.

## *II.3. Sedimentation Technique*

The applicability of the sedimentation technique to this project was inspired by the publication of Johansson and Bergenståhl (1992a). This simple technique potentially gives an indication of the strength of the interactions of sugar particles suspended in oils undergoing gravity settling. To facilitate separation, experiments were performed at low particle concentrations.

### *II.3.1. Rate of sedimentation*

Coulson and Richardson (1991) defined sedimentation as the process in which particles settle in a gravitational field, and each particle rapidly reaches its terminal velocity when the frictional force becomes equal to the net gravitational force. The magnitude of the net gravitational force is obtained from to the application of Newton's second law, i.e., as the mass (kg) multiplied by the acceleration due to the gravity (m. $s^{-2}$). Coulson and Richardson (1991) explained that all particles immersed in a fluid exhibit a buoyancy force. In reference to Archimedes` law: the buoyancy force is the upward force on an object which is equivalent to the weight of the replaced liquid or gas that is displaced by the object. Rhodes (1998) and Hiemenz (1986) showed that this force, associated with the drag and gravity forces, results in the net acceleration force acting to separate the particles from the liquid. Equation 2.4 represents the rate of sedimentation of particles suspended in a fluid:

**gravity – buoyancy – drag = acceleration force for particles to sediment (2.4)**

### *II.3.2. Factors Affecting the Rate of Sedimentation*

In this section we mention several parameters which can influence the rate of sedimentation. From a literature review, it has been noted that the properties of the particles, the particle-size distribution and the nature of the liquid medium play the most important roles determining the settling of particles (Coulson and Richardson, 1991; Walstra, 1996; Hiemenz, 1986).

Walstra (1996) has explained that sedimentation is enhanced by any growth of particle size, but it also affects the rate of aggregation of particles that are susceptible to aggregate. Coulson and Richardson (1991) explained that there exist two kinds of sedimentation, one for fine particles and the other for coarse particles. Sedimentation of fine particles will tend to be associated with a high degree of flocculation due to the high specific surface area of the particles. It was observed that generally the velocity of particles tends to decrease steadily as the particle concentration increases; this may be the result of networks of clusters of particles in very dilute systems. They also noted that the formation of aggregates is achieved in well-defined streams.

Hiemenz (1986) reported that larger particles tend to aggregate faster than small particles. Coulson and Richardson (1991) showed that, in flocculating systems, the larger are the aggregates, the faster they will overtake the smaller ones, thereby becoming even larger. They explained that this behaviour would tend to enhance the rate of sedimentation.

Walstra (1996) has explained that particles which are not homogeneous will tend to decrease the rate of settling of particles since the interstitial liquid in the aggregate causes the effective density difference to be smaller. If the volume fraction of particles ($\varphi$) is large, sedimentation is hindered. In an example given by Walstra (1996), for $\varphi = 0.1$, the sedimentation rate is reduced by 60%.

Moreover sedimentation rate is also dependent on the properties of the medium and slight disturbances in the flow (for example, the convection currents) can affect the sedimentation of particles (Coulson and Richardson, 1991; Walstra, 1996; Hiemenz, 1986; Rhodes, 1998). Coulson and Richardson (1991) observed that stirring consolidates the final sediment, in which spanning bridges can be formed amongst the particles. They explained that stirring tends to squeeze out the liquid and the settling becomes even more compact.

Coulson and Richardson (1991) showed that changes in the solution's environmental conditions, such as the salt content or the pH concentrations, will affect particle stability in aqueous media. The stability against aggregation of dispersions is determined by the interactions between the particles during collision. In absence of macromolecules, the stability is mainly defined by the repulsive interactions between similarly charged electrical double-layers. Israelachvili (1992) explained that steric repulsion which affects the stability of polymer-stabilised systems depends on the interactions between the polymer molecules and the medium. In the absence of these repulsive forces, van der

Waals forces will aggregate particles by means of attraction forces (Coulson and Richardson, 1991).

### *II.3.3. Sedimentation Mechanisms*

Coulson and Richardson (1991) reported that concentrated suspensions of fine particles sediment by two different types of mechanisms: type 1 settling and type 2 settling. The settling of particles in the type 1 process is represented by the formation of clearly defined sediment layers at constant sediment rate. Coulson and Richardson (1991) stated that this type of settling is the one most commonly encountered in the industry. The settling type n° 2, which generally occurs with a wide particle distribution, shows no apparent difference in the layers of the particle sediment and no obvious difference in sediment packing.

These two mechanisms are illustrated in Figure 2.4.

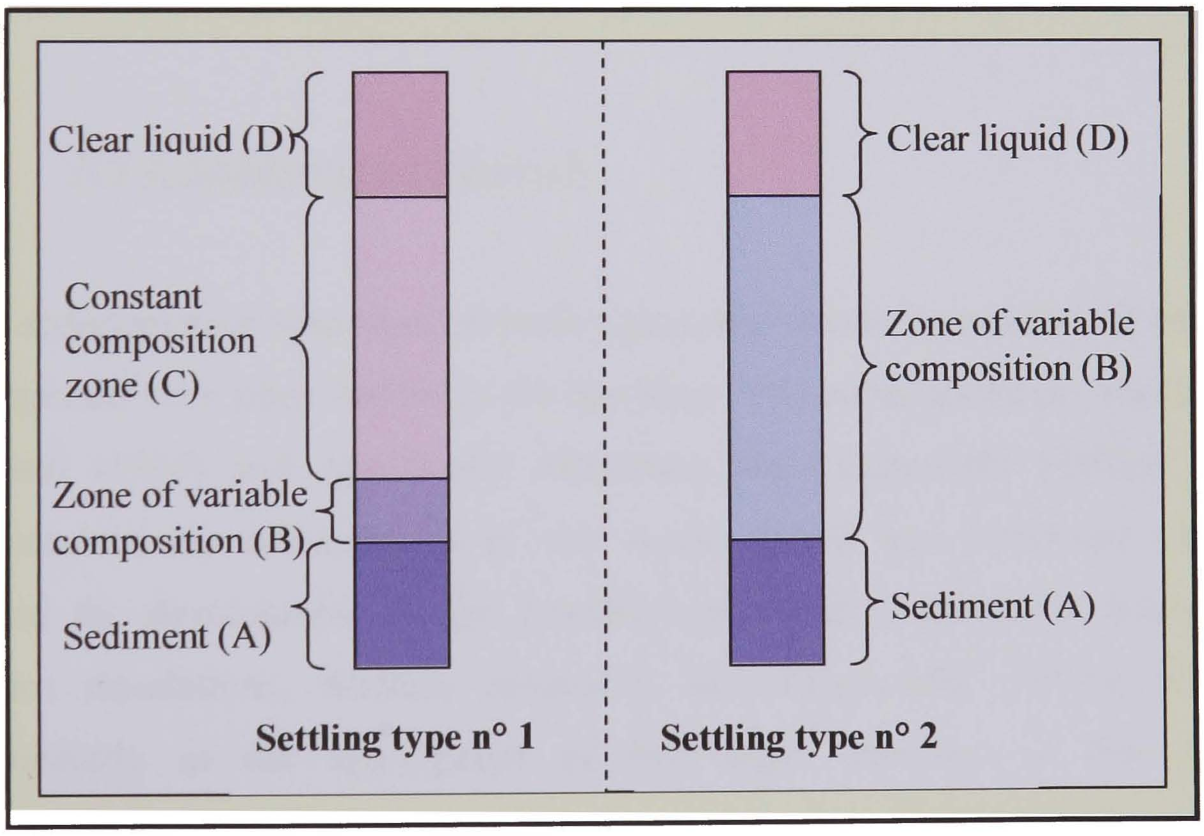


**Figure 2.4:** Different settling mechanisms for particles in liquids. Type n° 1 has been reported as the most common sedimentation behaviour, whereas type n° 2 is rare (Figure inspired by Coulson and Richardson, 1991).

In process type n° 1, the settling occurs as the liquid is pushed out from the packed particles. The formation of sediment is loose at the beginning and the rate of sedimentation remains constant until the upper interface corresponds to the upper zone of variable composition (B). Sedimentation is completed when the constant composition zone (C) and the zone of variable composition (B) disappeared.

The settling type n° 2 is rare, and it only occurs when the width of the size distribution of the particles is large. In this case, there is no zone of constant composition and the rate of sedimentation progressively decreases throughout the whole operation. Finally, the zone of variable composition (B) extends until reaching the top of the interface of the layer of sediment.

The sedimentation mechanism is important in determining the rate at which particles are settling (Coulson and Richardson, 1991). However, the use of sedimentation technique in this project has been inspired by the work of Johansson and Bergenståhl (1992a), in which they consider only sediment volume differences.

### *II.3.4. Sedimentation Research*

The sedimentation behaviour of dense particulate slurries in mineral oil has not been reported very often before in the literature. The publications of Ansell and Dickinson (1986) and specifically Johansson and Bergenståhl (1992a) have contributed to the development of this work. Ansell and Dickinson (1986) modelled the development of the formation a model sediment by Brownian dynamics simulations, whereas Johansson and Bergenståhl (1992a) looked experimentally at the aggregation of fine sugar particles at low sugar concentrations.

The work of Ansell and Dickinson (1986) relates to the formation of gel-like sediment by dynamic simulation. This can be represented by Figure 2.5

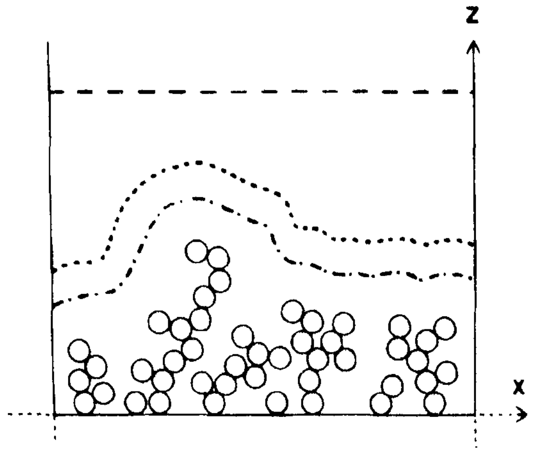


**Figure 2.5:** Representation of simulation reaction box of unit cell x and y. Spherical particles are randomly introduced from the top of the box. (- - -) is the highest sediment volume, (.......) and (-·-·-) represent the Brownian motion and the DLVO interactions (from Ansell and Dickinson, 1986).

They considered three different particles' settling modes: pure ballistic, pure Brownian, and gravity-driven Brownian. The first model is based on linear spherical particle settling without the interaction with the other particles. The second model refers only to the addition of a pure Brownian component. The final model also involves Brownian motion, but also includes the effect of colloidal and hydrodynamic interactions. They explained that the nature of the colloidal forces is extremely important and the presence of Van der Waals attractions promotes the coagulation of the particles. In the presence of large sedimenting forces, they showed that the sediment volume fraction increases as the field strength increases. Furthermore, they stated that the sediment structure for non-interacting particles approaches the randomly packed hard spheres at a volume fraction of 0.64. Finally they explained that the particles are mainly affected by the short-ranged hydrodynamic interactions rather than the long-ranged ones, but they also suggested that particles may have multibody interactions between each other leading to a specific compactness of the sediment.

The publication of Johansson and Bergenståhl (1992a) showed the influence of surfactants on fat and sugar dispersions in oils. They studied different types of emulsifiers and compared their results with information obtained from rheological experiments. They noted that emulsifiers adsorb weakly on fat crystals and that this attraction is only observed at high emulsifier concentrations. In the presence of sugar crystals, the emulsifiers will adsorb more strongly. Tightly packed monolayers are formed and the sugar crystals are generally sterically stabilised as the emulsifier concentrations increase. This means that layers become more compact as the surfaces of the particles tend to become fully covered. When the emulsifier concentrations are diminished, the opposite behaviour occurs. Johansson and Bergenståhl (1992a) also observed that monoglycerides interact in a different manner from others emulsifiers. Such emulsifiers tend to increase the attraction of the sugar crystals at all the different studied concentrations, whereas phospholipids reduce the attraction between the sugar particles and lead to denser sediments. In the case of saturated monoglycerides they noted that a network based on flocculation, occurs involving fat or sugar crystals.

In summary, we have observed that the rate of sedimentation cannot be easily measured (Coulson and Richardson, 1991; Walstra, 1996). We have noted that this sedimentation rate is influenced by several parameters, *e.g.*, properties of the particles and the properties of the medium. We also reported different types of particles aggregation in the sedimentation of fine particles. In this project, we are interested in understanding further the type of particles interaction occurring by determining the adsorption of emulsifier on the sugar particles dispersed in different oils.

## *II.4. Surface Adsorption Technique*

The third technique applied in this investigation relates to the surface adsorption of surfactant in our sugar + oil systems. It may allow us to understand and define the types of interactions present at the surface of the sugar particles in contact with different media. Over recent years, the types of interfacial interactions encountered in different media have been studied (Cleasson *et al.*, 1997). Different methods, such as interfacial tension and surface force measurements have been used to understand and quantify these interactions.

Shaw (1992) has explained that a colloidal system is mainly affected by the particles in a medium regarding the surface properties of: particles, and particle/particle interactions, or particle/solvent interactions. In reality, the surface property of the particles, which can be physical (adsorption) or electrical (double-layer effects), determines the strength of the different interactions present in the system. Surface-active components are amphiphilic entities which can bind to both organic and mineral media simultaneously. The strength of these surface-active compounds can be measured by surface adsorption.

Surface adsorption may be investigated by different analytical methods. These may be static, detachment or dynamic studies. However, Padday (1969) has explained that the analysis of surface measurement accounts for numerous errors due to poor experimentation and misinterpretation of the results. Nevertheless, some advanced techniques allow us to calculate surface activity of emulsifier within a precise range of data. Heimenz (1986) emphasizes on the fact that the surface adsorption and the surface free energy are directly linked to each other. The most common technique used for the determination of the surface activity is the ring method with the Wilhelmy plate or the De Noüy ring. This method enables the calculation of the surface tension whilst in principle it determines the adsorbed quantity of surfactant at the surface of a particle by applying the Gibbs adsorption equation.

$$\Gamma_2 = -(1/RT)d\gamma / d\ln x_2 f_2 \qquad \textbf{(2.4)}$$

where $\Gamma_2$ represents the surface excess concentration, $d\gamma$ is the derivative of the surface tension $\gamma$, $x_2$ is the solute mole fraction, and $f_2$ is the solute activity coefficient.

By the means of this equation it is possible to calculate the amount of adsorbed from the measurement of the variation of surface tension with different solute concentrations. We will now describe the experimental basis of this method.

### *II. 4.1. Surface Tension with the De Noüy Ring*

This detachment method determines the surface tension of two immiscible liquids or the surface at air/liquid by measuring the force required to pull the ring out from the liquid surface. Shaw (1992) explained that this force is calculated by the suspension of the ring or via the torsion arrangement, also known as the De Noüy tension. Calculations for the determination of the detachment force involve equation 2.5:

$$\gamma = \frac{\beta \times F}{4 \times \pi \times R} \qquad \textbf{(2.5)}$$

where $F$ represents the maximum force which can be applied to pull on the ring, $R$ is the mean radius of the ring, $\gamma$ is the surface tension, and $\beta$ is the correction factor. This latter parameter depends on the dimension of the ring and the nature of the interface.

Padday (1969) also explained that at the maximum force, surface tension acts vertically to the plane with the assumption of both outer and inner radii of the ring being equivalent. Figure 2.6 represents the detachment of De Noüy ring at the liquid/air interface.

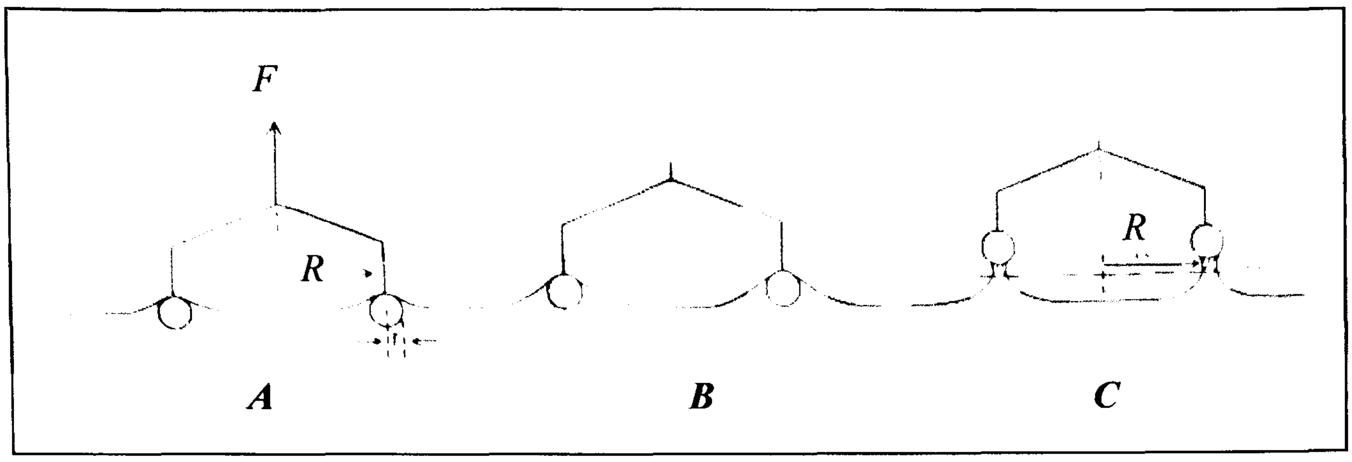


**Figure 2.6:** Force detachment measurement of De Noüy ring at liquid/air interface. *R* is the radius of the De Noüy ring; r is the radius of the cross-section of the De Noüy ring and *F* is the pulling force. ***A***, ***B*** and ***C*** represent three stages at different pulling forces on the De Noüy ring.

Picture *A* in Figure 2.6 represents the position where the De Noüy ring is totally immersed in the liquid and shows the force *F* starting to pull the ring out. As the force increases, the ring is lifted higher along with the surrounding liquid (picture ***B***). Picture ***C*** shows the maximum force required to lift the De Noüy ring out of the liquid. At this stage, the force, which is measured, can be defined as the surface activity of the liquid. Furthermore, this force can be calculated as a surface tension of the liquid via the equation 2.5. If the force is further increased, rupture of the ring in the medium will occur.

Padday (1969) explained that, the measurement of surface tension can lead to misinterpretations of the results due to several errors, such as high density liquids or low surface tension liquid. Therefore, correction factors adapted to the experiments are commonly used (see $\beta$ in equation 2.5). In order to determine the surface activity of oils accurately, different methods have been considered involving direct surface force measurement or indirect determination of the precise surfactant quantity in a media in the presence of various adsorptive particles.

### *III.4.2. Surface Force Measurements*

This section reviews three relevant publications based on the determination of the surfactant adsorption via surface force measurements. This technique is recording the interactions as the force is recorded as a function of the surface separation between two mica plates. The distance of the resolution is approximately 2 Å. The surface of separation is then directly related to the free energy of interaction per unit area.

Claesson *et al.* (1997) studied the interactions of triolein molecules at the surface of a mica plate. Force measurements showed interfacial ordering when anhydrous triolein molecules were used. Triolein was found to be deposited on the mica plates in two molecular layers at variable distances. The presence of water at different concentrations, defined as the water activity ($a_w$), shows different effects like capillary formations with the linkages of hydrogen bonds and ester groups of the triacylglycerol molecules ($a_w \approx 1$); or re-orientation and repulsion at the mica plate ($a_w \approx 0.2$).

Dedinaite and co-workers (Dedinaite *et al.,* 1998; Dedinaite & Campbell, 2000) discussed the interactions between mica plates or sugar particles in the presence of various surfactants (water, PGPR: polyglycerol polyricinoleate, PE: phosphotidylethanolamine, DDOA: dimethyldioctadecylammonium bromide or OPPE: L-α-oleylpalmitoylphosphotidylethanolamine) in oil media. They observed that as the sugar particle surface was not uniform, adsorption of emulsifier occurred only at specific areas of the sugar surface. The adsorption sites are mainly on areas of fractures or cracks in the sugar. They also noted that there was a phase separation of the emulsifier, which would saturate the surface of the particles. The remaining emulsifier would be dispersed into the oil without the formation of multiple layer adsorptions.

In 2000, Dedinaite & Campbell compared the surface interactions of PGPR and PE and the combination of PGPR/PE on mica plates. Both emulsifiers seemed to react differently at the interface: whereas PE acts on the structurally at the surfaces of the mica plates, PGPR acts as a steric barrier, and the combination of both surfactants leads to a complex adsorption at the surfaces of the mica plates. In both publications they also noted that the presence of water has a great influence on the dispersibility of colloidal systems at different water concentrations.

These three publications have shown that surface force technique permits the determination of the extent of surfactant adsorption in oil media. They have also been able to differentiate the levels of adsorption for different emulsifiers under several sets of conditions. After consideration with our sponsor, it was decided to determine the adsorption of surface-active components by other techniques. The surface adsorption may also be determined by techniques involving chromatographic analysis.

### *II.4.3. Adsorption Measurement via Other Methods*

This section shows the development of chromatographic techniques involved in the determination of the extent of adsorption for surfactants present in oils. Hemming and Hawthorne (1996) showed that the determination of lipids via chromatography was fully developed by using three methods: Thin Layer Chromatography (TLC), High Pressure Liquid Chromatography (HPLC) and Gas Liquid Chromatography (GLC). Recent developments mostly include work done with GLC. Christie (1982) explained that all the different lipid classes can be separated via adsorption chromatography. The lipids are generally separated due to their different solubilities in the solvent, which depends on their polarities compared to the adsorbent. Based on the principle developed by Stein and Slawson (1966) the most polar lipid will be eluted first due to its stronger binding to the adsorbent.

The quantification of the surface adsorption by the indirect chromatography method is based on the reference work of Beare-Rogers *et al.* (1992). They suggested a technique to determine the amount of phospholipids of lecithin present via high pressure liquid chromatography. This procedure is now known as the AOCS Official method Ja 7b-91. By making use of this technique, different types of surfactants such as mono- and diglycerides can be studied. Their quantities in oil media can be determined by HPLC in reference to the work done by Berner & Dieffenbacher (1999). This procedure is also known as the AOCS Official method Cd 11d-96.

In this section, we have described how the quantity of adsorbed surfactant can be measured using three different techniques. Firstly, we have shown the determination of the surface adsorption from the De Noüy ring technique. Then we noted that two other techniques could be used for determining the adsorption of surfactants. The first technique refers to the surface force measurements with Claesson *et al.* (1997) publication and the second method refers to the HPLC technique in reference to the AOCS official methods Ja 7b-91 and Cd 11d-96.

## *II.5. Confocal Laser Scanning Microscopy Technique*

Finally, we have investigated another technique for determining the state of aggregation and the strength of interactions of sugar particles dispersed in oils. Pawley (1995) explained that the principle of Confocal Laser Scanning Microscopy (CLSM) is based on the emission of the fluorescence light by a specimen due to irradiation from a laser beam. Firstly, a laser beam is focussed to a small spot by an objective lens, which in turn is focused onto the sample. The laser causes the sample to fluoresce. The emitted fluorescent light is collected back through the same objective lens. A beam splitter directs the collected light to a photo-detector. The photo-detector analyses the signal. The originality of this light microscope is due to a pinhole (confocal aperture) being placed in front of the photo-detector to reduce or eliminate any out-of focus signal.

With the CLSM, the choice of staining agent for the studied samples should be carefully chosen. Greespan *et al.* (1985) explained that several reasons exist for selecting the appropriate dye. He stated that the dye should be strongly coloured and highly soluble in the substances it intends to show. The characteristics of our samples we attempted to analyse are mixtures of sugar particles dispersed into oils. Greespan *et al.* (1985) noted that Nile red was a good staining agent for lipids, phospholipids, cholesterol and triacylglycerols. Nile red, which is also known as benzophenoxazone, is a side product of the reaction based on Nile blue reagent (phenoxazine). Nile red exhibits an intensive fluorescence in all organic solvent at a wavelength range between 570 to 590 nm. From this publication, it was concluded we should use this colour agent for staining our studied oils.

So far, no work to date has been published on the Confocal Laser Scanning Microscopy. However, there are several publications related to the characterisation and the microstructure of the fat crystals in oil media such as cocoa butter (Heathcock, 1985; Hicklin *et al.*, 1985; Narine and Marangoni, 1999). These publications are based on microscopy techniques such as transmission Electron Microscopy (TEM) for the work of Hicklin *et al.* (1985) or Polarised Light Microscopy (PLM) for the work of Narine and Marangoni (1999). Hicklin and co-workers (1985) study the effects of cocoa butter and vegetable fat blends by combining the results between three techniques: electron microscopy, the differential scanning calorimetry, and X-ray diffraction. They correlated the three techniques by knowing the molecular structure and morphology in the different polymorphs created. Narine and Marangoni (1999) differentiated the fat crystal network of two confectionery fats. The microscopic results were also combined with another technique: rheology. Unfortunately, they could not interpret the strength of their fat networks by the PLM technique. However, no work has yet already been presented with Confocal Laser Scanning Microscopy (CLSM).

Consequently, first attempts on the Confocal Laser Scanning Microscopy will be undertaken in order to characterise the interactions of sugar particles in oils. This may allow us to measure the distances between sugar particles and compares the strength of the different sugar networks dispersed in different oils. These results may also be interpreted by combining these results with the ones of sedimentation or rheology techniques.

## *II.6. Methodology Summary*

This chapter has reviewed the background to the four different techniques which have been used in this project. The main emphasis is on the rheology method, as this technique is currently applied routinely in the chocolate confectionery industry (Nelson and Beckett, 1999) and it allows a sensitive indicator of the strength of the interactions amongst the sugar particles dispersed into oils. The two other main research techniques which have been selected, sedimentation and surface adsorption, have arisen from a review of key previous publications (Johansson and Bergenståhl, 1992a) in the attempt to characterise better the nature of the sugar/oil interactions. Finally, a preliminary attempt has been made to use the emerging technique of Confocal Laser Scanning Microscopy.

## *II. 7. Materials' Reference*

In this chapter we listed the common materials which have been used in this project. The different specifications of eight oils, one type of sugar, three surfactants and three adsorbents are given in the following sections.

### *II.7.1. Common material for experiments*

**Sunflower oil** was purchased in a supermarket. Previously Table 1.5 represented the composition of fatty acids for commercial grade sunflower oil (O'Brien 2004).

**Refined Soybean oil** was purchased from Lamotte (Bremen, Germany) (reference USP 24/ BP 98/ Ph. Eur. 3, supplement 2001, lot 2934002.). The specification of this oil, given by the supplier, is shown in Table 2.2.

**Table 2.2:** Physical properties of common refined complies soybean oil (supplier's specification)

| Typical values | |
|---|---|
| Specific Gravity (20 °C) | 0.920 kg m$^{-3}$ |
| Refractive Index (20 °C) | 1.474 |
| Acid value | 0.15 |
| Iodine value | 130 |
| Saponification Value | 192 |
| Peroxide value | Complies |
| Unsaponifiable Matter | 0.84% |
| Heavy Metals | < 0.001% |
| Cottonseed Oil | Complies |

Table 2.3 represents the composition of the fatty acids in the supplied soybean oil.

**Table 2.3:** Fatty acid composition of common refined soybean oil from Lamotte specification (2001)

| Fatty acid composition | % (detection limit 0.05%) |
|---|---|
| < C14:0 | < 0.05 |
| C14:0 | 0.1 |
| C16:0 | 10.4 |
| C16:1 | 0.1 |
| C18:0 | 3.5 |
| C18:1 | 25.3 |
| C18:2 | 53.0 |
| C18:3 | 6.2 |
| C20:0 | 0.4 |
| C20:1 | 0.3 |
| C22:0 | 0.5 |
| C24:0 | 0.2 |
| others | < 0.05 |

**Cocoa butter** was supplied by Nestlé UK Ltd (York) and was originally purchased from ADM Cocoa. No specification or fatty acids composition were given at this stage.

**Palm kernel oil** (ID number: 602123) was obtained from the Nestlé R&D Centre (Marysville) and the product was called spearpoint SCKK Fat Blend.

**Concentrated butter** (box number: 0375) was purchased from Bodfari Producers Limited (Marlston-cum-lache, England) with a minimum fat content of 99.8%. No specification of fatty acids composition was given by the supplier.

**Olein** and **Stearin** were gifts from Lactalis (Petit-Fayt, France). Their specifications given by the supplier are shown in Table 2.4.

**Table 2.4:** Physico-chemical properties of Olein and Stearin

| Typical values | Olein | Stearin |
|---|---|---|
| Moisture content & non fatty components | 0.2% maximum | 0.2% maximum |
| Milk fat content | 99.8% minimum | 99.8% minimum |
| Free fatty acids (% oleic acid) | 0.35% maximum | 0.30% maximum |
| Peroxide value<br>meq active $O_2$ / KG maximum | 0.5 | 0.5 |
| Iron | 0.2 ppm | 0.2 ppm |
| Copper | 0.05 ppm | 0.05 ppm |
| Iodine value | 33.5 | 25.4 |
| Melting point (°C) | 21.4 | 41.3 |

The data for the percentage of solids present at different temperatures are summarised in Table 2.5. This information was also given by the supplier.

**Table 2.5:** Percentages of solid fat for both olein and stearin at different temperatures

| Temperature (°C) | % solids | |
|---|---|---|
| | Olein | Stearin |
| 0 | 54.4 | 76.1 |
| 5 | 47.3 | 74.4 |
| 10 | 38.7 | 71.6 |
| 15 | 20.7 | 59.3 |
| 20 | 6.5 | 47.5 |
| 25 | / | 37.2 |
| 30 | | 25.9 |
| 35 | | 15.2 |
| 40 | | / |

**Sucrose** was used as the sugar referred to with an added 2% cocoa butter. It had a density of 1.230 g $cm^{-3}$, a specific surface area of 0.8417 $m^2$ $g^{-1}$. Diameter of the volume of the mean particles are D [3, 2] = 5.80 µm and D [0,9] = 36 µm. (given by Nestlé UK Ltd. York).

Four different types of surfactants have been used throughout this project. These are known as phosphatidylcholine (PC), linoleic acid, polyglycerol polyricinolate (PGPR) and monoolein. Each surfactant specification has been tabulated in table 2.6.

**Table 2.6:** Surfactant names and specifications used in this project

| | Phosphatidylcholine | Linoleic acid | PGPR | Monoolein |
|---|---|---|---|---|
| Trade name | Epikuron 200 | cis-9,cis-12-octadecadienoic acid | Polyglycerol polyricinolate | Rylo MG 19 Pharma, glycerol monooleate |
| Manufacturer | Degussa Texturants | Sigma-Aldrich (Dorset, England) | Nestlé York (a) | Danisco |
| Reference: | 119040 | L 1376 | - | - |
| Lot | - | 61K1147 | - | 2119/83 |
| Molecular weight (g.$mol^{-1}$) | 773 | 280.4 | 2500 - 3500 | 356 |
| Extra information | PC content: 92.7 % | Density: 0.90 g $mL^{-1}$ | - | Glycerol monoester content: 97.6 % |

*Note*: (a): PGPR was obtained from Nestlé PTC York. The molecular weight has been calculated a different manufacturing company.

Three kinds of adsorbents have been used in this project. Their specifications are given in table 2.7.

**Table 2.7:** Different adsorbents used in this project and their specification.

| | Activated charcoal | Magnesium silicate | Phosphorous pentoxide |
|---|---|---|---|
| Trade name | - | Florisil | |
| Manufacturer | Chemviron "Pittsburg" | BDH (Poole, England) | Sigma-Aldrich (Dorset, England) |
| Reference | - | 15026 | 43141-9 |
| Lot | - | ZA8301542503 | 05708BA |
| Molecular weight (g mol-1) | - | - | 141.95 |
| Extra information | - | Particle size: 100 to 200 US mesh | Purity level: 99.99% |

The water used to perform rheological and sedimentation measurement was single-distilled water.

### *II.7.2. Other materials and methods*

Materials which have been used with specific methods are detailed in the different chapters. Moreover, all the methods are described in the following chapters.

# *Chapter III Adsorption at the sugar particle-fat interface*

## *III.1. Introduction*

Surface adsorption is commonly used for the determination of particles' interactions dispersed in a liquid (Shaw, 1992). This technique can quantify and estimate the strength of different interactions present in specific systems. In the present case only two ways have been selected to measure the strength of particles interactions: the direct and the indirect methods. The first method measures the interactions directly without previous sample treatment. Direct methods include surface tension determination (Shaw, 1992) or surface force measurement (Cleasson *et al*, 1997). In our specific systems, the determination of the rate of adsorption via the indirect method refers mainly to the chromatographic analysis. The rate of adsorption of some surfactants is estimated by defining the level of surfactants present in the oil phase before and after the addition of sugar particles. After separation treatment of the dispersed spheres from the continuous phase, both can be analysed *via* TLC, HPLC and also GLC. As an example, several methodologies have been designed to determine the amount of lecithin (AOCS Official method Ja 7b-91) or free fatty acid (AOCS Official method Cd 11d-96) which have been present in oil samples.

In this section, we have studied the surface adsorption of samples based in eight varieties of oils at three different sugar concentrations with and without the presence of emulsifiers. Firstly, we determined the surface adsorption of our samples *via* surface activity measurement with the De Noüy ring. Secondly, the rate of surfactants adsorption was estimated *via* the indirect method. The surfactants quantities were identified and analysed *via* chromatographic analysis of the oils with HPLC and potentiometer. This work will be presented in this section.

## *III.2. Materials for surface adsorption experiments via HPLC*

The name and specifications of the materials used in the HPLC analysis are given in table 3.1.

**Table 3.1:** Materials specification for HPLC experiments

| Product name | n-Hexane | 2-Propanol | Ethyl acetate |
|---|---|---|---|
| Manufacturer | Merck | Merck | Merck |
| Reference | 1.04391.1000 | 1.09634.1000 | 1.009623.1000 |
| Molecular weight (g mol$^{-1}$) | 86.18 | 60.10 | 88.10 |
| Density (g mL$^{-1}$) | - | 0.78 | - |

| Product name | Formic acid | Acetic acid | Triethylamine |
|---|---|---|---|
| Manufacturer | Merck | Merck | Fluka |
| Reference | 1.00264.1000 | 1.00063.1000 | 90340 |
| Molecular weight (g mol$^{-1}$) | 46.03 | 60.05 | 101.2 |
| Density (g mL$^{-1}$) | 1.22 | 1.05 | 0.727 |

**Petroleum spirit** (40– 60 °C “AnalaR”) was obtained from VWR International Ltd (Leics., England) (reference 10178 6H). It has a density of 0.64 g mL$^{-1}$.

## *III.3. HPLC Instrumentation*

In this section, we list the different pieces of equipment required to run surface adsorption experiments via HPLC chromatography.

*Chromatography column for free fatty acid determination:* **LiChrocart** supplied by Merck (Switzerland). Its reference number was 1.51352.0001. It had silica sorbent with a pore size of 60 Å and a particle size of 10 μm Column dimensions were 250×4 mm.

*Chromatography column for phospholipid determination:* **LiChrospher** supplied by Merck (Switzerland). Its reference number was 1.550836.0001. It had diol sorbent with a pore size of 100 Å. Column dimensions were 125×4 mm.

*Column guards* were used with both columns. These were supplied by Macherey–Nagel and had a product designation cc 8/4 Nucleosil 100 – 5. Their reference number was 721872.40.

*A UV lamp detector* (Osram, 50 W) was used for both methods.

*Chromatographic system* was supplied by Biotek and the software used was Kroma 3000.

*The centrifuge system* used was from Beckman Coulter. Its reference number was J2–HS. The fixed angle rotor was type JA-14 with wide-mouth polycarbonate bottles (reference number: 356013).

## *III.4. Methods*

### *III.4.1. Sample preparation for HPLC analysis*

Dispersions of 10, 30 and 70 wt % sugar in melted fat were prepared for the extraction experiments. A100 g portion of mixture was then mixed with 100 mL petroleum spirit. The sample was mixed for 30 minutes at a speed of 550 rpm on a magnetic plate, and then centrifuged for 10 minutes at 5000 rpm. The solvent part was filtered on Whatman paper (n°1) and kept in a closed round bottom flask to avoid solvent evaporation. The sugar mixture was treated with 100 mL of petroleum spirit and mixed on the plate at 550 rpm for 15 minutes. This procedure was normally repeated twice in order to extract the maximum amount of oil into the solvent.

Finally, the solvent was evaporated using a rotary evaporator at 35 °C. The oil phase, which may contain a small trace of solvent, was then kept in a dark storage area (cold room, -30 °C). The sugar samples were kept in a dark box at room temperature. The oil samples were then ready for analysis.

## *III.5. Results and Discussion*

The physical properties of the oil may change depending on the level of surfactant present in it. In order to measure the differences in the oil properties and to determine the quantity of surfactant adsorbed at the sugar surface, we first decided to measure the surface tension of the oil. Then we measured the quantity of surfactant in the oil phase.

### *III.5.1. Surface activity measurement via tensiometer and De Noüy ring*

Preliminary experiments were conducted with the Krüg tensiometer at 40 °C. Using this technique the surface tension of oil of commercial grade was analysed with and without the presence of emulsifier prior to the involvement of sugar particles in the mixtures. Experiments were conducted with sunflower oil with the presence of phosphatidylcholine as surfactant. Presumably because of the low amount of emulsifier in the oil (0.1 % w/w), no significant difference was observed in the detachment force for samples with or without emulsifier. Hence it was not possible to determine the presence of emulsifier in our oils by this technique, and certainly the quantification of the surfactant adsorbed at the sugar particles surface would not have been possible based on surface tension measurements.

Consequently, it was decided to re-direct our research in another method of surfactant concentration measurement involving high pressure liquid chromatography referred to as the AOCS methods Ja 7b-91 and Cd 11d-96.

### *III.5.2. Free fatty acid determination*

#### **III.5.2.1. Determination of free fatty acids as oleic via potentiometric method**

Based on the standard AOCS analytical method Ca 5a-40, experiments were carried out first on the oils prior to the addition of sugar and surfactant. This allowed us to determine the inherent quantity of free fatty acids in each oil by titration with alkli.

*Method*

A mass of oil from 3 g up to 6.5 g was weighed out in a beaker within an accuracy of ±0.02 g of oil. The oil was then mixed into 50 mL of solvent mixture composed of 94 % ethanol and diethyl ether 1/1 (v/v). Some drops (4 to 5) of lithium chloride (LiCl) saturated in ethanol were then added to this solution. Neutralisation of the oil acidity was achieved by adding alkali solution (0.1 N KOH).

The experiment was repeated twice in order to obtain a good reproducibility. This was done for all the oils with the exception of the fat mixture, due to the lower available quantity of the latter.

### *Calculation*

It is conventional to calculate the percentage of free fatty acids in terms of oleic acid, as this acid is the most commonly occurring free fatty acid in food oils. The percentage of free fatty acids as oleic is given by the formula:

$$ffa = \frac{V \times N \times 28.2}{m} \qquad \textbf{(3.1)}$$

here $V$ is the volume of potassium hydroxide; $N$ represents the exact normality of the standardised potassium hydroxide solution; $m$ is the mass of analysed sample, and the factor 28.2 corresponds to the mean average weight percent composition of oleic acid in a specific reference sample of cocoa butter.

### *Results*

Table 3.2 lists the percentage of free fatty acids as oleic obtained for the eight different studied oils. The experiments were repeated at least twice, and the results were obtained with a confidence interval of ± 0.01 %.

**Table 3.2:** Percentage of free fatty acids in the commercial triglyceride oils

| Type | Free fatty acid as oleic, % |
|---|---|
| Sunflower oil | 0.05 |
| Soybean oil | 0.17 |
| Cocoa butter | 1.94 |
| Palm kernel oil | 0.32 |
| Fat mixture (*) | 1.66 |
| Milk fat | 0.265 |
| Olein | 0.255 |
| Stearin | 0.19 |

*Note*: (*) The fat mixture is made up of 83.5 % of cocoa butter and 16.5 % of milk fat.

### *Conclusion*

It can be observed from Table 3.11 that cocoa butter has the highest content of free fatty acids amongst this set of oils. The next highest value is for the fat mixture. This was expected, as the fat mixture is a blend of cocoa butter and milk fat. We can estimate the expected free fatty acid percentage in the fat mixture from:

**ffa in fat mixture = [(1.94 × % cocoa butter in blend) + (0.265 × % milk fat in blend)]**

We thereby obtain the value of 1.66 % free fatty acid in the fat mixture, confirming the experimental result in Table 3.11.

It can be noted that sunflower oil has the lowest content of free fatty acids (0.05 % ffa as oleic), whereas each of the remaining oils has a quantity of free fatty acids in the range from *ca.* 0.2 % to *ca.* 0.3 %.

### III.5.2.2. Determination of free fatty acids via a chromatographic method

The chosen method to elucidate the quantity of free fatty acid by HPLC is the AOCS official method Cd 11d-96.

*Procedure*

A schematic diagram of the apparatus and procedure is shown in Figure 3.1.

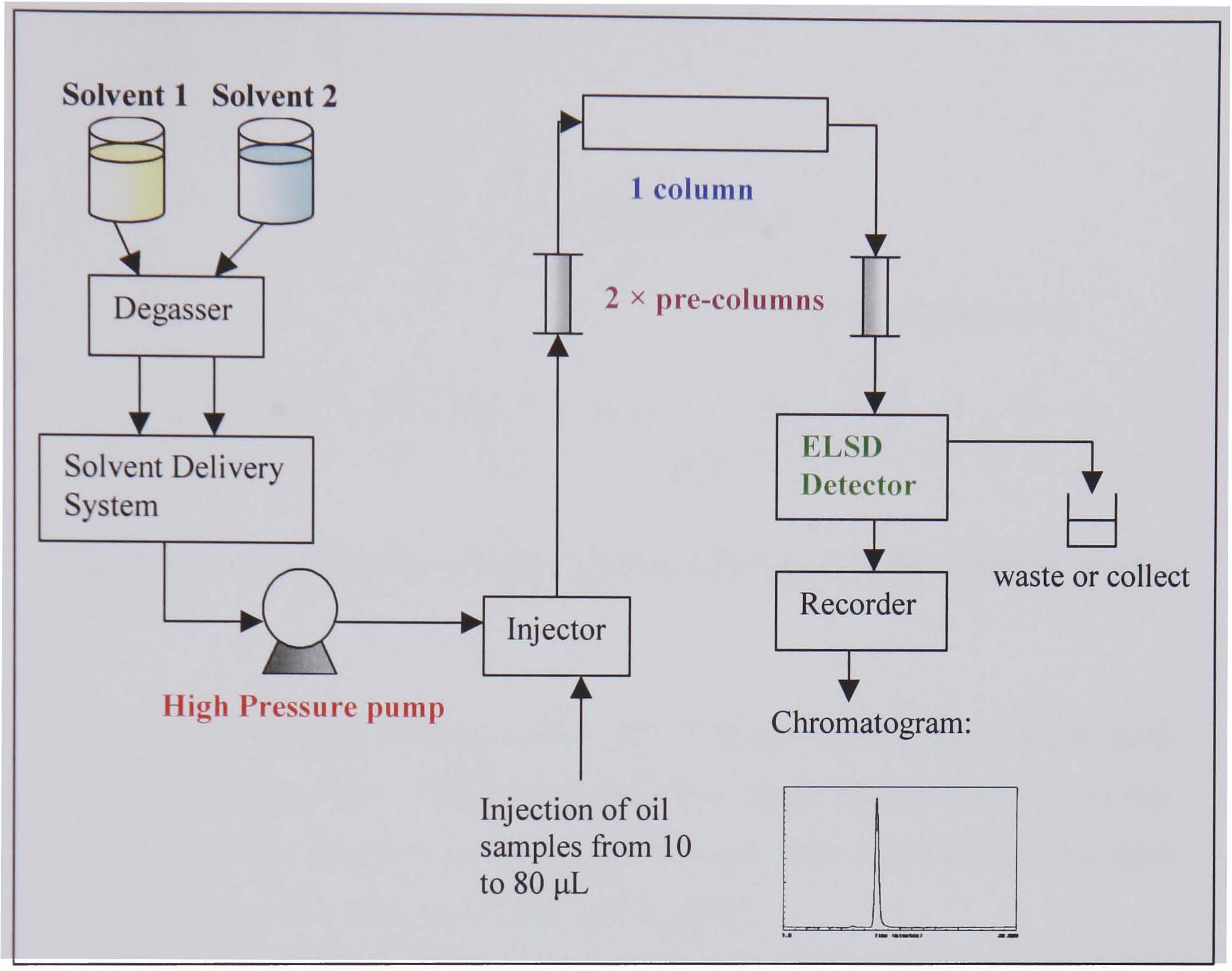


**Figure 3.1:** Schematic diagram of the HPLC procedure used at the NRC, Switzerland

Two different solvents were used for the mobile phase. Solvent 1 contained n-hexane and solvent 2 was a mixture of n-hexane/2-propanol/ethyl acetate/10% formic acid (80:10:10:1, v/v/v/v). The time taken to run each experiment was 19 minutes.

*Peak identification*

Figure 3.2 shows a chromatogram of a cocoa butter sample supplied by Nestlé Research Centre Switzerland (NRC) for preliminary study.

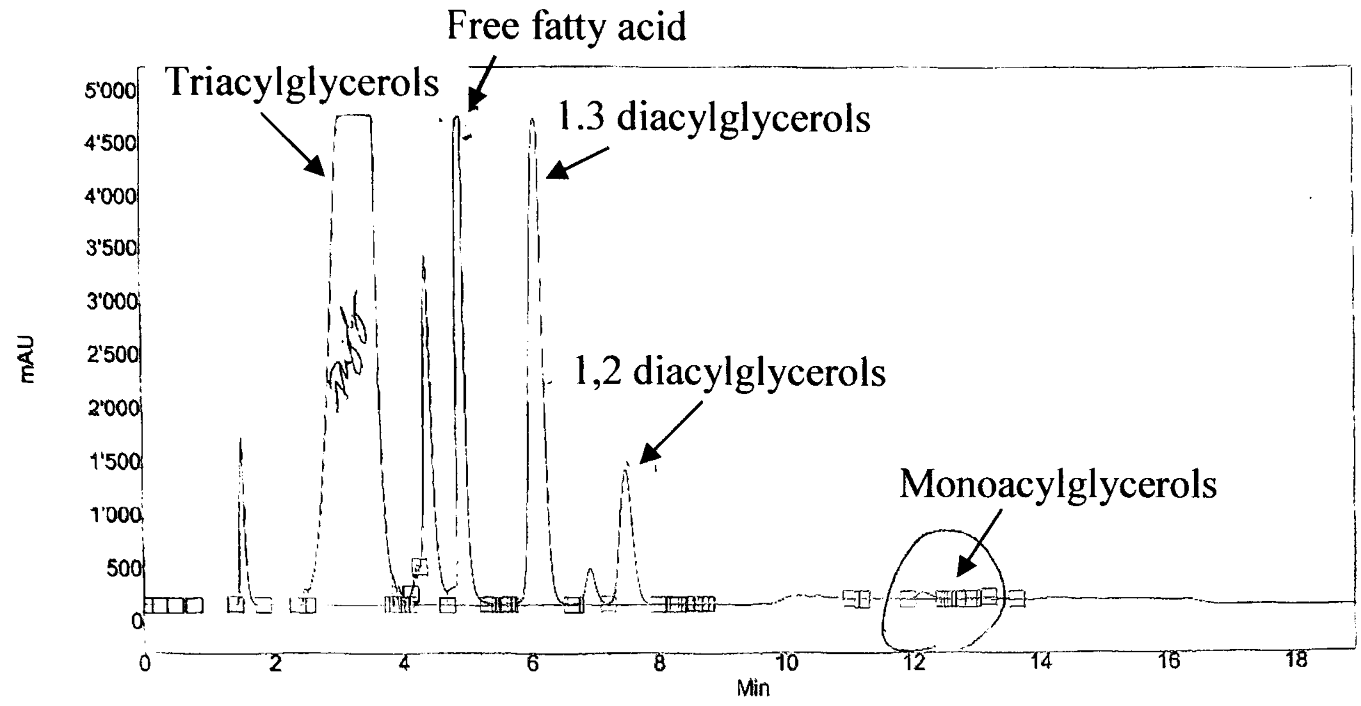


**Figure 3.2:** Chromatogram of cocoa butter at 429.22 mg/ 10 mL, injection volume: 50 μL.

Figure 3.2 indicates various peaks with different elution times. Each peak represents a species with a different polarity from each other component. In this chromatogram we observe that the components which are eluted first are the least polar and those eluted last are the most polar species.

It can be noted that triacylglycerols have a saturated broad peak at a retention time of Rt≈ 3 min. Such behaviour is expected as the triglycerides are generally the most abundant single lipid class in every oil and fat. The free fatty acids exhibit a peak at Rt 5 minutes, whereas the retention times for 1,3 and 1,2 diacylglycerols are at Rt≈ 6 and Rt ≈ 7.8 minutes. It can be observed that there are several minor peaks such as minor 1,3 diacylglycerols and/or phytosterols at Rt ≈ 7 minutes and the peak of monoacylglycerols at Rt ≈ 12 minutes.

Based on such chromatograms work has been developed on the quantification of free fatty acids and diacylglycerol determinations for each oil.

## Cocoa butter example

In order to have a clear understanding of the HPLC analysis procedure adopted, the analysis method used for one characteristic sample (i.e. cocoa butter) is described in detail first, pre summarising all of the results. Using this chromatographic method it has been possible to determine the quantity of free fatty acids in the oil, and also the amounts of 1,3 and 1,2 diacylglycerols. Unfortunately, the concentrations of free fatty acids and diacylglycerols are substantially different, and so different amounts of oil need to be injected for the optimum analysis of these two kinds of components.

The oil which has been analysed was previously separated from the sugar particles (see Chapter V.4.1). The quantity of oil injected for optimum analysis conditions corresponds to a peak surface height between 2000-3000 μV min. For free fatty acids analysis we needed to add approximately 250 μg, of cocoa butter, whereas for the analysis of diacylglycerols some 1000 μg of oil was required to obtain the optimum peak height.

### Free fatty acid determination

After defining the optimum quantity of cocoa butter to inject, 250 μg, we estimated an effective calibration range which would contain this quantity of oil in a dispensing volume of 20 μL. Knowing this mass/volume relationship we defined cocoa butter concentration to make of 12.5 mg mL. However an oil solution of 123.86 mg dispersed into 10 mL of solvent was prepared. From the results obtained in section III.5.2.1, we observed that cocoa butter contained 1.94 % of free fatty acid; consequently the effective concentration of free fatty acid in the present solution is 240. 28 μg mL. Then we injected known volumes of the present solution and measure the peak surface area. The Table 3.3 shows the different volumes used for making the calibration curve as well as the equivalent quantity of free fatty acid added per injection and the corresponding surface area of each peak.

**Table 3.3:** Calculations for the free fatty acid calibration curve of cocoa butter sample.

| Volume injection (µL) | Quantity of ffa per injection (µg µL) (a) | Mean peak surface area (µV min) |
|---|---|---|
| 10 | 2.4028 | 88.1 |
| 20 | 4.8057 | 254.9 |
| 30 | 7.2086 | 389.8 |
| 40 | 9.6115 | 459.6 |
| 50 | 12.014 | 496.3 |

*Notes*: (a) ffa: free fatty acid.

From the Table 3.3, it is possible to plot the quantity of free fatty acids injected versus the peak surface area. Three representative injections were done in order to establish the calibration curve. Figure 3.3 represents the calibration curve of free fatty acids for cocoa butter.

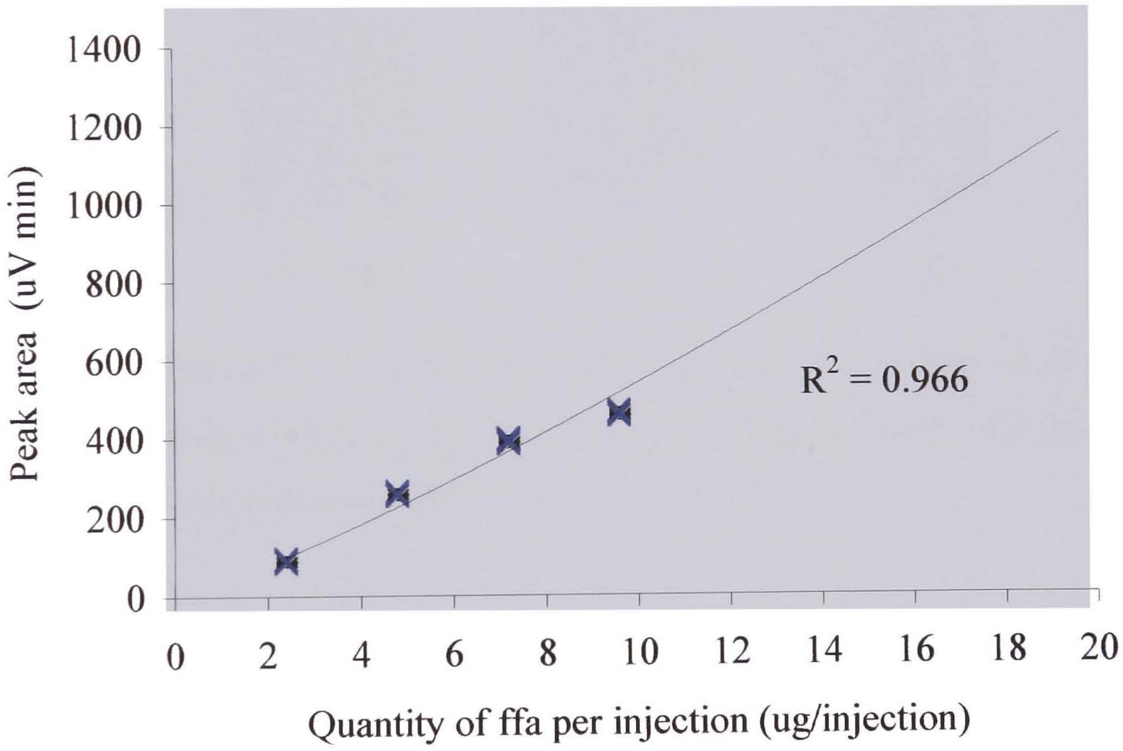


**Figure 3.3:** HPLC Calibration curve of free fatty acids in cocoa butter (experimental errors ± 6.6 µV min)

As previously mentioned, the same calibration curve was used for the determinations of free fatty acids and diacylglycerols contained in cocoa butter.

Consequently, only 4 points were selected to create the calibration curve of free fatty acid contained in cocoa butter. Above 10 µg / injection, the free fatty acid peak was saturated and no more analysis on this compound could be done.

To obtain the data in Figure 3.4, a sample of approximately 250 µg of cocoa butter from various sugar particles dispersions (10, 30, 70 wt % sugar) was injected into the column to determine the effect of added phosphatidylcholine on the measured value of free fatty acids. The graph shows the effect of the added emulsifier (0.3 % PC) on the determined content of free fatty acids at 10, 30 and 70 % (w/w) sugar concentrations.

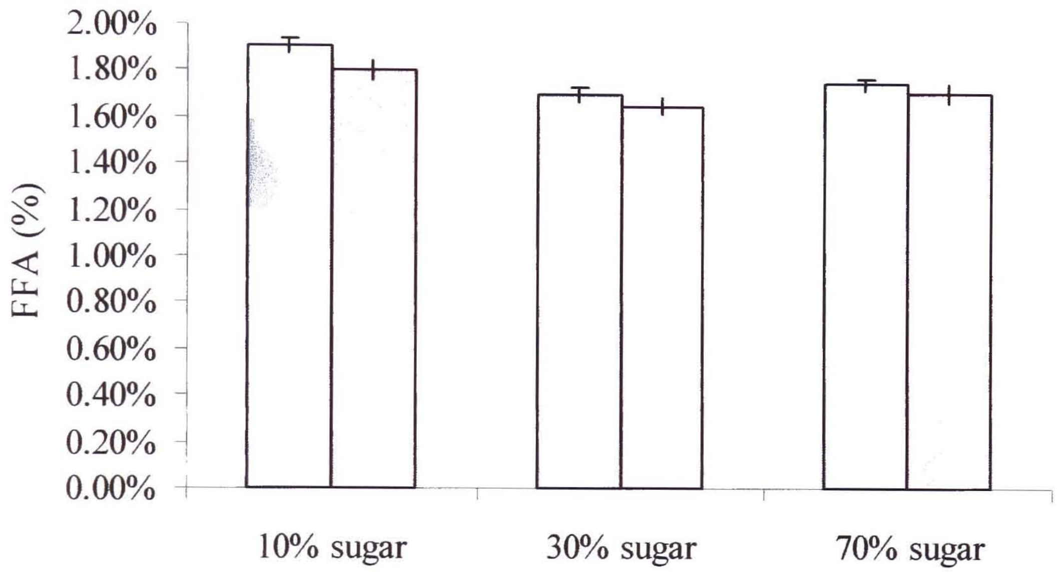
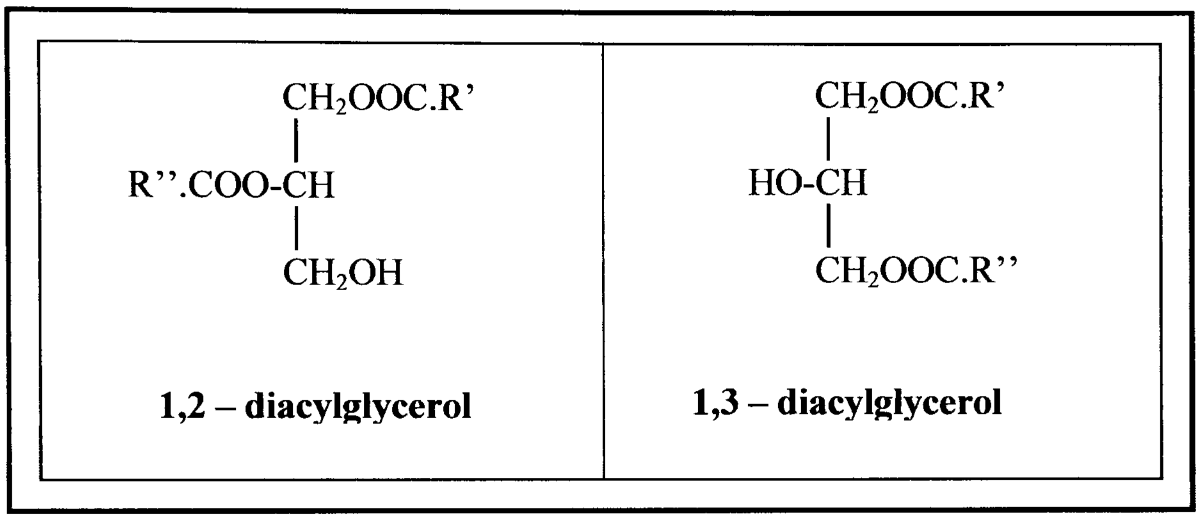


**Figure 3.4:** Effect of 0.3 % added phosphatidylcholine on free fatty acid content in cocoa butter dispersions containing 10, 30 and 70 wt % sugar particles. Samples prepared with emulsifier ( ) and without emulsifier ( ).

It can be observed that the measured level of free fatty acids in the oil phase decreases slightly from the original quantity (1.94 ± 0.01 %) as the sugar concentration increases in the system. On the other hand, it can be noted that the presence of the emulsifier does not interfere much with the quantity of free fatty acid determined in the different samples. Such a result may be explained by the fact that the cocoa butter already contains some phospholipids and the addition of slightly more phosphatidylcholine has no significant effect on the quantity of adsorbed free fatty acids. However this trend could be different for the less polar diacylglycerols; and so we have investigated the effect of phosphatidylcholine on these other minor species contained in the oil (1,3 and 1,2 diacylglycerols).

## 1,3 and 1,2 diacylglycerol determinations

Diacylglycerols are formally diesters of glycerol with fatty acids. They are made from two moles of fatty acids per mole of glycerol. The difference between the 1,3 and 1,2 diacylglycerols lies into their isomeric of configuration. Figure 3.5 represents the two chemical structures of 1,3 and 1,2 diacylglycerols.



**1,2 – diacylglycerol** **1,3 – diacylglycerol**

**Figure 3.5:** Chemical structures of 1,2 and 1,3 diacylglycerols. Both R' and R'' represent fatty acyl groups derived from condensed acids.

No direct chemical determination of the diacylglycerols was carried out. Therefore, it was not possible to determine the absolute quantity of diglycerides in the oil initially. Consequently, the adsorbed quantity of diacylglycerols was calculated as a function of relative peak area obtained initially in the samples. Knowing this mass/volume relationship we defined the cocoa butter concentration to make of 12.5 mg mL. However an oil solution of 127.17 mg dispersed into 10 mL of solvent was prepared. The Table 3.4 shows the different volumes used for making the calibration curve as well as the equivalent quantity of diacylglycerols added per injection and the corresponding surface area of each peak.

**Table 3.4:** Calculations for the 1,3 and 1,2 diacylglycerols calibration curves of cocoa butter sample.

| Volume injection (μL) | Quantity of cocoa butter per injection (μg μL) | Mean peak surface area 1,3 diglyceride (μV min) | Mean peak surface area 1,2 diglyceride (μV min) |
|---|---|---|---|
| 10 | 127.17 | 23.3 | 6.0 |
| 20 | 254.34 | 69.0 | 18.5 |
| 30 | 381.51 | 130.3 | 28.9 |
| 40 | 508.68 | 203.8 | 45.9 |
| 50 | 635.85 | 270.9 | 60.0 |
| 60 | 763.02 | 361.7 | 80.9 |
| 70 | 890.19 | 433.2 | 97.4 |
| 80 | 1017.36 | 520.6 | 119.9 |

The following graph represents the calibration curves for both 1,3 and 1,2 diacylglycerols as a function of the quantity of oil injected into the column.

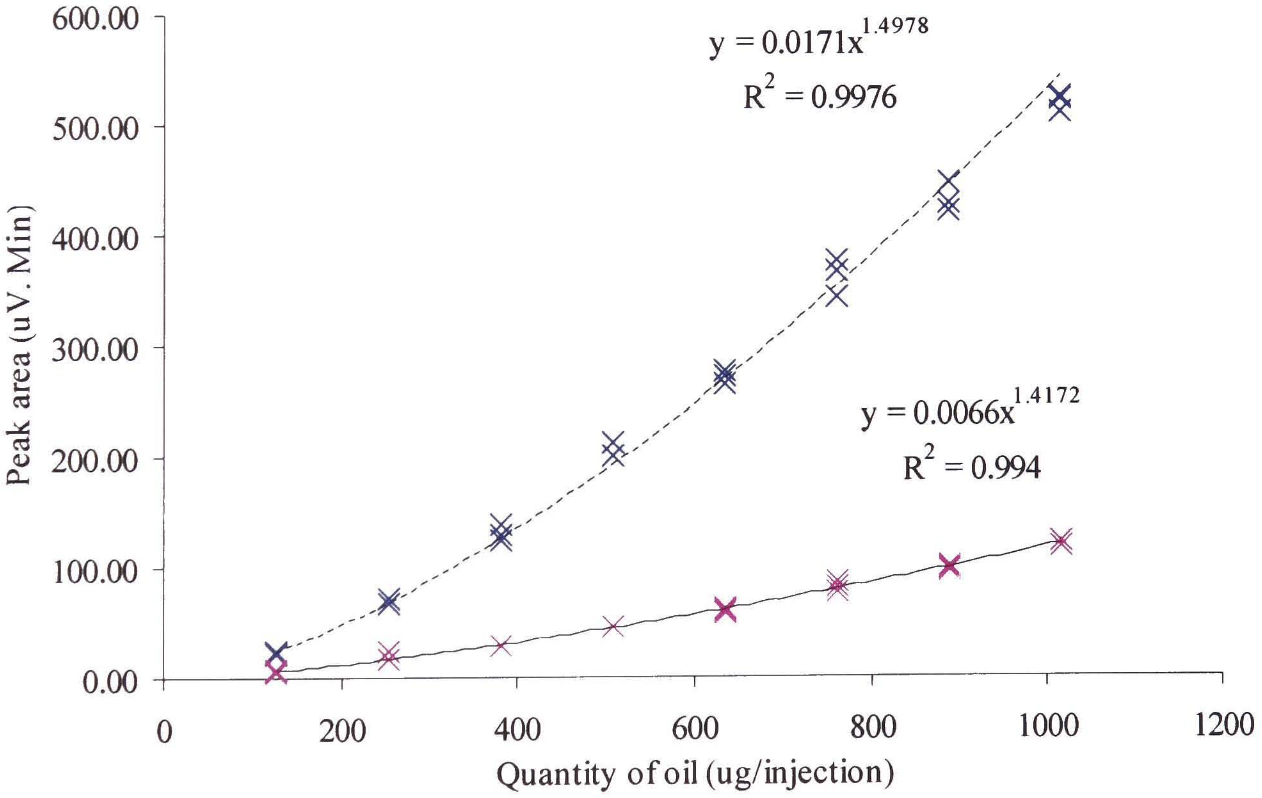


**Figure 3.6:** HPLC Calibration curves for 1,3 and 1,2 diacylglycerols in cocoa butter. (×) and (×) represents 1,3 and 1,2 diacylglycerols respectively.

From Figure 3.6, it can be noted that cocoa butter contains more of the 1,3 diacylglycerol than the other isomer. Some 1000 μg of cocoa butter was injected into the column in this specific experiment. This amount was needed in order to obtain a good response in the analysis of both these components. The analysis of both diacylglycerols was determined at the different sugar concentrations. Figure 3.7 shows the behaviour of the two diacylglycerols with or without the presence of phosphatidylcholine at different sugar concentrations. A 100 % response represents the initial content of both diacylglycerols in pure cocoa butter.

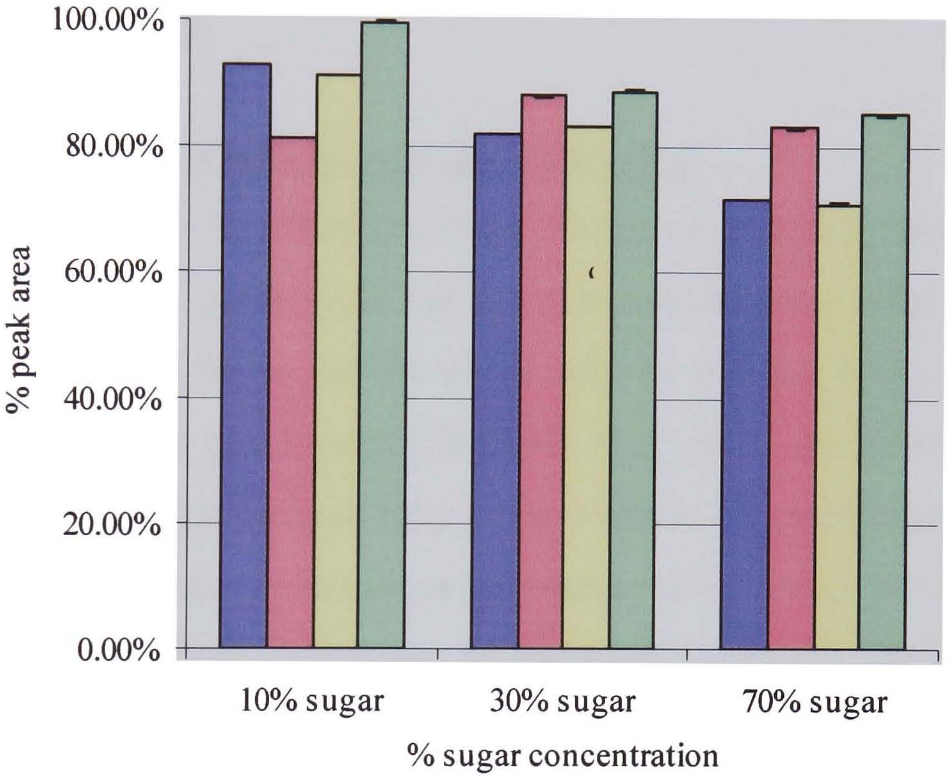


**Figure 3.7:** Percentage peak area of 1,3 & 1,2 diacylglycerols in function of different sugar concentrations. (■) and ( ) represent the percentage of peak area for 1,3 diacylglycerols with and without emulsifier (PC) respectively. ( ) and ( ) are the percentage of peak area for 1,2 diacylglycerols with and without emulsifier respectively (errors ± 0.1%).

It can be observed that, in the presence of sugar and prior to the addition of emulsifier, the amount of both diacylglycerols decreases as the sugar concentration increases. On the other hand, it can be noted that the range of adsorption of both diglycerides remains similar with the addition of sugar. On adding some phosphatidylcholine at a constant PC / sugar ratio, it has been observed that the quantity of both diacylglycerols apparently increases in the oil phase. As a consequence of these results, it can be said that for the cocoa butter samples, the emulsifier displaces the diacylglycerols from the sugar surface and consequently increases their concentration in the oil phase.

### *Summary*

Similar experiments have been carried out also for the following oils: sunflower, soybean, palm kernel, fat mixture and milk fat. A summary of these results is presented below.

### Free fatty acid determination

From table 3.5, it can be observed that a fraction of the free fatty acids have generally been adsorbed at the sugar surface at a level between 10 to 20%. In the particular case of milk fat, it can be noted that its content of free fatty acids remaining in the oil is the lowest (50-60%). For each individual oil, it can generally be observed that the free fatty acids content, for samples containing no added emulsifier, decreases as the sugar concentration increases from 10 % up to 70 % sugar (w/w). This behaviour is expected, because when the sugar concentration increases in the blend, more sugar surface is proportionately available for the binding of small surface-active molecules such as the free fatty acids.

**Table 3.5:** Effect of phosphatidylcholine (PC) on the percentage content of free fatty acids measured in the oil phase at different sugar concentrations.

| | 10 % sugar | | 30 % sugar | | 70% sugar | |
|---|---|---|---|---|---|---|
| | with PC | without PC | with PC | without PC | with PC | without PC |
| Sunflower oil | 85 | 86 | 88 | 82 | 81 | 69 |
| Soybean oil | 81 | 81 | 85 | 79 | 73 | 59 |
| Cocoa butter | 93 | 98 | 85 | 88 | 88 | 90 |
| Palm kernel oil | 73 | 75 | 78 | 90 | 99 | 100 |
| Fat mixture | 77 | 79 | 78 | 76 | 85 | 78 |
| Milk fat | 62 | 58 | 58 | 58 | 54 | 54 |

*Note:* PC / sugar ratios at 10, 30 and 70 wt % sugar were: 0.04/10, 0.12/30 and 0.30/70. Experimental errors: ± 5 %.

The main aim of these experiments was to determine the effect of added emulsifier on the free fatty acids contents in oils. It can be observed in table 3.3 that, at 10 % sugar and for all the oils, the addition of emulsifier does not significantly affect the free fatty acid concentration in the oil. As the sugar concentration increases up to 30 % (w/w), however, the free fatty acid content increases for some of the oils, namely, sunflower, soybean, fat mixture and milk fat. On the other hand, the free fatty acid concentration apparently decreases when emulsifier is added to systems containing cocoa butter and palm kernel oil. At 70 % sugar (w/w), the effect of phosphatidylcholine is rather more pronounced. It can be noted that free fatty acids are more concentrated in the oil phases in the presence of the emulsifier. This behaviour was observed for sunflower, soybean, fat mixture and milk fat. In the case of cocoa butter and palm kernel oil, however, the free fatty acid concentration was found to be almost constant despite the addition of emulsifier. For the palm kernel oil samples, experimental errors from the chromatographic column may have occurred in the integration of the free fatty acid peaks. As an example, after integration calculations we obtained a concentration in free fatty acids in palm kernel oil of exactly 100 % in the oil which seems unlikely.

## Diacylglycerol determination

In this section, two tables, Table 3.6 and Table 3.7, will respectively summarise the effect of added phosphatidylcholine on the adsorption of 1,3 and 1,2 diacylglycerols. Due to erroneous experiments, the data for samples made with sunflower and soybean oils have been deliberately omitted.

**Table 3.6**: Effect of phosphatidylcholine on the percentage content of 1,3 diacylglycerols determined in the oils at different sugar concentrations

| | 10 % sugar | | 30 % sugar | | 70% sugar | |
|---|---|---|---|---|---|---|
| | with PC | without PC | with PC | without PC | with PC | without PC |
| Cocoa butter | 81 | 93 | 88 | 82 | 83 | 72 |
| Palm kernel oil | 60 | 57 | 84 | 61 | 90 | 85 |
| Fat mixture | 32 | 33 | 30 | 29 | 27 | 27 |
| Milk fat | 98 | 101 | 97 | 108 | 99 | 99 |

*Note:* PC / sugar ratios at 10, 30 and 70 wt % sugar were: 0.04/10, 0.12/30 and 0.30/70. Experimental errors: ± 5 %.

From table 3.6, it can be observed that, for the various oil samples without emulsifier, the fat mixture contains the lowest amount of 1,3 diacylglycerols. The remaining oils have a percentage range between 60 to 90 %. In the case of milk fat, we observed a percentage higher than 100 % for the sample containing 30 % (w/w) sugar, which is impossible. For this particular set of samples, experimental errors from the equipement may have occurred in the integration of the free fatty acid peaks.

After the emulsifier has been added to the system, it can be observed that the quantity of 1,3 diacylglycerols increases in the systems as the sugar concentration increases. Therefore, it can be said that the emulsifier partially removes some of the 1,3 diglycerides from the sugar surface into the bulk oil, although the extent of the displacement is rather low.

The effect of emulsifier on the content of 1,2 diacylglycerols in the oil phase is presented in table 3.7. It can be observed that the fat mixture has the lowest percentage of 1,2 diglyceride in the oil phase amongst the studied oils. However, it seems that 1,2 dialyglycerols have not been significantly adsorbed at the sugar surface from the milk fat alone, as we obtained around 90% of diglycerides in the oils both with and without sugar.

**Table 3.7**: Effect of phosphatidylcholine (PC) on the percentage content of 1,2 diacylglycerols determined in the oils at different sugar concentrations

| | 10 % sugar | | 30 % sugar | | 70% sugar | |
|---|---|---|---|---|---|---|
| | with PC | without PC | with PC | without PC | with PC | without PC |
| Cocoa butter | 100 | 91 | 89 | 83 | 85 | 71 |
| Palm kernel oil | 65 | 74 | 42 | 55 | 82 | 90 |
| Fat mixture | 27 | 30 | 27 | 25 | 22 | 23 |
| Milk fat | 92 | 97 | 90 | 91 | 91 | 88 |

*Note*: PC / sugar ratios at 10, 30 and 70 wt % sugar were: 0.04/10, 0.12/30 and 0.30/70. Experimental errors: ± 5 %.

It can be observed that, in the presence of emulsifier, the various oils behave differently from each other. For the specific case of cocoa butter, adding phosphatidylcholine appears to remove the 1,2 diacylglycerols from the sugar surface. This is indicated by the fact that the concentration of diglycerides increases in oil in the presence of the emulsifier. On the other hand, the 1,2 diacylglycerol concentration in palm kernel oil, milk fat and fat mixture was found to decrease in the presence of emulsifier. It may be possible to infer that the presence of emulsifier leads to some rearrangements at the surface of the sugar particles involving these two surfactants. Nevertheless, the effect of emulsifier on 1,2 diacylglycerols on these oils is small, and any apparent interference could be due to some minor experimental anti-facts.

### *III.5.3. Determination of phosphatidylcholine*

The chosen method to determine the quantity of phosphatidylcholine by HPLC is the AOCS official method Ja 7b-91.

The same type of procedure as presented in Figure 3.1 was also used for this method. Two mixtures of solvents were used: solvent A was a mixture of n-Hexane / 2-propanol / acetic acid / triethylamine (1343.4/331.5/39.4/1.45, w/w/w/w) and solvent B was a mixture of 2-propanol / water / acetic acid / triethylamine (1646.2/350/39.4/1.45, w/w/w/w). The time taken for each experiment was 35 minutes. Figure 3.8 shows a typical chromatogram. It can be seen that triacylglycerols and diacylglycerols are positioned at the beginning of the chromatogram at Rt ≈ 2 minutes. The phosphatidylcholine has a peak around Rt ≈ 13–14 minutes. Figure 3.8 shows the presence of emulsifier in cocoa butter at Rt = 13.84 minutes. No other significant peak was observed.

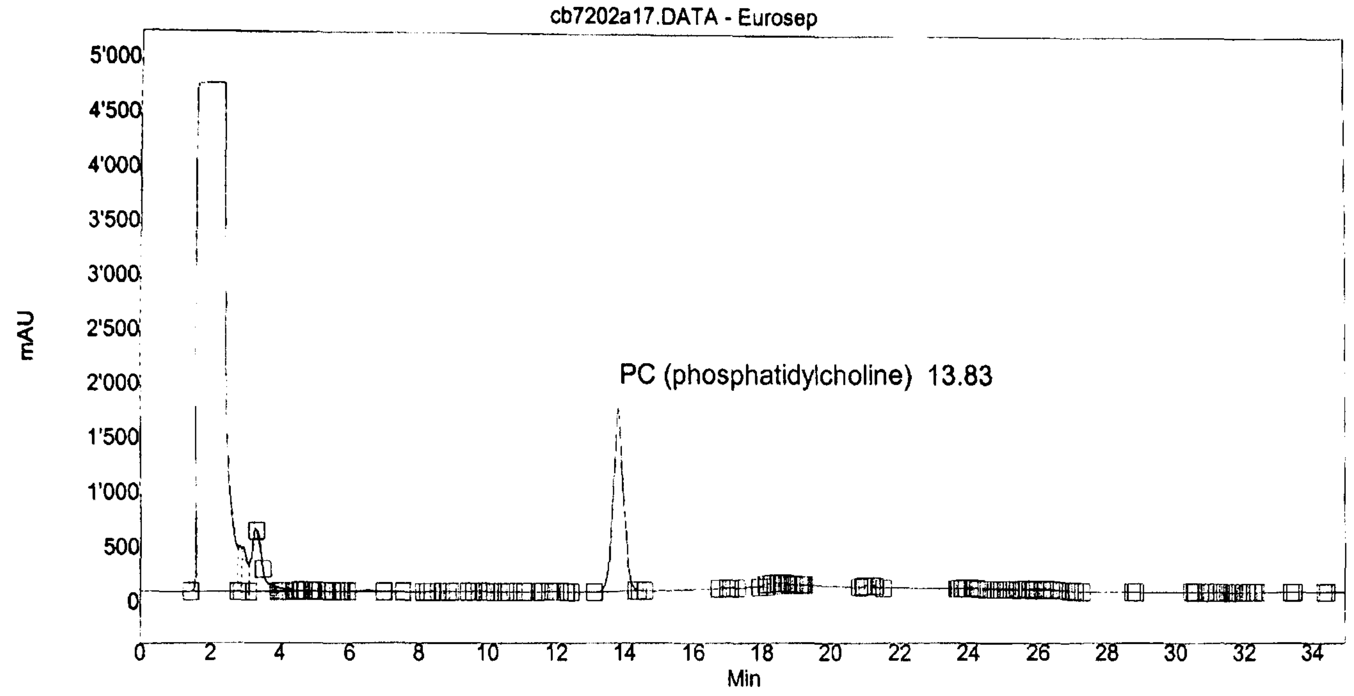


**Figure 3.8**: Typical chromatogram of cocoa butter at 4.33 mg / 10 mL, injection volume: 50 μL

Experiments have also been carried out without the presence of added phosphatidylcholine into the samples. The chromatograms show no significant peaks, which indicates the absence of phospholipids in the studied oils.

### *Example of 70 wt % sugar samples*

The initial quantity of emulsifier incorporated into these samples was 0.3 % (w/w). In order to keep the emulsifier/sugar ratio constant, the actual concentration depended on the amount of sugar present in the sample. The constant ratio which has been used is 0.3 % emulsifier for 70 % sugar (w/w). For samples containing 30 % and 10 % (w/w) sugar, therefore, 0.1285% and 0.0428 % (w/w) of phosphatidylcholine were incorporated.

Figure 3.9 shows the proportion of phosphatidylcholine left in the oils, and also the inferred amount which was adsorbed at the sugar surface, for samples containing 70 % (w/w) sugar.

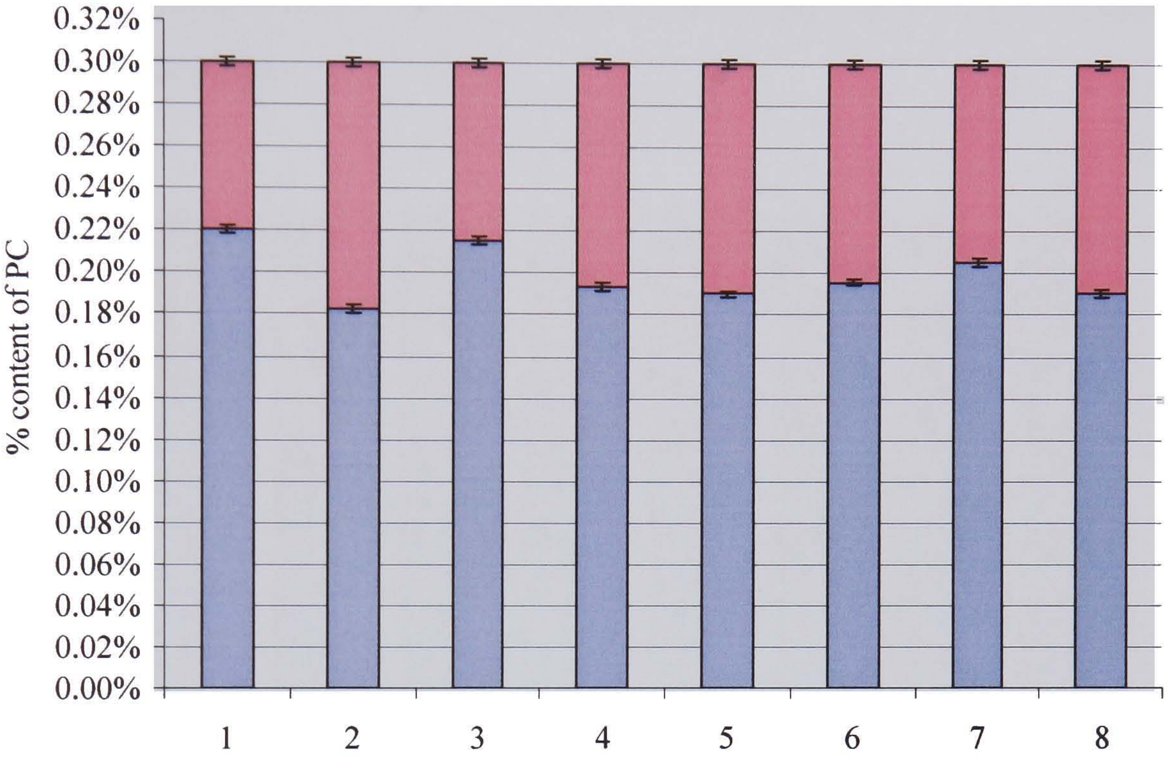


**Figure 3.9:** Percentage adsorption of 0.3 % phosphatidylcholine in different oil samples containing 70% (w/w) sugar. (🟦) represents the percentage of emulsifier in the oil and (🟥) is the percentage of emulsifier at the sugar surface. The following numbers represents the different oils, 1: sunflower oil; 2: soybean oil; 3: cocoa butter; 4: palm kernel oil; 5: milk fat; 6: fat mixture; 7: olein; 8: stearin.

It can be observed that roughly the same level of adsorption of phosphatidylcholine occurs with each oil. It seems that approximately 1/3 of the emulsifier present has been adsorbed at the surface of the sugar particles, leading to 2/3 of the phosphatidylcholine remaining in the oil. The amount of emulsifier left in the oil phase varies between 0.18 % for soybean oil up to 0.22 % for cocoa butter.

samples containing 30 % and 10 % (w/w) sugar, therefore, 0.1285% and 0.0428 % (w/w) of phosphatidylcholine were incorporated.

Figure 3.9 shows the proportion of phosphatidylcholine left in the oils, and also the inferred amount which was adsorbed at the sugar surface, for samples containing 70 % (w/w) sugar.

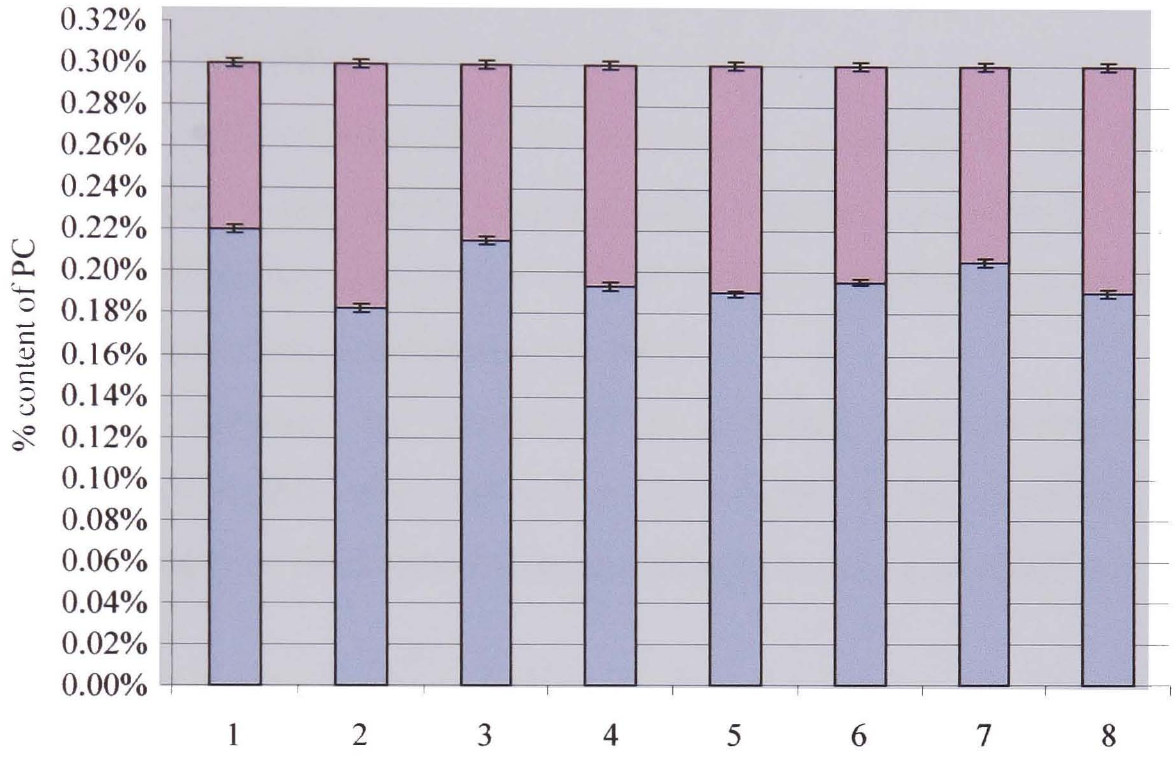


**Figure 3.9:** Percentage adsorption of 0.3 % phosphatidylcholine in different oil samples containing 70% (w/w) sugar. (■) represents the percentage of emulsifier in the oil and ( ) is the percentage of emulsifier at the sugar surface. The following numbers represents the different oils, 1: sunflower oil; 2: soybean oil; 3: cocoa butter; 4: palm kernel oil; 5: milk fat; 6: fat mixture; 7: olein; 8: stearin.

It can be observed that roughly the same level of adsorption of phosphatidylcholine occurs with each oil. It seems that approximately 1/3 of the emulsifier present has been adsorbed at the surface of the sugar particles, leading to 2/3 of the phosphatidylcholine remaining in the oil. The amount of emulsifier left in the oil phase varies between 0.18 % for soybean oil up to 0.22 % for cocoa butter.

The experiments carried out in sedimentation and rheology techniques ( to be repeated in chapters VI and VII) have shown substantial differences amongst the oils, thereby implying differences in the sugar/oil interactions. However, the level of adsorption of the surfactant is not strongly differentiated amongst the oils. This result implies that the surface coverage of the surface-active component was fairly constant. As the same quantity of emulsifier was added to each sample, and the total surface area of the sugar particles was generally constant, only a certain level of adsorption of emulsifier was needed to saturate the sugar surface. This behaviour was found essentially to be totally independent of the oil characteristics. From these results it is possible to determine the effective surface coverage of the emulsifier at the sugar surface. These calculations can be simply performed if the following assumptions are made:

(i) the sugar particles are considered as spherical and smooth with a corresponding low surface area, rather than as a highly porous material with a surface area as might be determined from gas adsorption using the BET equation. (Shaw 1992);

(ii) phosphatidylcholine has a typical surfactant monolayer coverage of *ca.* 1 mg $m^{-2}$.

Based on these two assumptions, we can estimate this quantity by calculating the mass of emulsifier adsorbed at the sugar particle with equation 3.2a.

**Mass of adsorbed surfactant = (total mass of adsorbent – mass remaining in oil phase)**

**(3.2a)**

Given the material amount, we observed that 2/3 of this quantity remained in the oils. Therefore, only 0.1 g of emulsifier has been adsorbed at the sugar surface.

The surface coverage of the emulsifier onto sugar particles is equal to the mass of emulsifier adsorbed at the sugar particle (mg) over the available sugar surface area ($m^2$). This is represented in equation 3.2b:

$$\textbf{surface coverage of emulsifier} = \frac{\textbf{mass of adsorbent}}{\textbf{available area}} \qquad \textbf{(3.2b)}$$

From the data given by Nestlé (PTC, York) on the specific surface area of the sugar particle (0.8417 $m^2g^{-1}$), we can estimate the area available in the system containing 70 % (w/w). This represents an area of 58.9 $m^2$. Equation 3.4b shows that the effective surface coverage is about ***1.6 mg $m^{-2}$*** at the sugar particle surface. This is of the order expected for a surfactant monolayer, although it is likely to be an overestimate because the specific surface area is probably an underestimate, due to deviation of the surface from the perfect smoothness assured in the calculation.

Calculations conducted for the 30 % (w/w) sugar samples, showed also a surface coverage of ***1.6 mg $m^{-2}$*** at the sugar surface particles. This result correlates with the previous calculations as the ratio of sugar/emulsifier remained constant in these experiments.

In order to differentiate better the level of adsorption in these oils, more work would be required. This might involve changing the quantity of emulsifier in the samples or changing the size of the sugar particles in the blends.

Two other experiments which have been carried out with this method looked at the effect of emulsifier adsorption on samples containing 10 % and 30 % (w/w) sugar, with proportionate additions of emulsifier.

### *Summary of samples containing 70, 30 & 10 % (w/w) sugar*

Figure 3.10 represents the percentage of phosphatidylcholine (PC) adsorbed at surface of the sugar particles or present in the oils. In this figure, it can be observed that the relative levels of emulsifier adsorption in the 70 % and 30 % (w/w) sugar samples are almost identical.

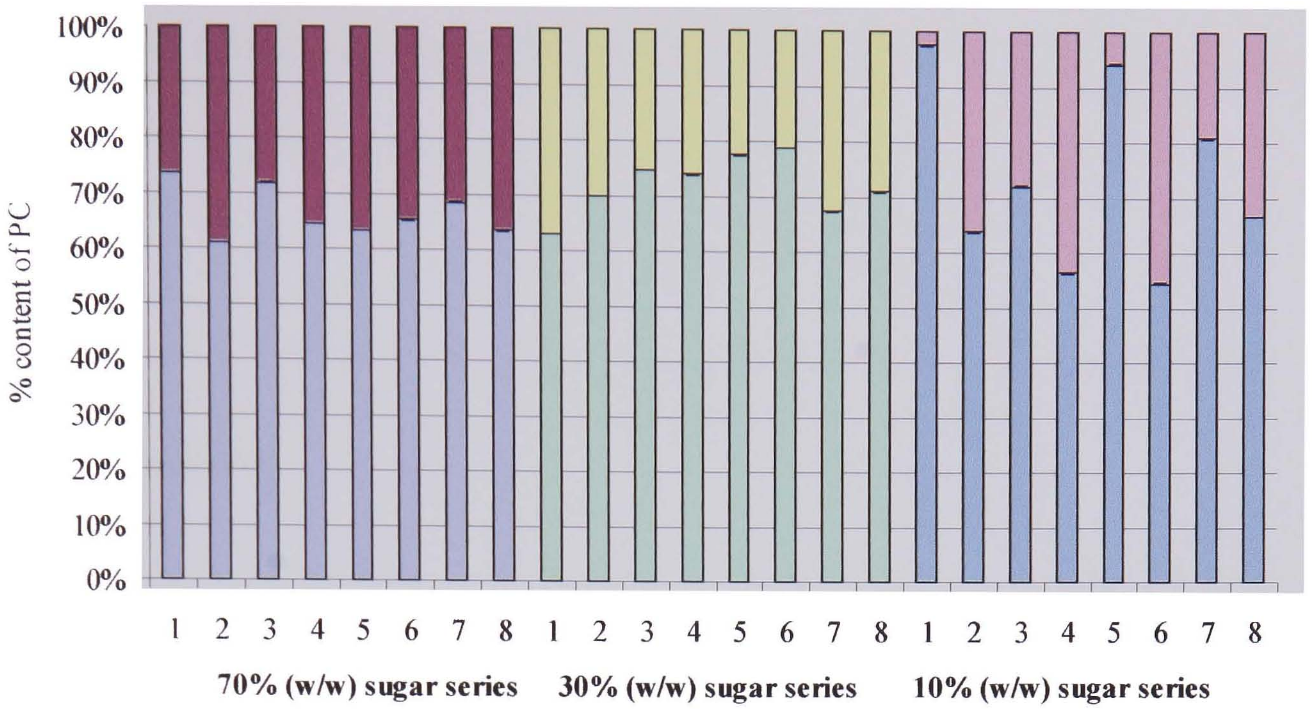


**Figure 3.10:** Percentage adsorption of phosphatidylcholine in different oil samples containing 70 % (■ in oil and ■ at sugar surface), 30 % ( in oil and at sugar surface) & 10 % (w/w) sugar ( in oil and ■ at sugar surface). The following numbers represents the different oils, 1: sunflower oil; 2: soybean oil; 3: cocoa butter; 4: palm kernel oil; 5: milk fat; 6: fat mixture; 7: olein; 8: stearin (errors ± 0.1%).

On average, the non-adsorbed quantity of phosphatidylcholine for the 70 % (w/w) sugar samples corresponds to about 65 % emulsifier left in the oil. In the case of samples containing 30 % (w/w) sugar, the level of non-adsorbed emulsifier is approximately 70 % phosphatidylcholine in the oil. As previously mentioned, we kept constant the emulsifier/sugar ratio at the different sugar concentrations. The difference in the results can be explained by a loss of precision in the calculations. Presumably the difference of results may account for the available surface area of sugar particles at both concentrations.

It can be noted from Figure 3.10 that the raw results for the 10 % (w/w) sugar samples seem highly scattered. This is probably due to the lower accuracy in the experimental procedure in this case while preparing the samples for the extractions. The amount of emulsifier required for such blends was just 0.0428 %. Blends of 100 g portions were prepared, but the balances available in the laboratory for these experiments had an accuracy of 0.001 g. Consequently, these results for the dispersions containing only 10 % sugar have larger experimental errors.

## *III.6 Conclusion*

The determination of the free fatty acids in the oils via potentiometric measurements is a straightforward technique. It gives a good level of reproducibility for all the different studied oils. With this method it was observed that cocoa butter contains the highest amount of free fatty acids in the oil. On the other hand, sunflower oil has the lowest amount of free fatty acids, with FFA as oleic = 0.05 %.

The chromatographic method used for the analysis of the free fatty acids in the oils has allowed us to define at the same time the quantity of minor components such as 1,2 and 1,3 diacylglycerols. These species were determined individually, being separated by their retention time.

Nevertheless, a calibration curve was required for each studied oil component and each specific oil. In the case of cocoa butter, it was observed that the free fatty acid content in the oil phase diminished as the sugar concentration increased. When adding some emulsifier, the level of free fatty acids diminished slightly, but this change was not statistically significant. It is suggested that, as cocoa butter contains a large quantity of free fatty acids and other surfactants in it, the presence of some more surfactant may not affect the sugar surface as it is already well covered by surfactants. When considering the diacylglycerol components, we have observed that 1,3 diglycerides were more present by a majority in the oils than their structural isomers. In this case, we noted that the addition of emulsifier leads to an increase of diacylglycerols in the oil. From these results, it may be possible to say that some minor components are removed from sugar surface due to the presence of more surface active components such as phosphatidylcholine.

As a general trend, we note that the presence of emulsifier (PC) leads to a release of free fatty acids and diacyglycerols from the sugar particle surface into the oils. This is likely to be due to the fact that phosphatidylcholine has greater surface activity, allowing it to displace other polar lipid compounds from the sugar surface.

Another chromatographic method has looked at the absolute adsorption level of phosphatidylcholine. In this case, only this specific surfactant could be determined as all minor species formed a mixed retention peak below 2 minutes.

In the samples containing 70 % (w/w) sugar, we noted that about a third of the emulsifier was generally adsorbed at the sugar surface. This represents a surface coverage of the emulsifier of 1.6 mg $m^{-2}$, assuming that sugar particles are spherical. This value is characteristic of a monomolecular layer adsorption of emulsifier at the sugar surface. This value was obtained for all the oils and it was not possible to differentiate the effects of this emulsifier with respect to specific oils. For the samples containing 30 % (w/w) sugar, we observed the same trend as for the 70 % (w/w) sugar blends. In the case of 10 % (w/w) samples, the experimental error was too large to be able to come to any reliable conclusions.

Although adsorption technique has been a powerful tool in determining the level of interactions at the sugar surface, it may be interesting in the future to investigate the presence of other minor compounds (monoacylglycerols). It may also be interesting to determine the number of layers of emulsifier surrounding the sugar particles by physical techniques (e.g. surface force measurement).

Consequently, the aggregates' formation has been studied at a molecular level based on the interactions of emulsifier at the sugar surface. However, Johansson and Bergenståhl (1992) showed that the level of interactions may be interpreted with an elementary technique such as the sedimentation. They analysed the sediment volumes of oil mixtures combined with different level of emulsifiers in the systems. Similarly to their project, the determination of sediment volumes in various oils in presence of singular or multiple additions of emulsifier seemed worth investigation.

# *Chapter IV Sedimentation, an indirect method to observe aggregate texture*

## *IV.1. Introduction*

The adoption of the sedimentation technique in this project was stimulated by the work of Johansson and Bergenståhl (1992a). They investigated sediment volumes of fine sugar particles dispersed in oils in the presence of emulsifiers or fat crystals. It has been reported in the literature that this technique is sensitive to the aggregation properties of particles dispersed in liquid media (Coulson and Richardson, 1991; Walstra, 1996; Hiemenz, 1986). The technique should allow therefore some interpretation regarding the relative strength of the interactions between sugar particles suspended in various oils. However, it does not quantify objectively the strength of these interactions as it is possible to do with the various kinds of rheology. Attempts to determine the nature of these same interactions via Confocal Laser Scanning Microscopy technique have not been reported before.

In the present chapter we report results from sedimentation and confocal microscopy. More experiments have been carried out with the sedimentation technique than with the confocal microscopy technique. In the sedimentation section, we have been able to explore the influence of several parameters, including the properties of the oils, and the addition of several types of surfactants and emulsifiers, on the sediment volumes of our samples. Moreover, we have looked at the effect of cumulative additions of surfactants on the sediment volume. In the confocal microscopy technique, simple qualitative evaluations of the sugar particles dispersed in different oils have been performed.

## *IV.2. Materials and Methods for Sedimentation and Confocal Microscopy*

Microscopy materials specifically relevant to this chapter are described in this section. Common materials used in experiments with the different techniques of this thesis, such as the oils and emulsifiers, have been previously mentioned in chapter V.

### *IV.2.1. Materials for Microscopic Measurements*

**Nile Red** (Nile Blue A Oxazone) was purchased from Sigma-Aldrich (Dorset, England) (reference N 3013). It is a fluorescent probe of intracellular lipids and hydrophobic domains of proteins.

**Poly (ethylene glycol) ($M_n$ *ca.* 400 daltons)** was obtained from Sigma-Aldrich (Dorset, England) (reference 20,239-8 lot 00524003). It had an approximate number of molecular weight of polymer of 400. Its viscosity at 210 °F was 7.3 cstokes.

### *IV.2.2. Methods*

#### IV.2.2.1. Preparation of Samples

*Preparation of Samples for Sedimentation*

Dispersions of 10 wt % sugar in liquefied fat were made up. Once the sugar was completely dispersed into the oil, 5 g of this mixture was carefully poured into a set of four calibrated test tubes (precision ± 0.1 mL). The samples were agitated at high speed with the help of Vibromixer. During this process, it should be brought to the attention of the reader that, the use of Vibromixer (or vortex) generates lots of air bubbles in the sample as it is mixed at high shear. At atmospheric pressure the release of all the air bubbles entrapped in the sugar-in-oil system cannot be achieved. Consequently, after being processed with the vibromixer, samples were left standing in a vacuum drier at 40 °C. The vacuum dryer was set at 400 mm Hg (~ 15 Hg) of negative pressure. Samples were left overnight in order to allow a complete settling of the particles. After different settling times (up to 72 hours), it was observed in preliminary experiments that the sediment volumes remained constant after 24 h. Therefore, sedimentation experiments whose results are reported here refer to data obtained after 24 hours' settling time. Figure 6.1 is a schematic representation of the procedure of the sedimentation experiments.

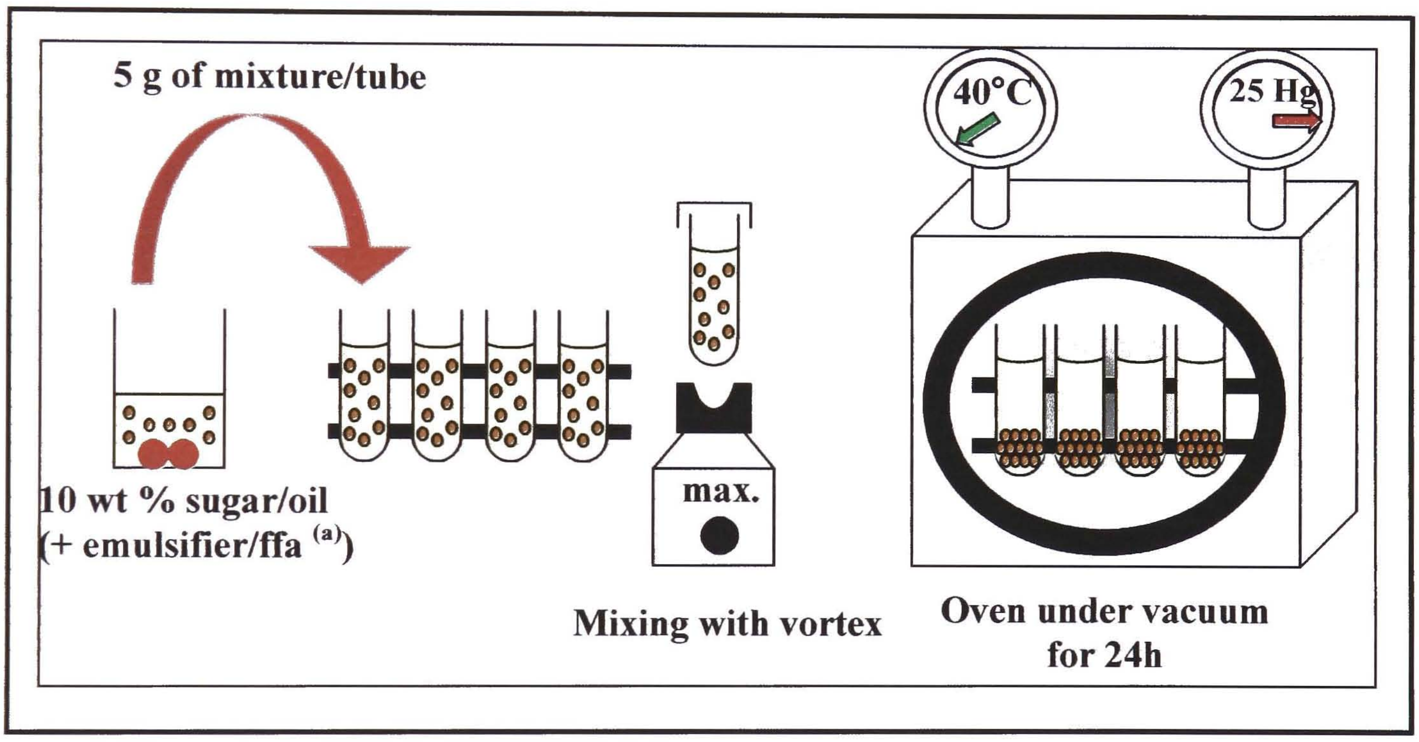


**Figure 4.1:** Representation of the methodology used in the sedimentation technique.
*Notes*: (a) ffa means free fatty acid

### *Preparation of Samples for Confocal Microscopy*

Confocal microscopy was carried out on the Leica confocal microscope TCS SP2 in a temperature-controlled room at 22 °C but at an experimental temperature of 40 °C. Eight sugar-in-oil dispersions were investigated at different concentrations: 30, 40 and 60 wt % sugar in oil. Samples were prepared via the "*refined mixing method*", as explained in Chapter V. The extent of dispersion was observed under the microscope with the use of Nile red which stains the fat phase of the different mixtures. The dye was diluted in polyethylene glycol 400. This method of staining has been described by Greenspan *et al.* (1985).

#### *Preparation of Samples Containing Emulsifier (Epikuron 200)*

A 1 mmol $L^{-1}$ addition of emulsifier was made to the liquefied fats. The sample was then placed in an oven to allow the dispersion of the emulsifier into the sample. The sample was left for two hours, at 60 °C, in order to have a complete dispersion. Once an effective dispersion was achieved, the sugar was added. Then addition of 10 wt % of sugar particles in the oils containing emulsifier was carried out and samples were prepared for sedimentation experiments.

#### *Preparation of Samples Containing Free Fatty Acid (Linoleic acid)*

Concentrated solutions (from 20 to 60 mmol $L^{-1}$ of free fatty acid in the liquefied fats) were prepared. Different dilutions were undergone in order to obtain a final concentration of free fatty acid in the liquefied fats of 1 mmol $L^{-1}$. Once the fat samples were prepared, 10 wt % of sugar particles were added to the oils which contained the free fatty acid. Samples were then prepared for the sedimentation experiments.

### **IV.2.2.2. Purification Stages**

#### *Purification with Activated Charcoal*

A 10 wt % portion of activated charcoal was added to the liquefied fat. The sample was left overnight in a cool dark place. The oils were then filtered on filter paper (Waltham n°1 filter paper) either at room temperature (soybean oil and sunflower oil) or in the oven at 60 °C (cocoa butter and palm kernel oil). Once filtered, sugar particles were added to these oils, and the standard sedimentation procedure was executed.

#### *Purification with Florisil*

First the florisil was dried in an oven overnight at 75 °C and put in a desiccator. A small sample of oil was added to 10 g of florisil in a beaker in order to have an effective swelling of the florisil into the oil. Once the adsorbent was fully impregnated, this suspension was carefully poured into a chromatographic column, and 150 g of oil was treated with the florisil. Purified samples were then ready for the different applications.

## *IV.3. Results and Discussion*

Experiments were performed with samples containing eight different oils: sunflower oil (commercial sample), soybean oil (purchased from Lamotte, GR), cocoa butter, palm kernel oil blend and milk fat (supplied by Nestlé UK Ltd, York), fat mixture (a blend of 83.5 % of cocoa butter and 16.5 % of milk fat), and two milk fat fractions (olein and stearin, provided by Lactalis Petit-Fayt, France). Low and intermediate sugar content samples have been studied, respectively, by the sedimentation and microscopy techniques.

We have compared the level of aggregation of sugar particles in the oil samples under several sets of conditions, depending on the fact that the oils may be purified under different conditions or submitted to the addition of different surfactants.

### *IV.3.1. Sedimentation Experiments*

This study is justified on the basis of the work published by Johansson and Bergenståhl (1992a). In this paper they compared the sediment volumes of sugar particles dispersed in oil samples containing various amounts of emulsifier (phosphatidylcholine, (PC) and monoolein) and fat crystals. The work was done with dodecane, decanol and soybean oil. We have performed similar sedimentation experiments with eight food oils: sunflower, soybean and palm kernel oils, cocoa butter, pure olein, pure stearin, milk fat, and a blend of cocoa butter and milk fat. Moreover, we decided to compare the influence of three emulsifiers (PC, monoolein and PGPR) and one free fatty acid (linoleic acid) on the sediment volumes. Based on the work of Johansson and Bergenståhl (1992a), it was decided to run experiments at the same concentration of surfactant, but to investigate the purification of the oils with two types of adsorbent (activated charcoal and florisil). Therefore, based on the original work of Johansson and Bergenståhl (1992a), we have extended the sedimentation technique to a range of common fat ingredients in the presence of multiple relevant additives.

These experiments were carried out at 40 °C in order to avoid complications due to the fat crystallisation of cocoa butter, palm kernel oil, milk fat, fat mixture, and stearin. Each series was prepared with four different samples having the same concentrations in sugar and/or surfactants. The concentrations of the systems were 10 % in sugar and 0.1 % (1mmol $L^{-1}$) in emulsifier and free fatty acids. Two different types of adsorbent were used in order to purify the oils: activated charcoal and florisil. A comparison of the efficiency of these two adsorbent was performed.

### IV.3.1.1. Effectiveness of the Purification Agents

The determination of the fatty acids contents in the oils was carried out at the Nestlé UK Regional Laboratory. For each analysed oil, eighteen free fatty acids were identified and quantitatively determined. The three main fatty acids found are: Palmitic (C16:0), Oleic cis (C18:1) and Linoleic cis (C18:2).

Figure 4.2 shows the percentage of the total free fatty acids (as Oleic) in five different oil systems (sunflower, soybean, palm kernel oils, cocoa butter and milk fat). Each oil had been purified by two different techniques: activated charcoal and florisil.

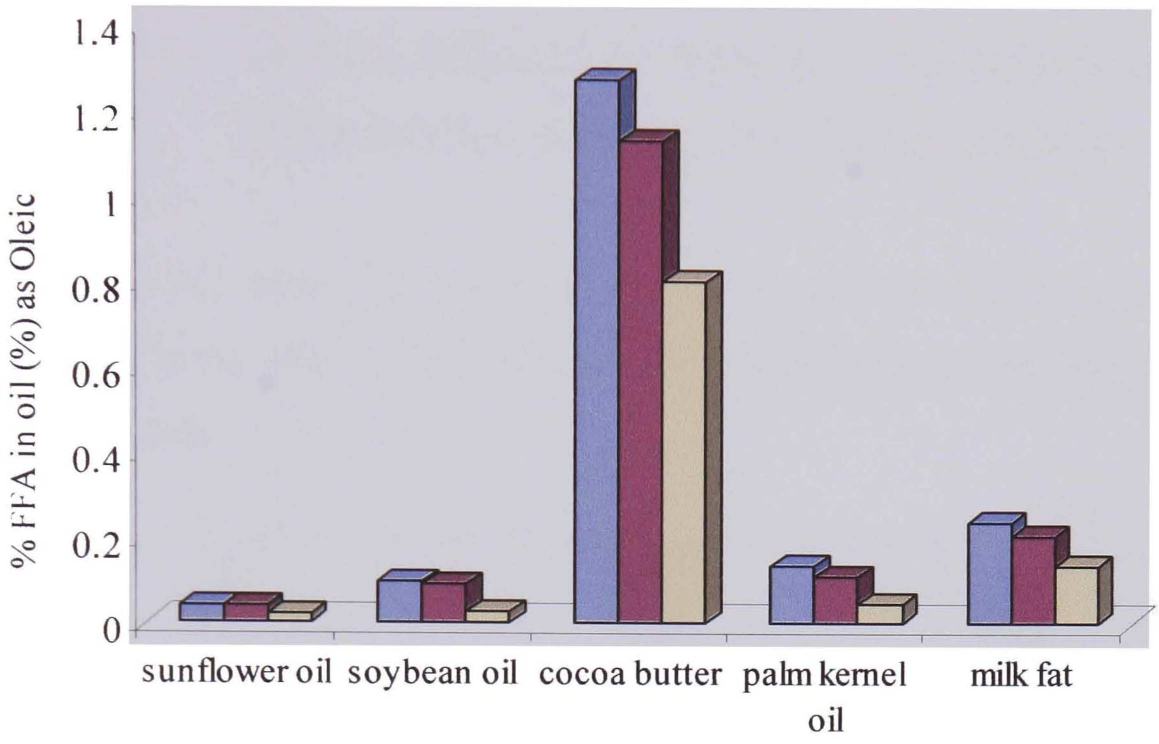


**Figure 4.2:** Free fatty acid (FFA) content of five purified and non-purified oils. In each case, (■) represents the original sample; (■) represents the charcoal purified sample and ( ) represents the florisil purified sample.

It can be observed that cocoa butter has by far the highest level of free fatty acids amongst all the oils tested (about 1 % FFA as Oleic). Milk fat contains a substantial amount of free fatty acids. On the other hand, sunflower oil has the lowest content of free fatty acids (less than 0.1 % FFA as Oleic). It can also be noticed that both oil purification methods reduce the detected content of free fatty acids in each oil, although Florisil appears more effective in the removal of free fatty acids for all the studied systems.

The next section describes the first set of sedimentation experiments involving oils containing 10 wt % sugar particles. We compared the measured sediment volume for sugar particles dispersed in the different studied oils.

The next section describes the first set of sedimentation experiments involving oils containing 10 wt % sugar particles. We compared the measured sediment volume for sugar particles dispersed in the different studied oils.

#### IV.3.1.2. Effect of different dispersing media on the sedimentation of sugar crystals after 24 hours

Figure 4.3 shows the sediment volume results for the dispersions of sugar particles in the oils at 40 °C without any oil purification or addition of emulsifier or fatty acids.

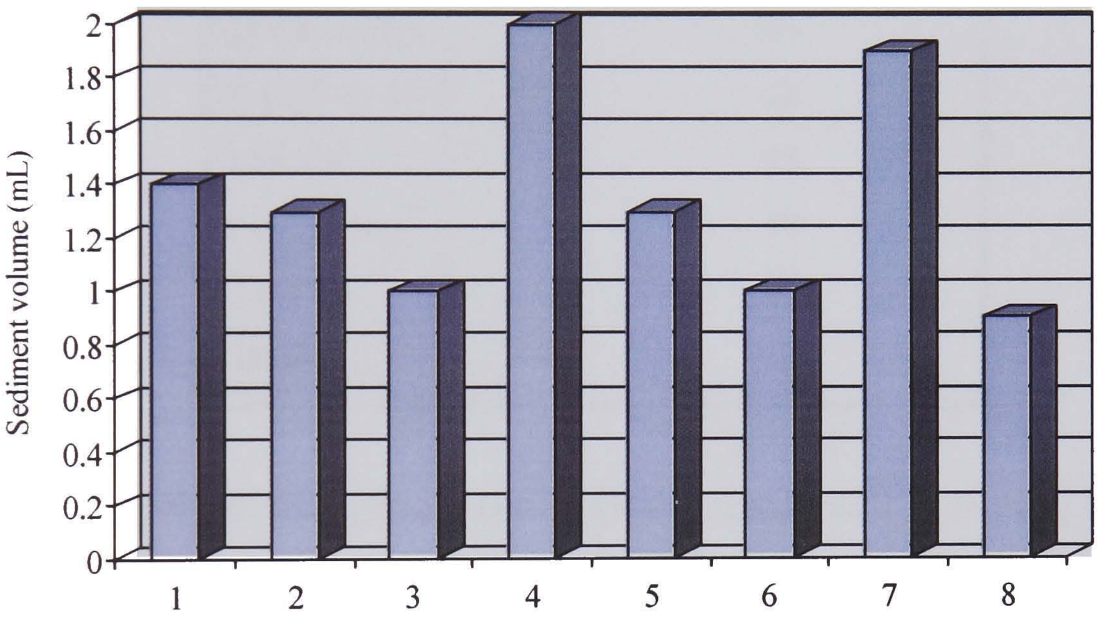


**Figure 4.3:** Comparison of sedimentation volume of sugar particles (10 wt %) in the different oils at 40 °C (total volume of dispersion: ~ 5 mL; ± 0.1 mL error). The sample numbers refers to the different oils, 1: sunflower oil; 2: soybean oil; 3: cocoa butter; 4: palm kernel oil; 5: milk fat; 6: fat mixture; 7: pure olein; 8: pure stearin.

From Figure 4.3, it can be observed that the sediment volume does not exceed 2 mL. This means that the maximum volume of sediment obtained for the sugar particles dispersed in all the oils is below 40 %. In order to have a clearer picture of the level of aggregation in each system, it is convenient to compare the percentage sedimentation volume of sugar particles in the different oils. The percentage sedimentation volume was calculated from:

$$\% \ Vs = \frac{Vs}{Vt} \times 100\% \qquad \textbf{(4.1)}$$

where $Vs$ is the sediment volume of sugar particles and $Vt$ is the total volume of mixed oil and sugar particles. These results are summarised in Table 4.1.

**Table 4.1:** Comparison of percentage sedimentation volume of sugar particles in the different oils at 40 °C (volume of dispersion: ~ 5 mL; ± 2 % error )

| Oil/sugar mixtures | % sediment volume |
|---|---|
| Sunflower oil | 26 |
| Soybean oil | 25 |
| Cocoa butter | 19 |
| Palm kernel oil | 38 |
| Milk fat | 25 |
| Fat mixture | 19 |
| Olein | 35 |
| Stearin | 17 |

It can be noticed that palm kernel oil and pure olein generate the largest sediment volumes (around 35 %). Sunflower oil, soybean oil and milk fat present an intermediate sediment volume (above 20 % and below 30 %). For cocoa butter, fat mixture and pure stearin, it can be noticed that these oils present the lowest sediment volume (below 20 %). Pure stearin has the lowest sediment volume (16.5 %) amongst all the oils.

From these results, it can be suggested that sugar crystals interact most strongly in presence of palm kernel oil and olein, as compared with stearin or cocoa butter. That is, the sugar crystals in the former oils have a strong tendency towards aggregation, and this results in less compact sediments. Such interactions may be associated with the presence of strong short-range van der Waals forces or hydrogen bonding forces and less effective steric repulsion forces between particles. On the other hand, the particles in stearin or cocoa butter have predominantly repulsive inter-particle forces, thus resulting in a more closely packed sediment. Intermediate interaction levels occur with sunflower oil, soybean oil and milk fat. From the results of Table 4.1, we can classify the different percentage sediment volumes for each oil into three arbitrary sediment packing categories. Figure 4.4 is a representation of the three levels of interactions illustrated schematically.

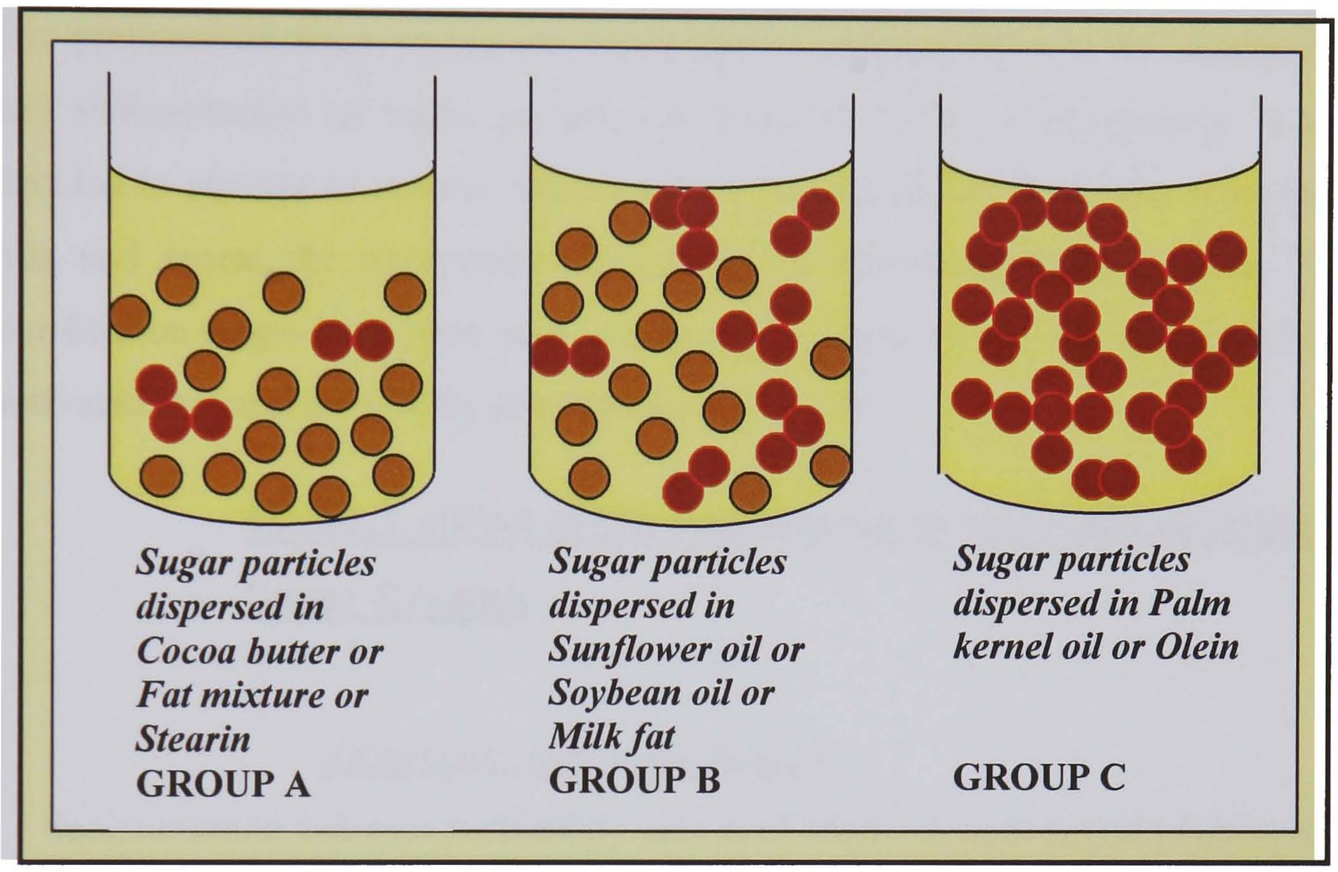


**Figure 4.4:** Types of interaction inferred with sedimentation technique. (●) represents a dispersed sugar particle and (●) represents an aggregated sugar particle.

The representations of the dispersion microstructures given in Figure 4.4 show that there are different levels of interaction between sugar particles in the different oils. It may be possible to separate them into three different categories. In the case of group **A**, the degree of flocculation is very low, the particles settle individually, resulting in a compact particle packing sediment. As a consequence, little or no attraction between the particles is inferred. For the group **B**, there is a limited state of aggregation. The flocculation of the particles results in a medium density sediment, and it could be inferred that weak attractions occur between sugar particles in these group **B** oils. In the case of group **C**, large aggregates settle and a high sediment volume is produced. In this case, the soybean is highly flocculated and highly space-filling aggregates are present. It can be suggested that strong attractive interactions exist between the sugar particles in this group **C** system.

Each oil has in it a non-negligible amount of minor surface-active components, such as free fatty acids, as determined in Chapter III and in the present chapter. The presence of these surfactants could have a strong effect on the aggregation and sedimentation of sugar particles in these food oils. Consequently, it was decided to attempt to remove some of these surface-active impurities from these oils and repeat the experiments based on the sedimentation technique. Two purification stages have been used, one involving charcoal and the other involving activated magnesium silicate (also known as florisil).

### IV.3.1.3. Effect of Oil Purification on the Sedimentation of Sugar Crystals

#### *Adsorbent: Activated Charcoal*

Sedimentation samples purified by activated charcoal were prepared following the experimental section IV.2.2.2. The measurements were not performed with the pure olein or pure stearin oils. Figure 4.5 shows the effect of charcoal purification on the percentages of sediment volumes of the 10 wt % sugar/oil systems.

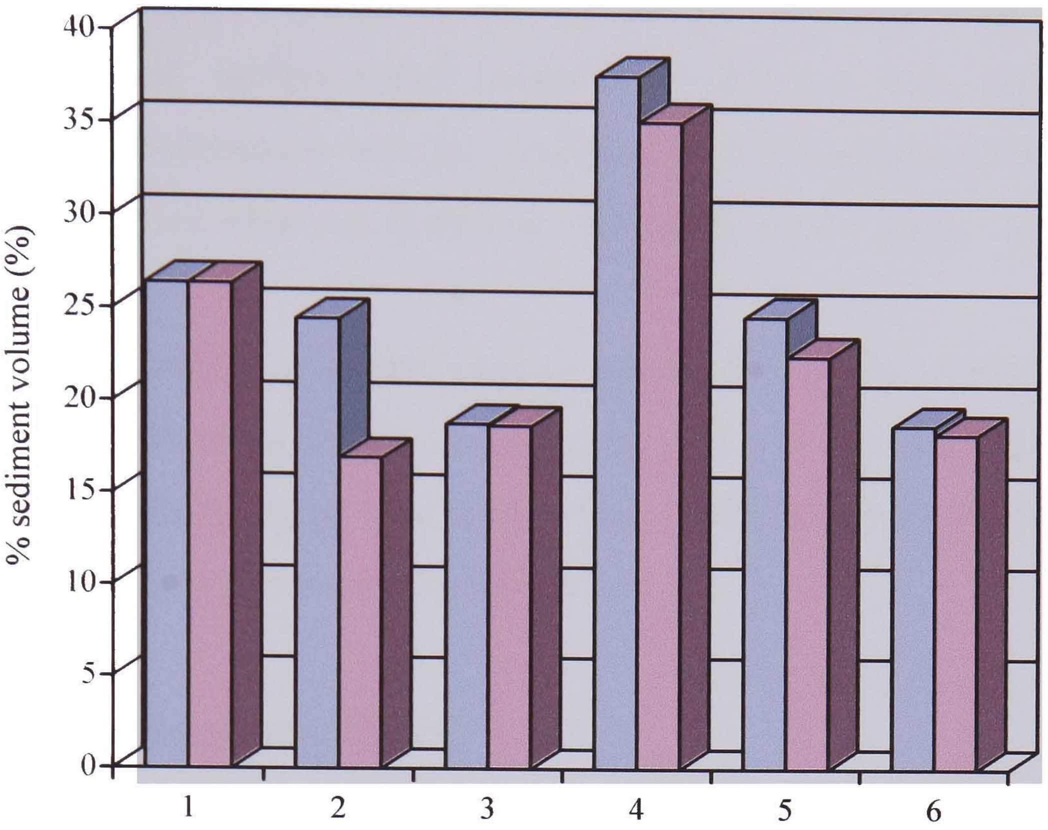


**Figure 4.5:** Effect of purification by charcoal on the sugar/oil sedimentations at 40 °C (volume of dispersion: ~ 5 mL; ± 2% errors): ( ) represents the original sediment volume and ( ) is the sediment volume after purification with activated charcoal. The sample numbers represent the different oils, 1: sunflower oil; 2: soybean oil; 3: cocoa butter; 4: palm kernel oil; 5: milk fat; 6: fat mixture.

It can be observed that the sediment volume measured for sunflower oil, cocoa butter and fat mixture remain constant. The volume of sediment for the soybean oil suspension has decreased by almost a third of its original volume.

From the results obtained in this experiment, two observations can be made. The first is that charcoal is not very efficient for the purification of some of the oil systems, and the second is that some systems may already be free from surface-active impurities thus limiting the action of the charcoal. Regarding the first hypothesis, charcoal purification generally is not considered so efficient as it can perhaps only remove residual components (like water) in the soybean oil and palm kernel oil. Gaonkar (1989) showed that charcoal has a limited efficiency for the purification of oily systems. On considering the second hypothesis, it seems unlikely that sunflower oil, cocoa butter and fat mixture are pure fats. These three oils are commercial products and there is high probability that the oils contain a significant level of surface-active impurities, such as free fatty acids. Consequently, in confirmation with the published work of Gaonkar (1989), it can be said that activated charcoal does not efficiently purify these sugar + oil systems.

Gaonkar (1989) also used another type of adsorbent (florisil) which was more effective in removing oil impurities. As a consequence, it was decided to purify our different oils with this same type of adsorbent. The florisil which was used for this purpose was 100 to 200 mesh of particles size.

### *Adsorbent: Florisil*

Sedimentation samples containing oils purified by florisil were prepared following the procedure described in the experimental section IV.2.2.2. Experiments were not performed with the olein and stearin oils. Figure 4.6 shows the effect of florisil on the percentage of sediment volume of sugar particles with the non-treated samples used as reference.

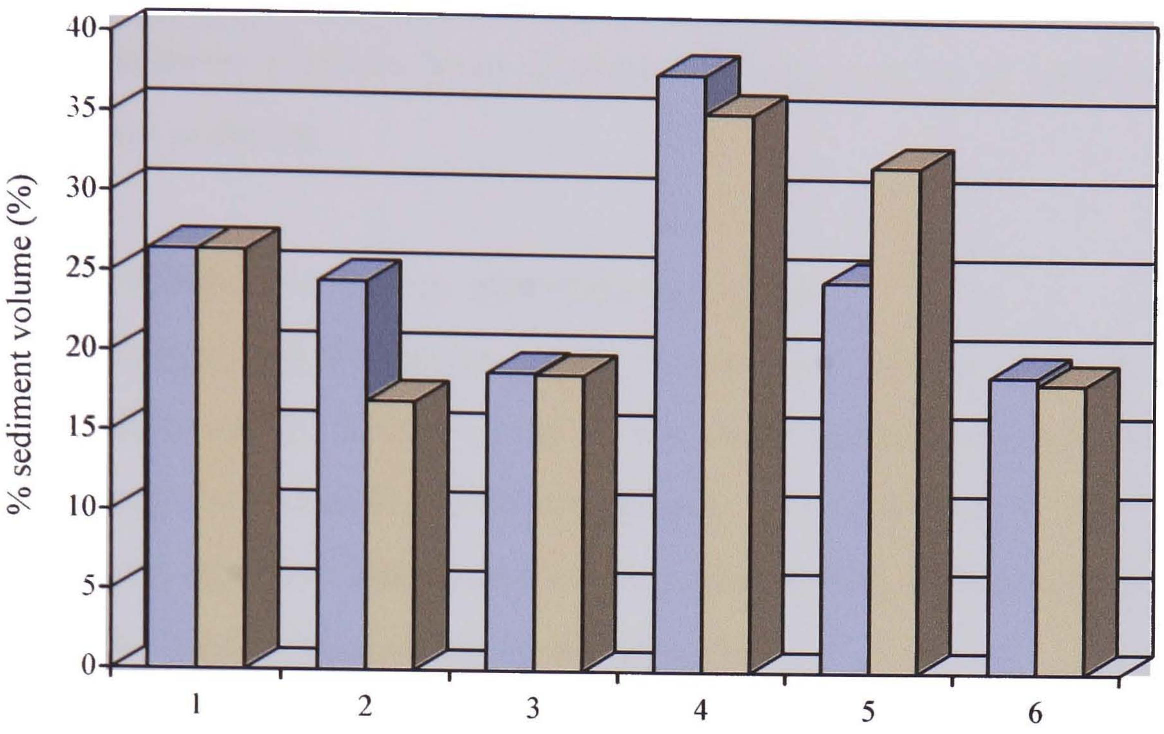


**Figure 4.6:** Effect of purification by florisil on the sugar/oil sedimentation results at 40 °C (volume of dispersion: ~ 5 mL; ± 2% errors). ( ) represents the original sediment volume and ( ) is the sediment volume after purification with florisil. The numbers sample represent the different oils, 1: sunflower oil; 2: soybean oil; 3: cocoa butter; 4: palm kernel oil; 5: milk fat; 6: fat mixture.

It can be seen that oil treatment with florisil has a substantial effect on the sediment volume in soybean oil, palm kernel oil and milk fat, whereas for sunflower oil, fat mixture and cocoa butter the volume of sediment remains almost constant. Percentages of sediment volumes were constant at 16% for fat mixture and cocoa butter.

It could be suggested that the cocoa butter and fat mixture do not contain a large quantity of impurities, as their sediment volumes did not change after the two different purifications. The samples containing milk fat or soybean oil are interesting as their sediment volumes are diminished in the presence of charcoal by 2 %, but are increased in the presence of florisil by 2 %. It can be therefore assumed that the two adsorbents may be adsorbing different impurities contained in the milk fat and soybean oil samples. The presence of charcoal may not completely remove the water, and therefore repulsive forces due to counter ions may remain. After purification with florisil, it appears that most of the repulsive interactions between particles have disappeared. Aggregation is enhanced and leads to a looser sediment.

For these different oils, we can generalise as follows:

*In cocoa butter and fat mixture*, both oil samples behave similarly. Their sediment volumes are the lowest of the series. Sugar particles are the most fully dispersed in comparison with the others systems. This behaviour is likely to be due to the strong repulsive interactions of the particles. This implies a more stable system with the least predominance for aggregation.

*In sunflower oil*, the sediment volume is intermediate, and the sugar particles are partially dispersed in the oil. This system may be considered as an almost stable system with less repulsive forces between the surface layers of the particles.

*In palm kernel oil*, the sediment volume is the highest of the series. Particles tend to aggregate extensively. This attraction between the particles may be the result of van der Waals forces. It results in a loosely packed sediment. The effect of purification tends to reduce the level of aggregation between the particles, leading to a slight reduction in the sediment volume.

*In soybean oil and milk fat*, there are undoubtedly some impurities which are not completely removed with charcoal. After the filtration with florisil, most of the surface-active impurities are apparently removed and aggregates are formed. This tends to give a loose high-volume sediment. The aggregation of sugar particles may be due to the presence of strong attractive forces, such as van der Waals forces and residual water which may form water bridges.

As a conclusion to this section, we note that the activated charcoal and florisil purification results are different. This feature is consistent with the work of Gaonkar (1989) who explained that activated charcoal is not a particular good adsorbent for purification of oil, in comparison to florisil. From Figure 4.6, samples purified with activated charcoal have commonly low volume of sediment, whereas non-purified samples tend to have a larger sediment volume. This confirms the observations of Johansson and Bergenståhl (1992c) who explained that water molecules will generally be adsorbed at the surface of sugar crystals, leading to the formation of aggregates. This means that, in presence of water (in non-purified samples), poorly packed sediments will be preferentially formed, and so larger sediment volumes will be obtained

Finally, in agreement with the work carried out by Johansson and Bergenståhl (1992a), these findings show that the sedimentation behaviour is strongly dependent on the specific nature of the studied oil, especially the presence of contaminating surfactants even at small concentrations. In order to confirm this statement, different sets of experiments were carried out in the presence of added emulsifier. Consequently, it was decided to add a known amount of pure phospholipid emulsifier (phosphatidylcholine, PC) to treated and non-treated systems in order to analyse the effect of the surface-active lipid on dispersions of sugar particles in the four different oils: sunflower oil, soybean oil, cocoa butter and palm kernel oil.

### IV.3.1.4. Effect of Emulsifier on the Sedimentation of Sugar Crystals in the Different Oils

#### *Effects on Purified Samples*

This type of experiment was carried out after the purification with activated charcoal only. The procedure was the following: purification of each oil, addition of the emulsifier (1 mmol $L^{-1}$ of Epikuron 200), and once the phosphatidylcholine (PC) was dissolved into the oil, addition of sugar crystals as for a standard sedimentation experiment.

Figure 4.7 shows the measured percentages of sediment volumes of particles in the different oils in the presence or absence of emulsifier for purified systems at 40 °C.

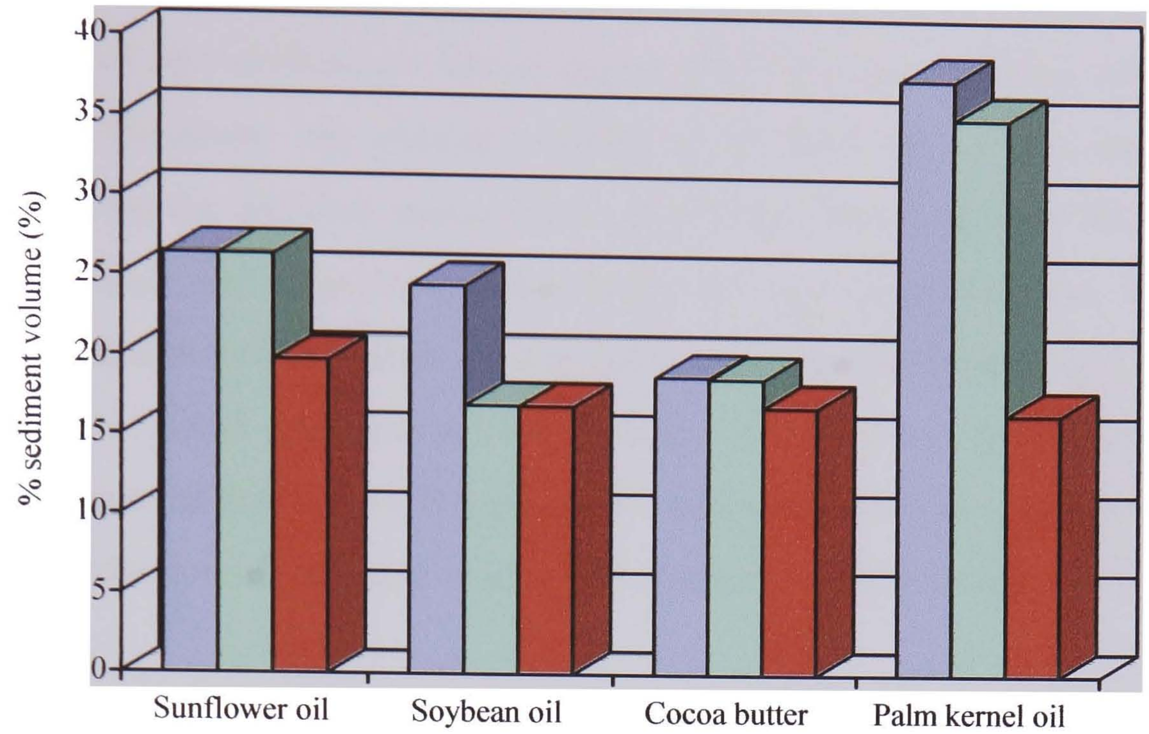


**Figure 4.7:** Effect of Epikuron 200 on the sugar sedimentations at 40 °C after purification with charcoal (volume of dispersion: ~ 5 mL: ± 2 % errors): ( ) represents the original and non purified sediment volume; (■) represents the original charcoal purified sediment volume containing emulsifier; and ( ) is the sediment volume after purification with activated charcoal.

Figure 4.7 shows that the presence of emulsifier reduces the volume of sediment in all the systems with the exception of the soybean sample. In this latter case, the volume of sediment remains constant. The drop in percentage of sediment volume is small for cocoa butter, but very substantial for palm kernel oil, whereas for sunflower oil it decreases slightly in comparison with the original sample. It can be observed that the addition of emulsifier tends to reduce the sediment volume towards a limiting value, which is ~ 16.9 %. In the case of sunflower oil, it can be suggested that the slightly higher value of the sediment volume (19.8 %) is still the same within the experimental errors (± 2 %).

The presence of emulsifier makes the sugar particles better dispersed in the oils, and consequently this improved stability results in the formation of tightly packed sediments. This result is in agreement with the observations made by Johansson and Bergenståhl (1992a). They noted that the presence of phosphatidylcholine in sugar + oil dispersions reduced the sediment volume by

30 %. In our experiments we note that, in presence of phosphatidylcholine, sunflower oil and soybean oil had an approximate sediment volume reduction of 30-40 %. However, the volume reductions of sediment in the presence of emulsifier for the purified cocoa butter and palm kernel oil were found to be negligible and very substantial respectively. By way of explanation, it may be suggested that the cocoa butter already contains emulsifier which gives the sugar particles a compact sediment volume already. In the case of palm kernel oil, it may be supposed that this oil is initially free of emulsifier, and so it would greatly favour a reduction of sediment volume in presence of added surfactant.

In order to confirm the veracity of these results and their interpretation, it was decided to study the effect of emulsifier on the non-purified samples, and to compare the results with the ones described above.

### *Effects on Non-Purified Samples of Four Oils*

Emulsifier was added to the oil. Once it was fully dispersed, a known amount of sugar was added into the mixture and the sedimentation experiment was carried out. Figure 4.8 shows the effect of emulsifier on the sugar particle sedimentation at 40 °C.

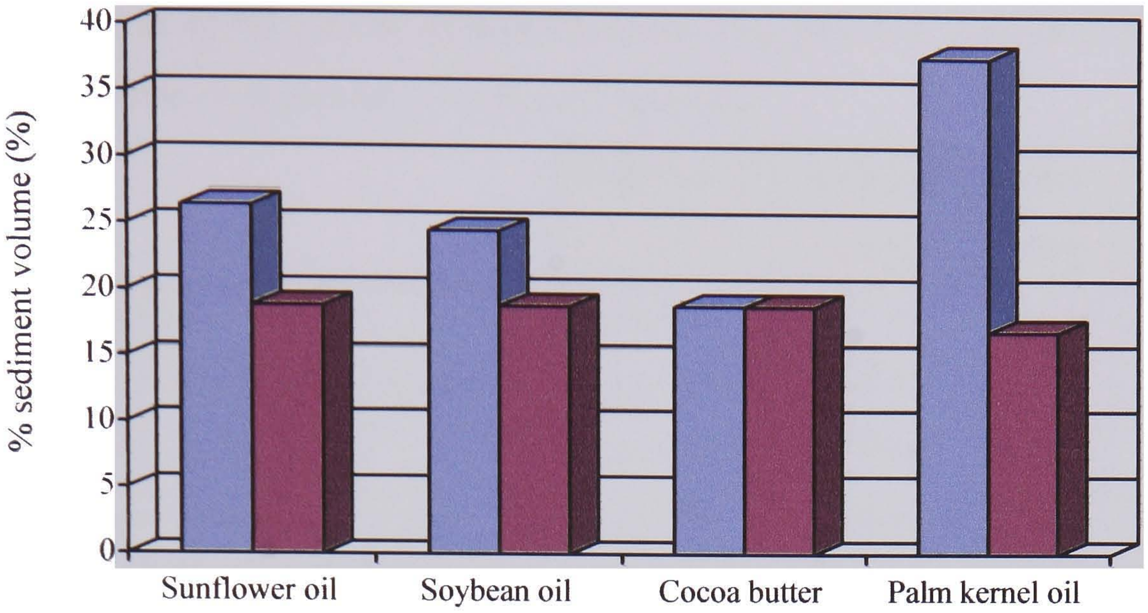


**Figure 4.8:** Effect of Epikuron 200 on the sugar sedimentations at 40 °C in non-purified oils (volume of dispersion: ~ 5 mL; ± 2 % errors). (▨) represents the original sediment volume and (■) is the sediment volume after emulsifier addition.

In Figure 4.8, it can be observed that the presence of emulsifier leads to a general decrease in the volume of sediment. This pattern confirms the trends found in the previous experiment done on purified systems. With the exception of palm kernel oil, it can be suggested that the limiting value in this series is no longer 16.9 % but 18.9 %. In the case of palm kernel oil, the volume of sediment is 16.9 %; but this is still within the experimental error. On the other hand, the presence of emulsifier in cocoa butter has no apparent effect on the sediment volume. The various results of sedimentation volume, with and without purification, and in the presence or not of emulsifier, are summarised in Table 4.2.

**Table 4.2:** Comparison of percentage sedimentation volume of sugar particles in the different oils at 40 °C with or without treatment with activated charcoal and emulsifier addition (volume of dispersion: ~ 5 mL; ± 2 % errors)

| | Sunflower oil | Soybean oil | Cocoa butter | Palm kernel oil |
|---|---|---|---|---|
| Original samples | 26.4 % | 24.5 % | 18.9 % | 37.7 % |
| Samples (original) with emulsifier (a) | 18.9 % | 18.9 % | 18.9 % | 16.9 % |
| Samples purified only (b) | 26.4 % | 16.9 % | 18.9 % | 35.8 % |
| Samples (purified) with emulsifier | 20.7 % | 16.9 % | 16.9 % | 16.9 % |
| % difference with emulsifier | 7.5 % | 5.6 % | 0 % | 20.8 % |
| % difference with purification | 0 % | 7.6 % | 0 % | 1.9 % |
| % difference with purification & emulsifier | 5.7 % | 7.6 % | 2.0 % | 20.8 % |

*Notes:* (a): Addition of 1 mmol $L^{-1}$ of phosphatidylcholine (Epikuron 200)
(b): Purification *via* activated charcoal

The Table 4.2 shows the following features:

*With sunflower oil*, the purification of the oil without emulsifier has no effect on the sediment volume; it remains constant at 26.4 %. A small reduction is observed for the samples containing emulsifier, from 26.4 % to 18.9 %, but this is hardly outside the experimental uncertainty. The presence of emulsifier decreases significantly (by 5.7–7.5 %)the volume of sediment with or without treatment with activated charcoal.

*With soybean oil*, the purification process has already lowered the sediment volume to its minimal value, i.e. that for a fully dispersed sample. So the emulsifier has no effect on the sediment volume (it remains at 16.9 %). When the sample is not purified, the volume of sediment is larger due to more aggregation amongst sugar particles (24.5 %). But the presence of emulsifier in the non-purified sample still reduces the volume to 18.9 %.

*With cocoa butter*, the only significant effect is the slight decrease of sediment volume when the sample is purified and contains emulsifier. At this stage, the volume drops by 2.0 %, the standard sediment volume for cocoa butter being

18.9 %. This implies that the sugar particles in cocoa butter are already well dispersed in the medium.

*With palm kernel oil*, the presence of emulsifier has a strong effect on the sediment volume as it reduces the value by almost one half without emulsifier. The effect is present for both purified and non-purified samples (20.8 % drop and 18.9 % drop, respectively). The purification stage has a marginal effect in decreasing the sediment volume from 37.7 % to 35.8 %. Consequently, palm kernel oil has a strong tendency to make sugar particles aggregate together and form a loose sediment. In the presence of emulsifier, the sediment volume is reduced to its smallest value, indicating a fully dispersed stable system.

To summarise these results, we can note that a single addition of emulsifier in both purified and non-purified samples decreases the sediment volume down to that of a tightly packed sediment. With the exception of cocoa butter, the emulsifier is effective in all the studied oils. In comparison with the effect of oil purification *via* charcoal, it has a slight effect in decreasing the sugar particles' sediment volume. The maximum difference obtained is about 8 % whereas the addition of emulsifier can lead up to a difference of 21 %.

Hence, the combination of these two adjustments to the oil composition has shown a reduction of sedimentation volume similar to that already obtained by a single operation (e.g. palm kernel oil and soybean oil). In the case of cocoa butter, we noted that the combination of purification and emulsifier addition leads to small reduction of sediment volume; whereas for sunflower oil, this cumulative adjustment has a less predominant effect than the single addition of emulsifier to the system.

Finally, it may be stated that the volume of sediment is mainly dependent on the nature of the oil, purified or not, as well as on the presence of surface-active components in the system.

As purified samples are relevant only in the field of research, and may be not practicable in the industry, it was decided to investigate further the sedimentation of our samples in the presence of combined minor oil components of different origins. We consider particularly the contribution of free fatty acids and lecithin emulsifier on the sedimentation volume.

#### IV.3.1.5. Effect of Free Fatty Acids on the Sedimentation of Sugar Crystals

After investigating the effect of phosphatidylcholine of the aggregation behaviour in these systems, it was decided to check the impact of free fatty acid on the sedimentation of sugar crystals dispersed in the oils. Linoleic acid was selected as the fatty acid. In order to have a 0.1 % free fatty acid solution in oil, a dilution was required. A known amount of this dispersion was used, and oil was added to complete the preparation of the sample. Then sugar crystals were added to the suspension and dispersed following the standard sedimentation procedure.

Figure 4.9 shows the effect of 0.1 % free fatty acid on the sedimentation of sugar crystals.

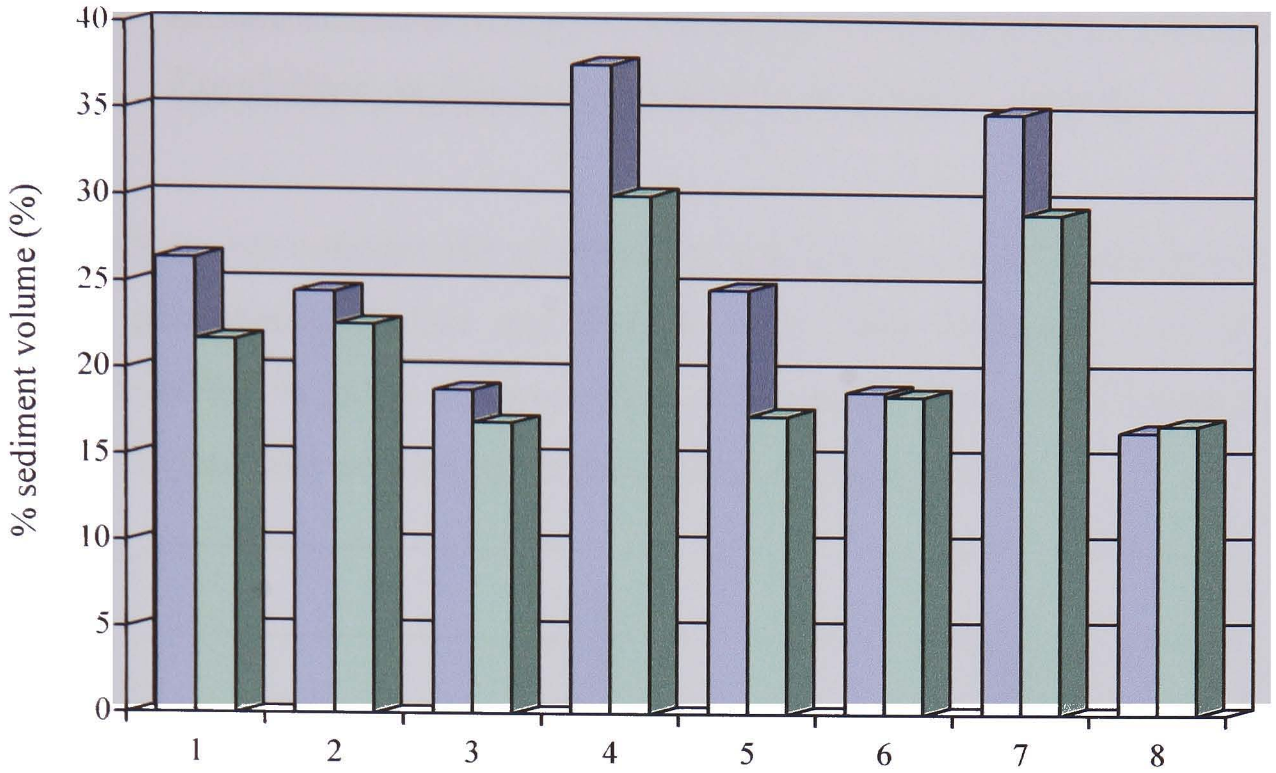


**Figure 4.9:** Effect of free fatty acid on the sugar/oil sedimentations at 40 °C (volume of dispersion: ~ 5 mL; ± 2 % errors). ( ) represents the original sediment volume and ( ) is the sediment volume after addition of free fatty acid. The sample numbers represent the different oils, 1: sunflower oil; 2: soybean oil; 3: cocoa butter; 4: palm kernel oil; 5: milk fat; 6: fat mixture; 7: olein; 8: stearin.

In Figure 4.9, we see that the presence of the free fatty acid, linoleic acid, reduces the sediment volume of each oil to some extent. Its presence has only the slightest or negligible effect on soybean oil and cocoa butter, and the fat mixture, respectively. It has a significant effect on sunflower oil and olein (a 4.7 % drop) and the largest effect with palm kernel oil and milk fat (a 7.5 % drop). In the case of stearin, it seems that the addition of emulsifier increased the sediment volume by 0.4 % (approximately 0.05 mL) but this is within the experimental uncertainty.

We see that in most of the cases the presence of free fatty acid enhances the stability of the dispersion by reducing the sediment volume. As a result of the presence of free fatty acid, the sediment is more dense or compact, suggesting that repulsive interactions between the particles are more predominant.

### IV.3.1.6. Comparison of the Effects of Free Fatty Acid and Emulsifier on the Sedimentation of Sugar Crystals

In this section, we compare the effect of the addition of two different types of surfactants: phosphatidylcholine and linoleic acid. Each surfactant was at a concentration of 0.1 % in the dispersed sugar/oil systems. Figure 4.10 shows the effect of 0.1 % free fatty acid on the sedimentation of sugar crystals.

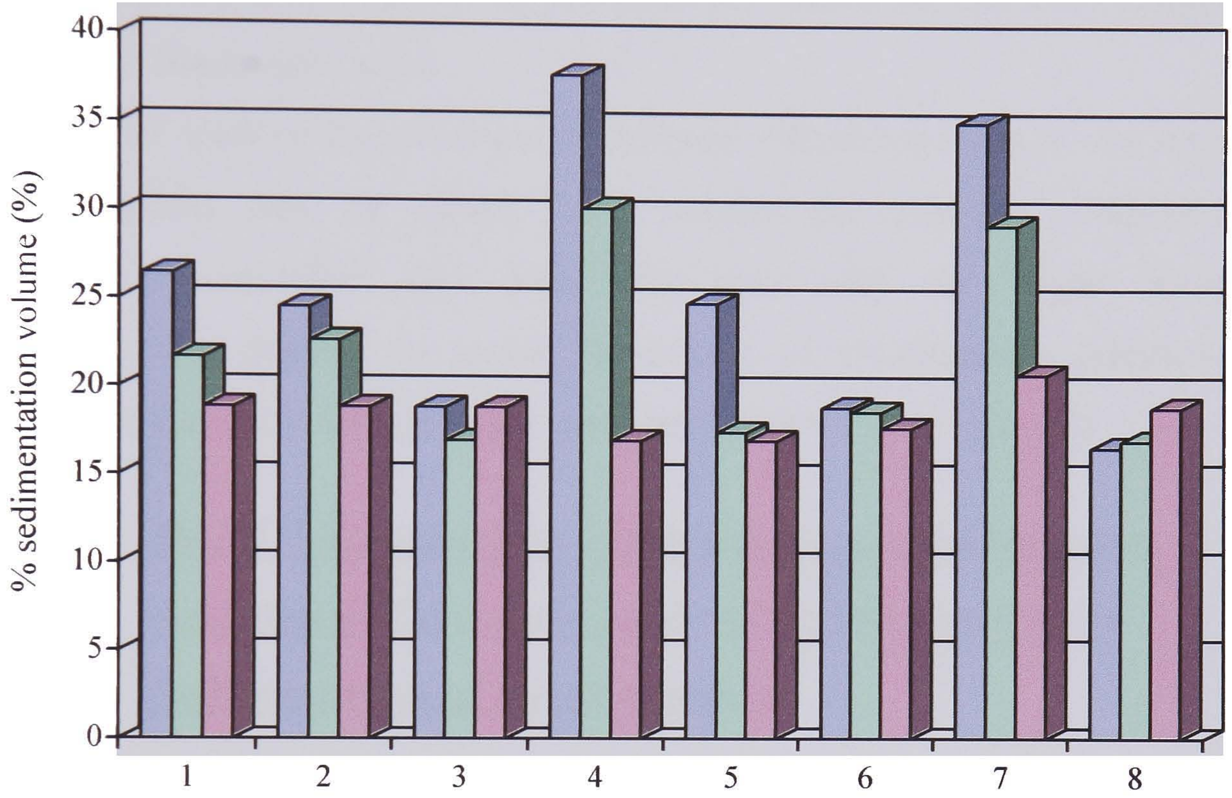


**Figure 4.10:** Effects of emulsifier (phosphatidylcholine) and free fatty acid on the sedimentation of sugar crystals in oil at 40 °C (volume of dispersion: ~ 5 mL; ± 2 % errors). ( ) represents the original sediment volume; ( ) is the sediment volume after addition of free fatty acid and (▪) is the sediment volume after addition of emulsifier. The sample numbers represent the different oils, 1: sunflower oil; 2: soybean oil; 3: cocoa butter; 4: palm kernel oil; 5: milk fat; 6: fat mixture; 7: olein; 8: stearin.

In Figure 4.10, we note that the addition of either free fatty acid or emulsifier decreases the volume of sediment to some extent with the exception of the stearin sample. It can also be observed that the presence of the emulsifier (lecithin) is more effective in reducing the sediment volume than is the presence of free fatty acid, with the exceptions of cocoa butter and stearin. In these latter cases, the volume of sediment with or without emulsifier either remained unchanged or increased by 2 %. This probably indicates that cocoa butter and stearin already contain some effective emulsifier within their own composition, due to the low sediment volume values in comparison with the others samples. Moreover the addition of further phospholipid may not be beneficial due to the large quantity of surfactant already surrounding the sugar crystals. On the other hand, the addition of linoleic acid may interact with the sugar crystals at some different areas on the surface of these crystals, whereas the phospholipid emulsifier will not necessarily be adsorbed in those same areas.

The point of view of the existence of different adsorbing areas on the surface of sugar crystals may be raised. This implies the possible simultaneous interactions of emulsifier and free fatty acid with the sugar crystals. Consequently, we decided to probe the effects of simultaneous addition of emulsifier and free fatty acid on sugar particles dispersed in several oils.

### IV.3.1.7. Comparison of the Effects of Simultaneous Addition of Free Fatty Acid and Emulsifier On the Sedimentation of Sugar Crystals

In this section, we describe the effect of the simultaneous addition of two surfactants: phosphatidylcholine and linoleic acid. Each surfactant was at the same concentration in each of the systems (0.1 % in sample). Figure 4.11 shows the percentage of sediment of sugar particles in the different oils. The amount of surfactant in the oil phase represents 1 mmol $L^{-1}$ (0.1 %) and it is independent of the sugar content in the mixtures.

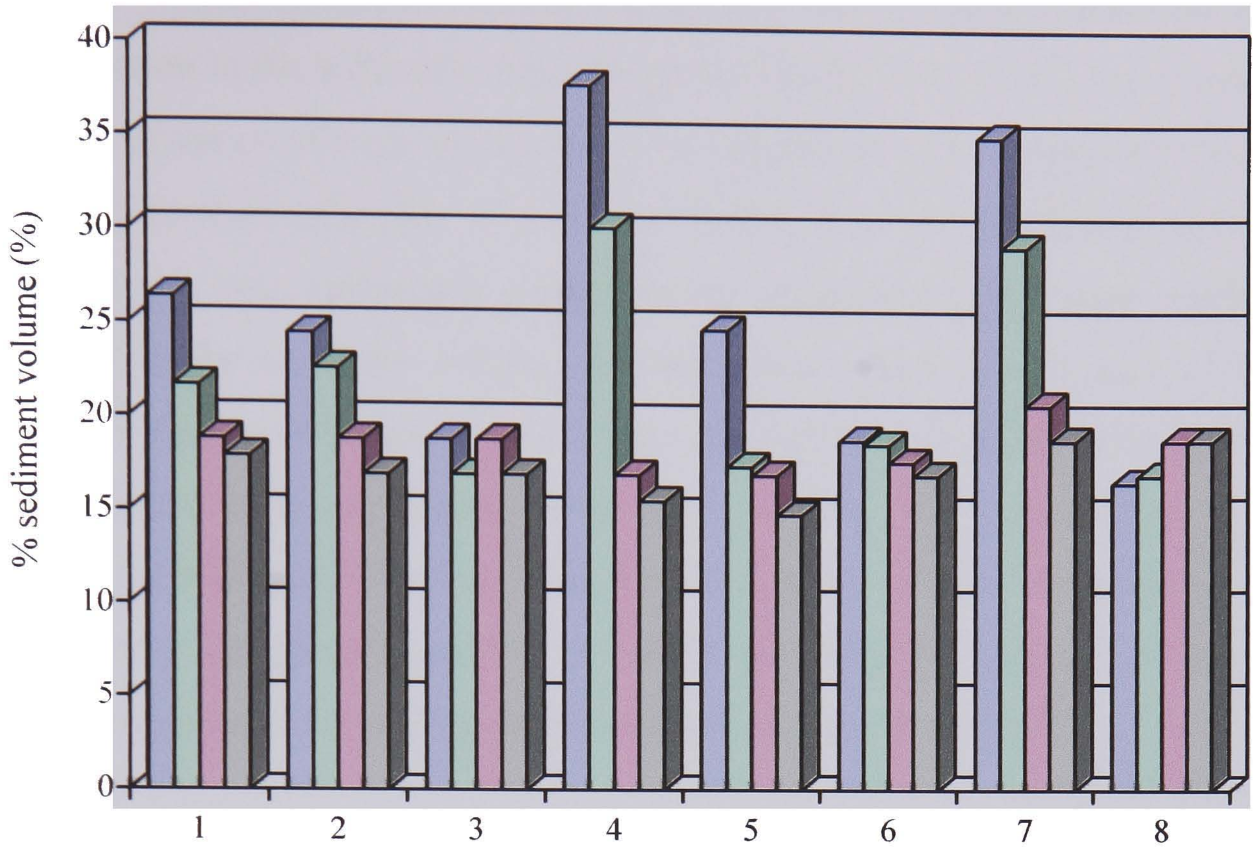


**Figure 4.11:** Effects of 0.1% emulsifier and/or 0.1% free fatty acid on the sedimentation volume of sugar for different oils at 40 °C (volume of dispersion: ~ 5 mL; ± 2 % errors). ( ) represents the original sediment volume; ( ) is the sediment volume after addition of free fatty acid; (■) is the sediment volume after addition of emulsifier and ( ) is the sediment volume after simultaneous addition of free fatty acid and emulsifier. The sample numbers represent the different oils, 1: sunflower oil; 2: soybean oil; 3: cocoa butter; 4: palm kernel oil; 5: milk fat; 6: fat mixture; 7: olein; 8: stearin.

From Figure 4.11, it can be seen that the addition of both surfactant species together into the mixtures reduces the sediment volume of the sugar particles. The type of surfactant added also influences the volume of sediment: the presence of emulsifier allows a greater reduction of sediment volume in comparison to free fatty acid addition. The percentages of sediment volume drop for samples containing emulsifier or free fatty acid are about 7 % and 3 % reduction respectively. Nevertheless, we note that in presence of either kind of surfactant, the sediment volume tends to have a limiting value of 0.9 mL (approximately 16 % volume sediment) for all the systems studied, with the exception of palm kernel oil. In this specific case, palm kernel oil has a minimum sediment volume of ~ 0.8 mL.

We observed that cumulative addition of lecithin and free fatty acid results in a further reduction in the sediment volume (approximately 8 %). As a consequence, the addition of the combined surfactants to all samples results in the formation of more closely packed sediments. From these results, it can be concluded that the presence of the two surfactants enhances the stability of the sugar particle dispersions, as the sediment volume decreases more than for the case of the addition of a single surfactant. Moreover the combined addition of surfactants of different origins, such as phosphatidylcholine and free fatty acid, seems more effective in the reduction of volume than for addition of each one alone. However, this statement is not clearly justified as we did not check the effect on sediment volumes of singular addition of emulsifier at different concentrations.

### IV.3.1.8. Comparison of the Effects of Three Different Emulsifiers on the Sedimentation of Sugar Crystals

New experiments were carried out involving the two following emulsifiers: polyglycerol polyricinoleate (PGPR) and pure monoolein. The choice of these emulsifiers was determined by the fact that PGPR is commonly used as an additive in chocolate confectionery due to its rheological ability to lower the yield value of chocolate much more effectively than lecithin (or phosphatidylcholine). Monoglycerides are commonly used emulsifiers in the food industry. Pure monoolein is considered here because of it use in the work of Johansson and Bergenståhl (1992a).

**Table 4.3:** Comparison of the effect of different emulsifiers (0.1 %) on the percentage sediment volume of sugar particles in the different oils at 40 °C (volume of dispersion: ~ 5 mL; ± 2 % errors).

| | % sediment volume with added emulsifier | | | |
|---|---|---|---|---|
| | original | PC (a) | PGPR (b) | Monool. (c) |
| Sunflower oil | 26.4 | 18.8 | 18.8 | 18.9 |
| Soybean oil | 24.5 | 18.8 | 14.8 | 18.9 |
| Cocoa butter | 18.8 | 18.8 | 16.9 | 17.4 |
| Palm kernel oil | 37.7 | 16.9 | 13.4 | 18.9 |
| Milk fat | 24.7 | 16.9 | 14.8 | 17.4 |
| Fat mixture | 18.8 | 17.6 | 16.7 | 17.9 |

(a) PC: phosphatidylcholine; (b) PGPR: polyglycerol polyricinoleate; (c) Monool: monoolein

In Table 4.3, we note that the presence of 1 mmol L$^{-1}$ of any type of emulsifier reduces the level of sediment in most of the cases, with the exception of phosphatidylcholine in cocoa butter. We can also note that the presence of any emulsifier lowers the sediment volume down to a value of ~ 16 % and this independently of the nature of the oil. In the present study, we have generally observed that the minimum value of sediment volume that can be obtained is around 16 % by volume. If sugar particles are represented as roughly spherical, this minimum sediment volume value corresponds fairly closely to the random close packing of mono-disperse hard spheres.

Moreover, in Table 4.3, we note the general pattern that PGPR is the most efficient emulsifier and monoolein the worst one with respect to reducing the sediment volume. But it cannot be concluded that such an emulsifier will always have better dispersing properties, as these stability properties are directly related to the specific characteristics of the oils and their own surfactant contents. As consequence, for cocoa butter and fat mixture, the percentage decrease of sediment volume is the lowest amongst the eight studied oils. But this is mainly due to the fact that sugar particles in these two oils are already better dispersed without any emulsifier addition. On the other hand, the percentage sediment volume reduction is the greatest in the palm kernel oil system because the sugar particles were the most strongly aggregated in this particular oil to start with.

In order to have a more complete picture of the interaction between emulsifiers and free fatty acids, we studied the effects of polyglycerol polyricinoleate and monoolein in samples already containing free fatty acid.

### IV.3.1.9. Comparison of the Effects of Three Different Emulsifiers on the Sedimentation of Sugar Crystals in Oils Containing Free Fatty Acid

Figure 4.12 shows the percentage sediment volume in the different oils systems containing added free fatty acid (1 mmol $L^{-1}$) and with the addition of 1 mmol $L^{-1}$ of PC, PGPR or monoolein.

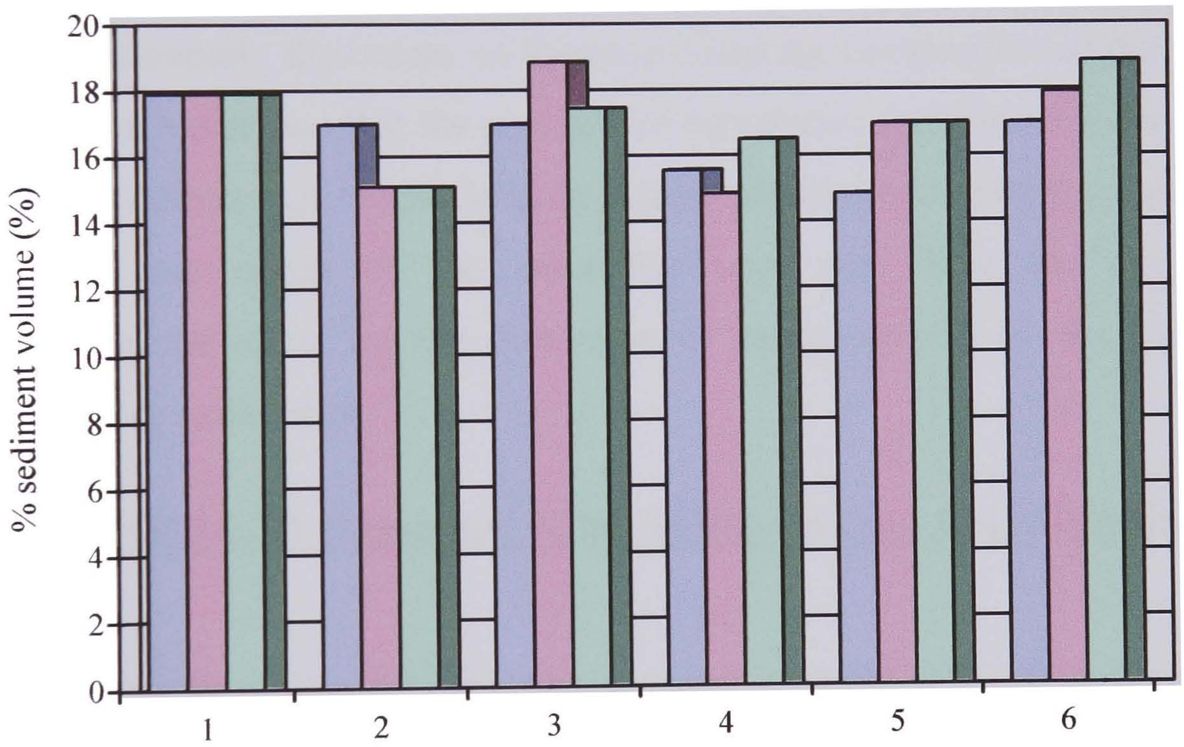


**Figure 4.12:** Effects of 0.1 % emulsifier on the sedimentation volume of sugar for different oils containing already 0.1 % linoleic acid at 40 °C (volume of dispersion: ~ 5 mL; ± 2 % errors). ( ) represents the original sediment volume after addition of phosphatidylcholine (PC); ( ) is the sediment volume after addition of polyglycerol polyricinoleate and ( ) is the sediment volume after addition of monolein. The sample numbers represent the different oils, 1: sunflower oil; 2: soybean oil; 3: cocoa butter; 4: palm kernel oil; 5: milk fat; 6: fat mixture.

In Figure 4.12, we see that the percentage sediment volumes lie in the narrow range between 15 % and 18 %. The effect of the emulsifier is only slightly variable amongst the different samples. The presence of emulsifier of different origins does not influence sedimentation in the sunflower oil samples. In soybean oil and milk fat samples, both polyglycerol polyricinoleate and monoolein give the same sediment volumes. Phosphatidylcholine seems more efficient in reducing the sediment volume of milk fat cocoa butter, palm kernel oil and fat mixture rather than with soybean oil; but PGPR strongly affects the sediment volumes of palm kernel oil sample.

As a conclusion to this section, we observed that single addition of emulsifier on samples which already contain free fatty acids leads to a reduction of sediment volume on samples containing already a known amount of free fatty acid. However, the differences between the sediment volumes are not significant and these remain extremely dependent on the nature and the composition of the oils. This experiment has shown that the presence of cumulative surfactants in the oils, influence the dispersion of the particles by further reducing the sediment volume. Unfortunately, the origin of the emulsifier does not allow for accurate characterisation the effect on the dispersion of sugar particles in the oils in presence of other surfactants.

### IV.3.1.10. Summary of the Sedimentation Experiments

The oil purification stage results in the apparent formation of more aggregates and this behaviour leads to a less compact sediment. It has been demonstrated that charcoal has a low efficiency for adsorbing surfactants and moisture present in the oils. On the other hand, samples purified with florisil have displayed a large volume of sediment. This after behaviour implies a more aggregated state of sugar particles, which may be the result of more predominant attractive forces (e.g. van der Waals forces) combined with water bridges formed at the sugar surface.

Our sedimentation results obtained with the purifications of activated charcoal and florisil were different for both adsorbents. We noted that activated charcoal was a poor adsorbent in comparison with the florisil. These results were consistent with the work of Gaonkar (1989). Moreover, this analysis has also shown that water, which forms water bridges, influence greatly the attraction of sugar particles in our system. These results confirm the observations made by Johansson and Bergenståhl (1992c) in which water tends to increase the adhesion between sugar crystals in oils.

The sedimentation results can be schematically summarised in Figure 4.13.

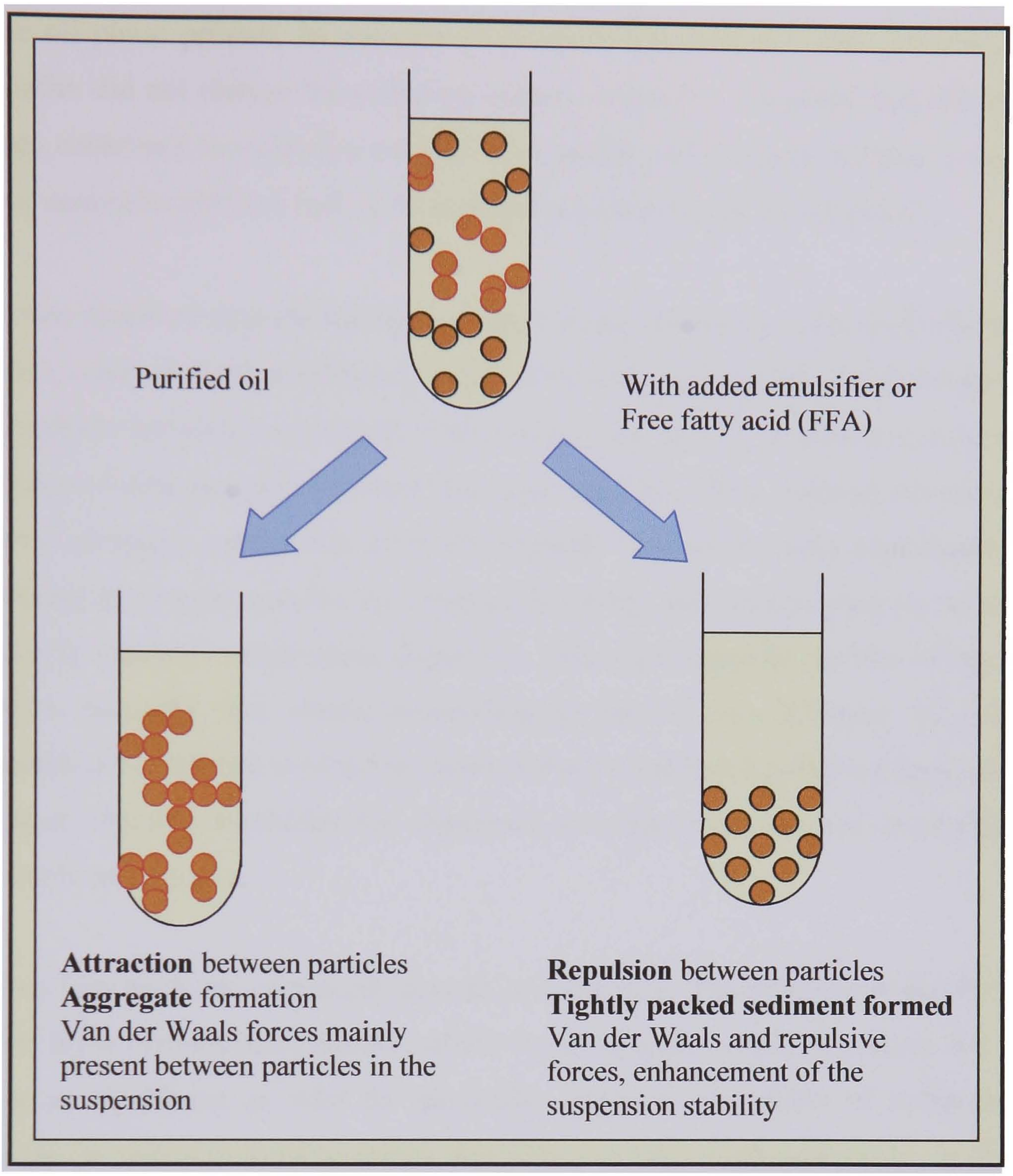


**Figure 4.13:** Schematic interpretation of the interactions present in the sedimenting of sugar/oil dispersions. ( ◉ ) represents dispersed (isolated) sugar particle and ( ● ) represents an aggregated sugar particle.

The presence of surfactants in the oils phase has a positive effect on the reduction of sediment volume. This behaviour was also noted by Johansson and Bergenståhl (1992c). They explained that the emulsifier strongly adsorbs at the surface of the sugar crystals. Such adsorption will tend to inhibit particle aggregation and thus reduce the sediment volume. The present study has further shown that the volume of sediment changes considerably depending on the nature of the oil phase present. In the case of cocoa butter, however, the addition of emulsifier did not change the sediment volume. It may be suggested that this fat already contains a considerable amount of emulsifier. Its sediment volume, which is low, cannot be affected further by addition of another type of surfactant.

It was observed that the sediment volume in the suspension of a total volume of 5 mL tends to reach a minimum value of 0.9 mL, presumably due to limiting steric effects between the particles. This controls the packing of particles until no rearrangement is possible between the sugar crystals. Close packing of mono-disperse particles corresponds to 16 % sediment volume in these experiments. Assuming that sugar particles are spherical particles, this random packing would be closely related to single phase dispersion. This result supports the idea of sugar particles behaving like simple mono-disperse spheres (see Chapter V). The comparison of different emulsifiers showed that polyglycerol polyricinoleate was the most effective emulsifier for dispersing the sugar particles; and monoolein was the least effective.

Free fatty acids are commonly present in edible oils. The addition of free fatty acid to these systems has a positive effect in reducing the sediment volume, but it is not as significant as with the emulsifier addition. The effect of surfactant addition on sediment volume varies depending on the oil characteristics. In the case of cocoa butter, it may suggested that free fatty acid is adsorbed on certain areas of the surface of sugar crystals where emulsifier is not adsorbed at all. This implies that it may be possible to perform multiple additions of different surfactants to reduce the aggregation state of particles in this fat. It was shown that such additions decrease even further the volume of sediment. It can be suggested that the surface of sugar crystals may present regions of different binding energy. It can be assumed that simultaneous addition of emulsifier and free fatty acid improves the stability of sugar dispersions in oil.

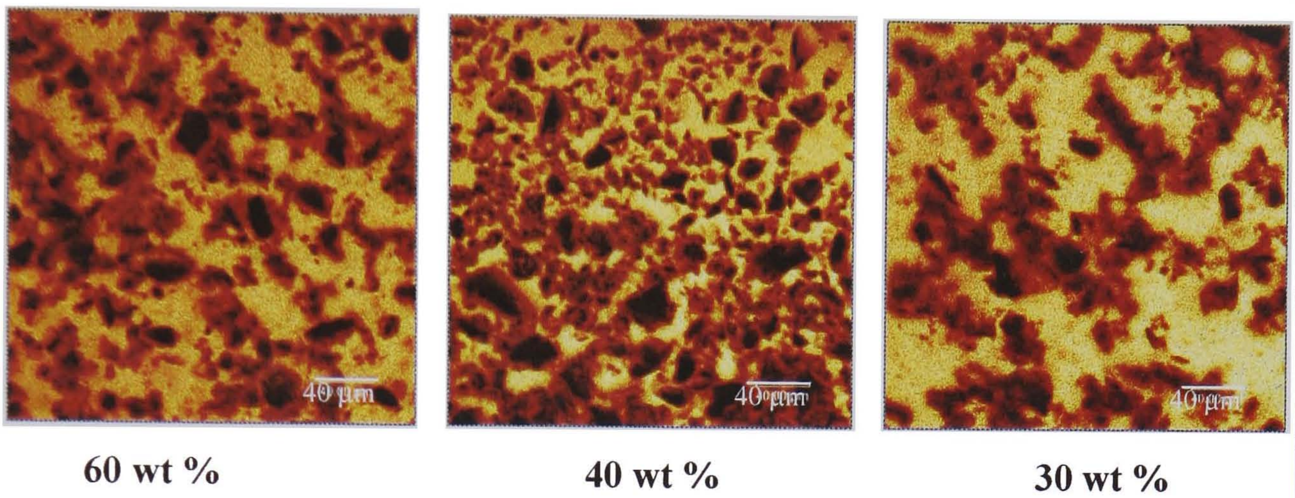
More work has been done on the dispersions of sugar particles mixed in oil which already contained a know amount of free fatty acid. Attempts have been made to characterise the influence of specific emulsifiers on the sediment volume with phosphatidylcholine, PGPR (polyglycerol polyricinoleate) and monoolein. We noted that the addition of emulsifier improves the dispersion of sugar particles in comparison with a singular addition of surfactant. However no specific effect has been observed from one emulsifier to the other.

Sedimentation remains a powerful technique for subjectively analysing and interpreting the behaviour and the interactions of sugar particles dispersed in oils. This technique has shown that the aggregation of sugar particles dispersed in oils accounts for different forces such as van der Waals and repulsive forces and hydrogen bonds. The predominance of these forces depends of the physical properties and chemical composition of the oils, the purification step of these oils and the presence of surfactants. In order to define further the influence of the concentration in sugar particles, more work has been developed with Confocal Laser Scanning Microscopy or rheology (Chapter V).

### *IV.3.2. Confocal Microscopy Experiments*

For confocal microscopy, samples were prepared with three different sugar contents (30, 40 and 60 wt %) dispersed in cocoa butter and palm kernel oil. In conformity with the work published by Greenspan *et al.* (1985), specimens were stained with a solution of Nile Red diluted into polyethylene glycol 400. The dye solution had a concentration of 0.01 % (g $mL^{-1}$). Different amounts of dye solution were added to the system in depending on the sugar concentration in the mixtures. Two levels of magnification (x 300, x 500) were used to investigate signs of aggregation amongst the sugar particles.

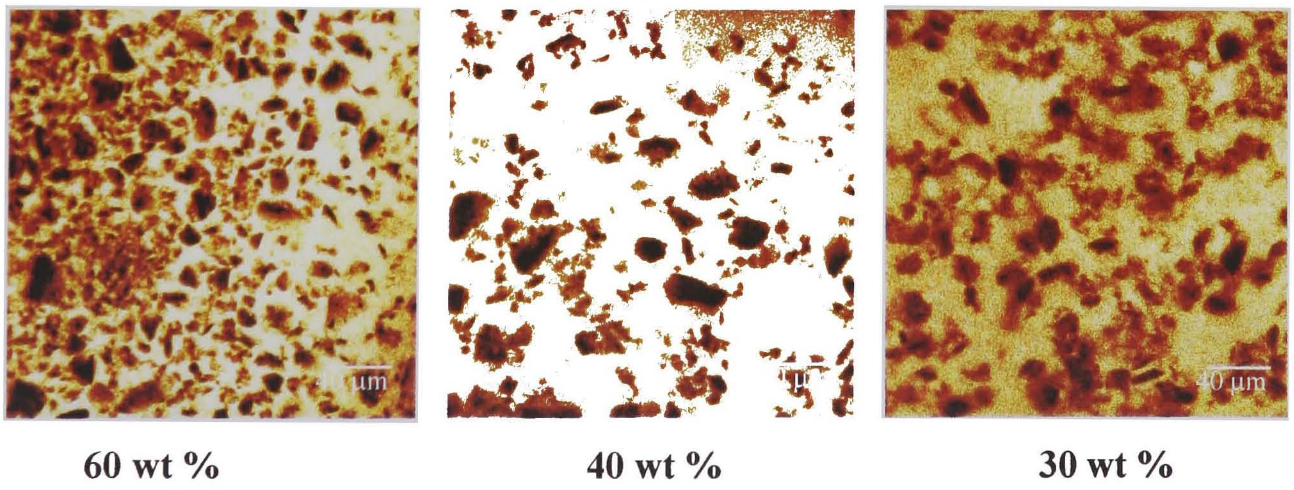
Figures 4.14a and 4.14b show the microscopic images for the three different sugar contents based, respectively, on cocoa butter and palm kernel oil at × 300 magnification. The series with × 500 magnification are presented in Appendix **VI-i**.



**60 wt %** **40 wt %** **30 wt %**

**Figure 4.14a:** Confocal microscopy images for 60, 40 and 30 wt % sugar particles dispersed in cocoa butter.

In Figure 4.14a it can be observed that the higher sugar content mixtures (60 or 40 wt %) do not easily permit assessment of the level of aggregation of the particles, as the systems are too concentrated. The sugar patches are more predominant at these higher concentrations, rather than at 30 wt %. At the lower concentration, discrete aggregates can be differentiated.



**60 wt %** **40 wt %** **30 wt %**

**Figure 4.14b:** Confocal microscopy images for 60, 40 and 30 wt % sugar particles dispersed in palm kernel oil.

In Figure 4.14b sugar patches were much more agglomerated at high sugar concentrations and distinctive aggregates were formed at lower sugar concentration in the samples. Despite careful micrograph examinations, the interpretation of the results via confocal microscopy was inconclusive. Therefore, it was decided to restrict our attention to the 30 wt % sugar content mixtures in order to compare the relative levels of aggregation of sugar particles in the two oils, cocoa butter and palm kernel oil.

A qualitative comparison of sugar interactions in cocoa butter and palm kernel oil has been attempted with the micrographs obtained in these series. We have observed that sugar particles surrounded by palm kernel oil appear more spread out. Sugar aggregation seems less strong than in sample made of cocoa butter and the system seems more disparate than in the case of sugar surrounded by cocoa butter.

These attempts with the confocal microscopy technique showed that no qualitative analysis on the aggregate structure could be performed and structure could be only defined by visual interpretation. So it was not possible to quantify the relative strengths of the interactions with this microscopy technique. Due to the disappointing results from this technique with our preliminary samples, we decided not to investigate further with all the remaining oil systems. Micrographs representing the remaining mixture samples at a concentration of 30 wt % sugar are represented in Appendix **VI-ii**.

As a summary to this section, we may say that the confocal microscopy technique permits a good visualisation of the sugar particle aggregates in the oils, but it remains insufficient for quantifying or modelling the structure of these aggregates. Based on our preliminary observations, we have managed to determine an effective sugar concentration in oil (30 wt %) to observe distinctive aggregates, but there are no reproducible significant differences between the samples made with cocoa butter, palm kernel oil or the other oils. This non-disruptive technique is therefore of little assistance in characterising quantitatively the strength of the sugar particle interactions.

As a future work it may be a good idea to consider the combination of the Confocal Laser Scanning microscopy method with any other physical techniques. (Hicklin *et al.*, 1985).

## *IV.4. Conclusion*

To conclude, the use of the subjective physical techniques such as sedimentation allowed the comparison of the level of interaction of sugar particles dispersed in oils. We have seen that the purification of the oils is not effective with the activated charcoal in comparison with the florisil. The singular addition of surfactant, emulsifier or free fatty acid, has reduced substantially the level of sediment volume. The effect of the emulsifier was more predominant on the reduction of sediment volume than the effect of free fatty acid. However, we observed that the cumulative addition of both surfactants reduces further the sediment volume and therefore improves further the stability of the suspension. We also observed that the minimum close packing has a value of 16 %. Considering the dispersion model of hard spheres suspended in a liquid, this value is characteristic of the mono-disperse sphere suspension. The interesting feature of this technique was the ability to understand and to characterise the influence of several parameters at low concentrated dispersions. It was not possible to quantify the strength of the dispersed sugar particles in the different oils. However, further more analysis have been undergone which allow the correlation of these results with the rheology technique.

Consequently, other techniques need to be further explored to understand better the factors affecting the interactions between sugar particles in the different oils and to measure quantitatively the effects of the different parameters conditions. Based on the work of Johansson and Bergenståhl (1992a), it may be interesting to correlate our sediment results with a technique like rheology.

# *Chapter V Rheological Study of concentrated solid/liquid systems*

## *V.1. Introduction*

Rheology is a well used technique for analysing the flow properties of particles dispersed in a continuous media (Dickinson, 1992). Moreover, in reference to the published work of Johansson and Bergenståhl (1992b), it was logical to perform some analysis with this method. In their publication, they investigated the rheological behaviours of fat and sugar particles dispersed in vegetable oils. Due to the popularity of this technique, several characteristic flow models have been reported at different occasions. Einstein equation is mainly encountered for diluted dispersions of hard spheres (Dickinson, 1992); whereas Casson equation has been commonly used in the chocolate industry (Chevalley, 1975; Nelson and Beckett, 1999). The flow properties may also be influenced by the presence of surfactant which favour the dispersions or the aggregations of particles in the media (Johansson and Bergenståhl, 1992b; Schantz and Rohm, 2005).

The present section will evaluate the physical aspect of the aggregation of sugar particles dispersed in eight oils. The viscosity of the sample was determined at variable stress. We have been comparing the level of interactions of the sugar particles within different oils at different sugar concentrations. Further analyses compared these interactions with theoretical models such as Einstein equation and Casson equations. The dispersions of sugar particles at laboratory scale remained insufficient in comparison with the products made in the chocolate industry. Therefore development of the dispersing technique has been performed and compared with the results obtained with the original dispersing process. Moreover, the separate effects of three emulsifiers on their interactions with sugar particles have been identified. We have also discovered the specific relationship between phosphatidylcholine and milk fat in aggregating the sugar particles together.

## *V.2. Materials and methods*

### *V.2.1. Materials*

Materials specifically used for the rheology work described in this chapter are detailed below. The other common materials have been described in chapter IV.

### *V.2.2. Methods*

#### **V.2.2.1. Preparation of samples**

*"Basic mixing method"*

Dispersions of 10 to 50 wt % sugar in oil were prepared for the sedimentation and rheological experiments. The oil was heated on a hot plate at 40 °C. Once the oil was at the right temperature, the sugar was added to the oil. In presence of emulsifier, this one was added first and prior the addition of sugar. After complete dissolution of the emulsifier, a defined amount of sugar was poured into the oil. After the mixture had been stirred for 24 hours, it was ready for analysis. Figure 5.1 represents the addition stage of emulsifier and sugar into the oil using the "*basic mixing method*".

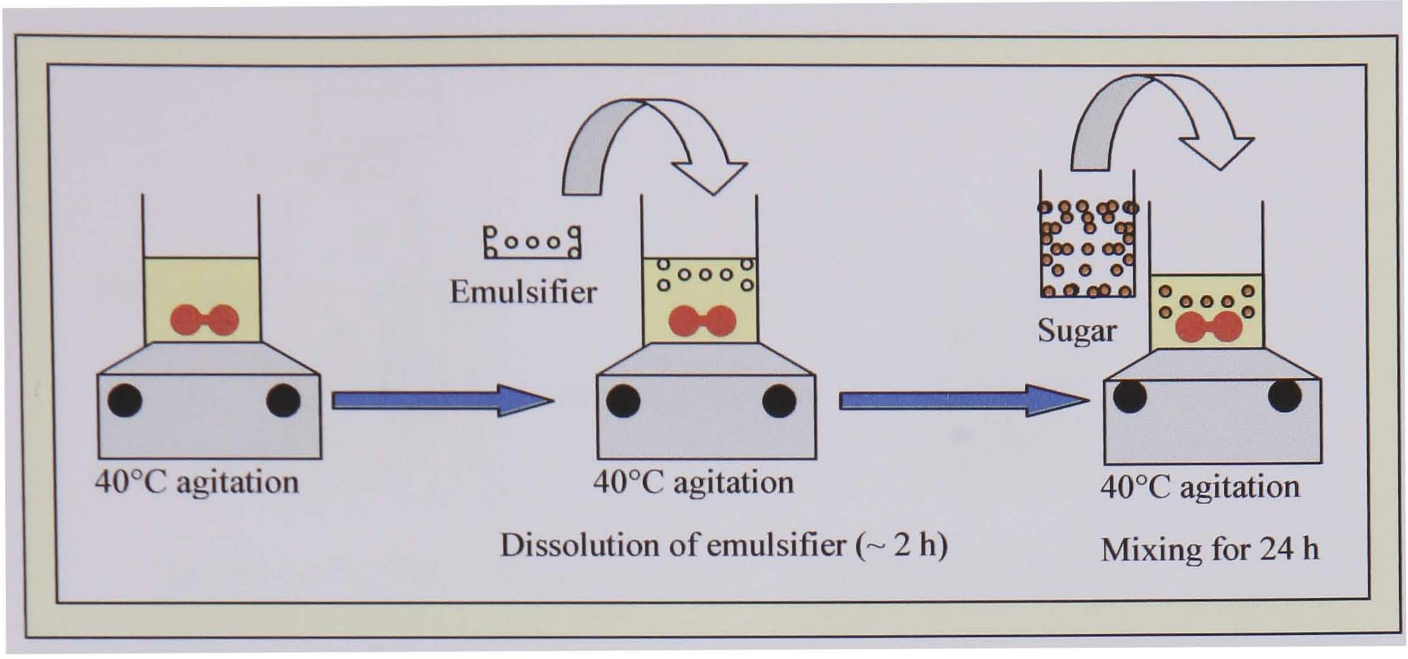
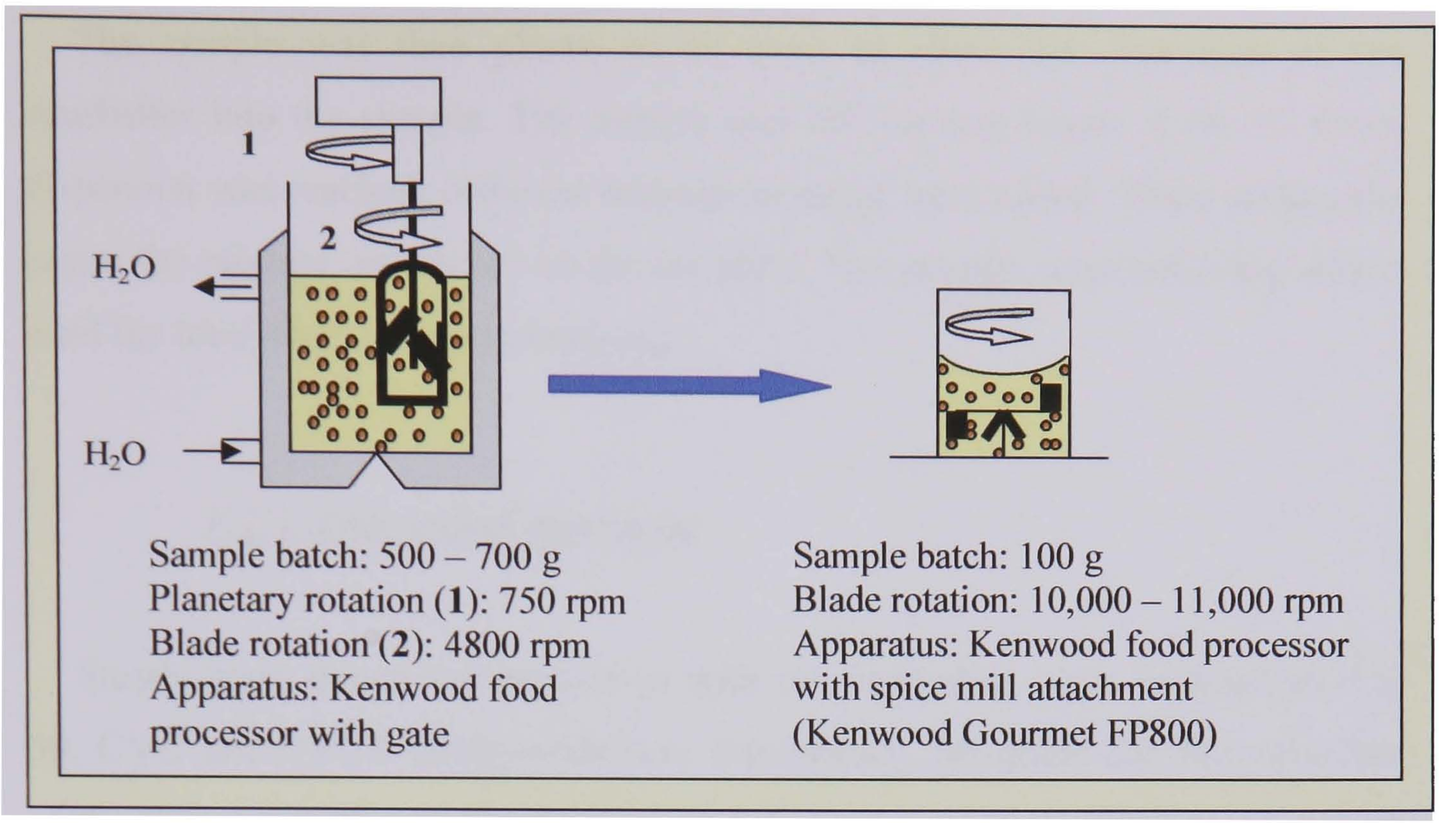


**Figure 5.1:** Schematic diagram of the "*basic mixing method*"

*"Refined mixing method"*

Highly concentrated dispersions of 50 to 75 wt % sugar were prepared into a two-stage process. This method was developed in order to replicate more closely the process used in the chocolate confectionery. It involved making larger quantities of sample (700 g of sample) by using industrial apparatus such as a Kenwood food processor and a Kenwood spice mill attachment.

A base of 70 - 75 wt % sugar in oil was first made using the Kenwood food processor with a mixing gate. The sample was mixed at the maximum speed for 10 minutes and was subjected to two different rotations, a blade and a planetary rotation. Once mixed, 100 g of sample was taken and further mixed in a Kenwood food processor with a spice mill attachment. At this stage, the sample was diluted and emulsifier added for all the concentrations. The spice mill attachment had a blade rotation speed of 10,000 to 11,000 rpm. The sample was mixed for 2 minutes under these conditions. Figure 5.2 illustrates the refined method for mixing sugar into the oil.



Sample batch: 500 – 700 g
Planetary rotation (**1**): 750 rpm
Blade rotation (**2**): 4800 rpm
Apparatus: Kenwood food processor with gate

Sample batch: 100 g
Blade rotation: 10,000 – 11,000 rpm
Apparatus: Kenwood food processor with spice mill attachment
(Kenwood Gourmet FP800)

**Figure 5.2:** Schematic diagram of the "*refined mixing method*"

### *Calculations of the amount emulsifier to be added into the samples*

Based on the ratio of 70 wt % sugar / 0.3 wt % emulsifier, a defined amount of emulsifier was added to the liquefied fats. Table 5.1 represents the different amounts added for the various sugar concentrations.

**Table 5.1:** Percentage of emulsifier added for different percentages amount of sugar

| % wt of sugar | % wt of emulsifier |
|---|---|
| 70 % | 0.300 % |
| 65 % | 0.278 % |
| 60 % | 0.257 % |
| 55 % | 0.236 % |
| 50 % | 0.214 % |
| 40 % | 0.171 % |
| 30 % | 0.128 % |
| 20 % | 0.086 % |
| 10 % | 0.043 % |

The sample was then placed in an oven to allow the dispersion of the emulsifier into the sample. The sample was left for two hours, at 60 °C. Once dispersion was reached, different amounts of sugar were added. While adding the sugar, the mixture was stirred on the hot plate. The samples were left being stirred until the total dispersion was achieved.

### *V.2.3. Rheological equipment*

Steady state rheometry performed with the controlled-stress rheometers, CS 50, CVO and CVOR (Bohlin-Malvern Equipment), permitted the determination of the flow properties of the samples. The viscosity was determined as a function of sugar mass fraction and shear-rate. The sample was analysed using a concentric cylinder measuring system: cup and bob (geometry C25, inner diameter 25 mm, outer diameter 27.5 mm; geometry C14, inner diameter 14 mm, outer diameter 15.4 mm) or cone-and-plate measuring system (geometry CP 4° / 40, inner diameter 40 mm, outer diameter 60 mm, cone angle 4.0 °). The cone-and-plate measuring system was mainly used for the most highly concentrated systems. The sample was carefully poured into the cup at 40 °C with a temperature control of ± 0.1 °C. Both concentric cylinder and cone and plate geometries are represented in Figure 5.3.

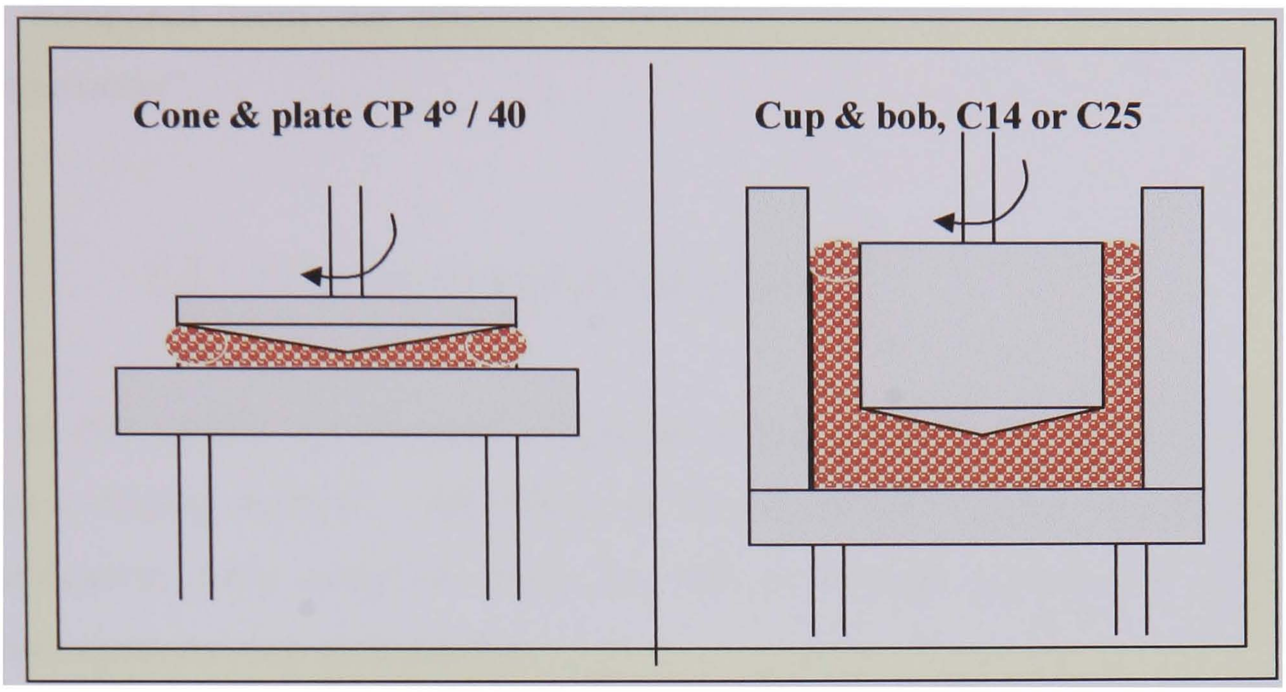


**Figure 5.3:** Schematic representation of the equipment used for rheology experiments

## *V.3. Results and Discussion*

The present section will evaluate the physical aspect of sugar particles dispersed in oil media. The level of interactions will be compared by determining the viscosity of the sample at variable force stresses. In the first part of the analysis, the results will be compared at different sugar concentrations for four oils: sunflower oil, soybean oil, cocoa butter and palm kernel oil. As the samples get more concentrated, work has been extended with the use of different oils. In the second part, the sugar suspensions of the first four oils will be further analysed via theoretical models such as Einstein and Casson equations, for respectively diluted and concentrated dispersions. Suspensions of sugar particles prepared at laboratory scale were not representative enough to compare them with suspensions developed industrially. Therefore, a dispersing technique was developed, which would mimic chocolate factory dispersions and thus results will be compared from the original dispersing process to the "*refined mixing preparation*".

### *V.3.1. Preliminary rheological experiments*

In this section the physical properties of the oils were investigated via the "basic mixing method". The effects of absence and presence of surfactant on purification were analysed. Then the effects of sugar particles at different concentrations were investigated.

#### **V.3.1.1. Inherent physical properties of the oils**

##### *Effect of purification on the viscosity of oils*

In these preliminary experiments, summarised by Figure 5.4, no sugar was added. The purification was carried out using activated charcoal with 10 % (w/w) of charcoal added to the oils. Samples containing adsorbent were left for 24 hours in a cool dark area. Filtration was done with filter paper. No purification was performed with florisil.

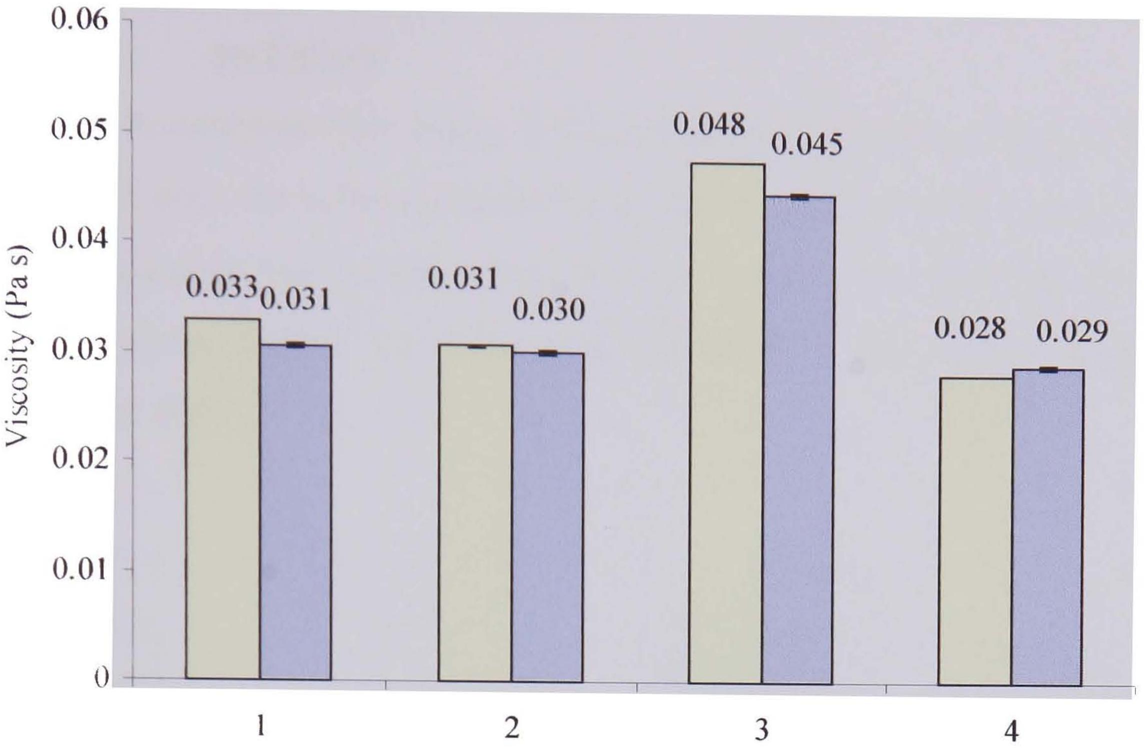


**Figure 5.4:** Effect of purification of oils on viscosity measured at a shear stress of 1 Pa at 40 °C (experimental errors: ~ $5 \times 10^{-5}$ Pa $s^{-1}$). ( ) represents the original sample viscosity without purification and ( ) is the original sample viscosity after purification with activated charcoal. The sample numbers represent the different oils, 1: sunflower oil; 2: soybean oil; 3: cocoa butter; 4: palm kernel oil.

From Figure 5.4, it can be observed that the purification stage slightly reduces the viscosity of the sunflower oil, soybean oil, and cocoa butter. This presumably means that the charcoal adsorbed some of the impurities which were contained in the oils, such as water and minor oil components. Such impurities may self-ensemble into small aggregates, and this may increase the viscosity of the sample. For example, Johansson and Bergenståhl previously reported that water molecules may impart on the dispersions of fat in oil by forming water bridges (1992c). This behaviour can be explained by the fact that palm kernel oil does not show signs of reduced viscosity due to it possibly being already pure enough without the charcoal treatment. Consequently, the purification of oils by activated charcoal has shown some viscosity reduction in most of the oils, with the greatest effect on cocoa butter. To confirm the results of these preliminary tests, the addition of surfactant to the purified and non-purified samples was performed.

### *Effect of purification on the viscosity of the oils containing surfactant*

In order to complete this study, 0.1 % emulsifier (Epikuron 2000) was added to the different oils. Once the surfactant was fully dispersed into the oil, 10 % (w/w) activated charcoal was added to the oils and samples were left for 24 hours in a cool dark place. Figure 5.5 shows the viscosity of each oil with and without surfactant at 40 °C.

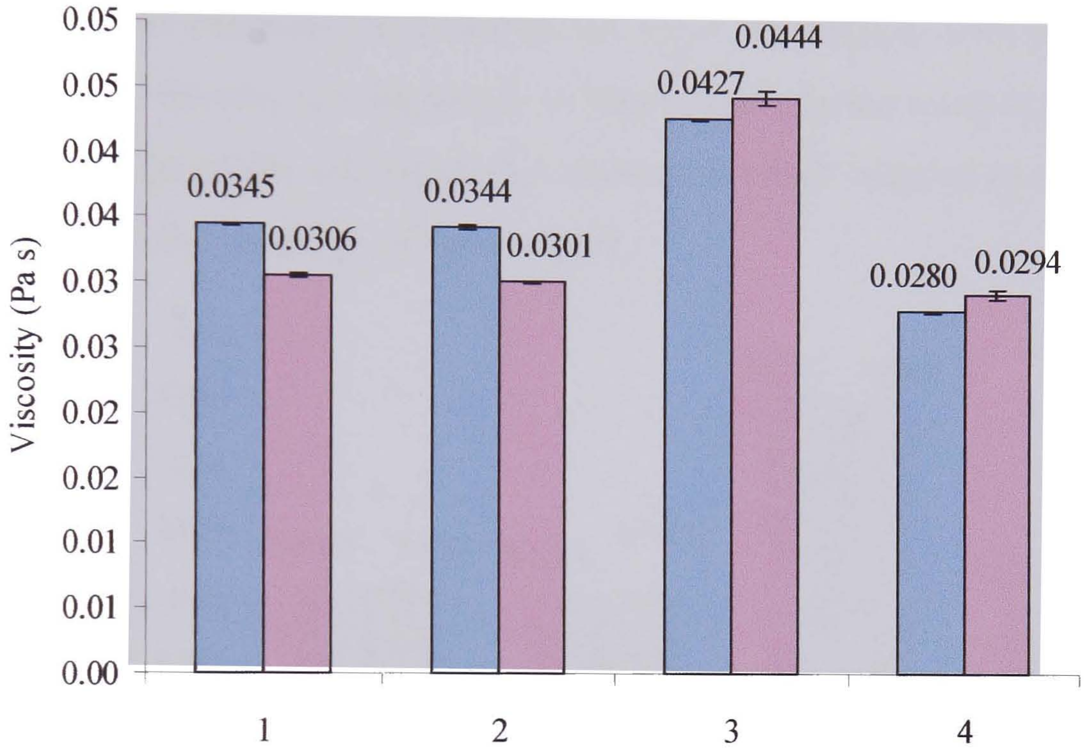


**Figure 5.5:** Effect of addition of 0.1% emulsifier and treatment with activated charcoal on the viscosity of oils at 40 °C. (■) represents the original sample viscosity without purification and (■) is the original sample viscosity after purification with activated charcoal. The sample numbers represent the different oils, 1: sunflower oil; 2: soybean oil; 3: cocoa butter; 4: palm kernel oil.

In Figure 5.5 it can be observed that the viscosity of non-purified sunflower and soybean oil is enhanced by the addition of emulsifier. Once these oils are purified, the viscosity decreases to a value similar to that of the purified oils. This means that the purification stage was effective in removing the minor components as well as the emulsifier too. In the case of non-purified cocoa butter, the addition of emulsifier reduces the viscosity.

For the purified sample, the viscosity remains approximately constant at the value for purified cocoa butter (0.044 Pa s) obtained from Figure 5.3. The loss of viscosity exhibited by the non-purified sample may be the result of different interactions between emulsifier and minor surface-active oil components, which are removed in the purification stage. Palm kernel oil samples show the same pattern as samples containing no emulsifier. The values obtained in these two experiments are almost identical. This means that the presence of emulsifier in the purified and non-purified palm kernel oil systems induces no viscosity change.

In order to continue the research on sugar dispersions with the presence of emulsifier in the oils, it is important to know precisely the viscosity change due to the purification of the oil. Figure 5.6 shows the effect induced by the presence of emulsifier on the rheology of the samples.

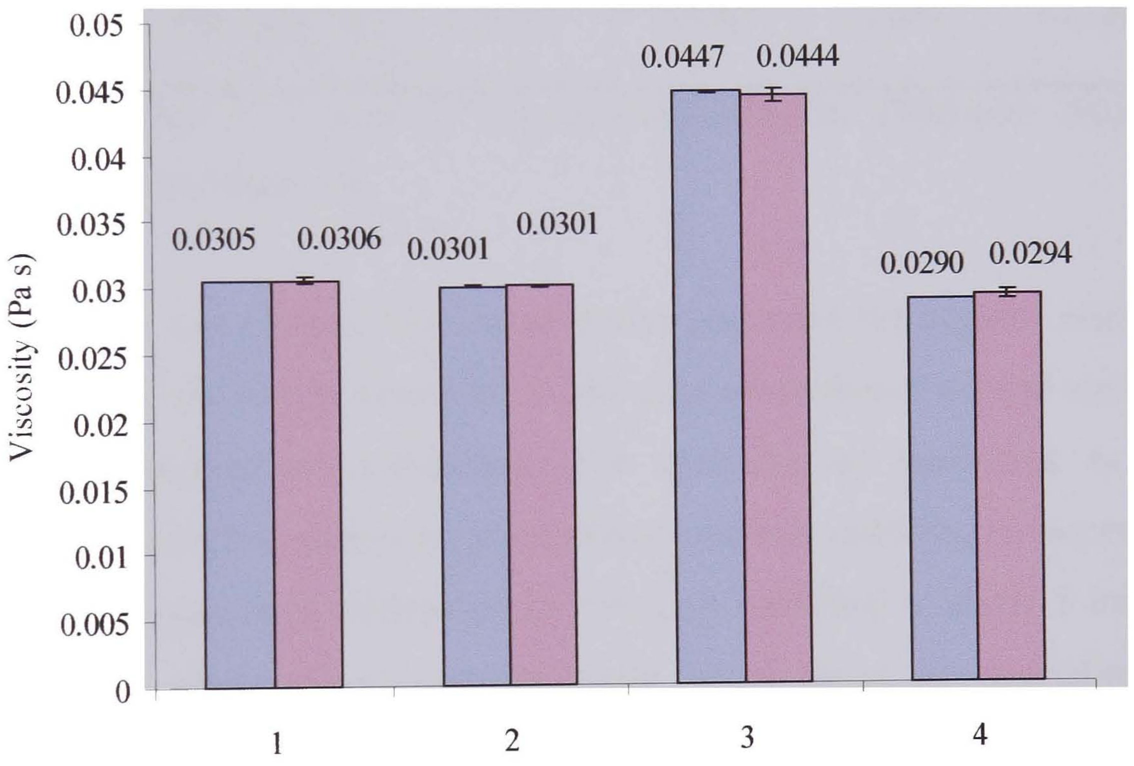


**Figure 5.6:** Effect on the oil viscosity by addition of emulsifier to purified oils at 40 °C. ( ) represents the original sample viscosity without addition of emulsifier and ( ) is the original sample viscosity with addition of emulsifier. The sample numbers represent the different oils, 1: sunflower oil; 2: soybean oil; 3: cocoa butter; 4: palm kernel oil.

From Figure 5.6 it can be observed that the presence of emulsifier in the purified oils has no specific effect on the viscosity of the oils themselves.

Consequently, all the rheological differences encountered with the oils in the absence of sugar particles can be considered as negligible. All the viscosities observed from the individual oils due to the presence of surfactant and purification are summarised in Table 5.2.

**Table 5.2**: Comparison of viscosity in the different oils at 40 °C in presence or not of purification stage and emulsifier addition

| | Sunflower oil | Soybean oil | Cocoa butter | Palm kernel oil |
|---|---|---|---|---|
| Original samples (Pa s) | 0.033 | 0.031 | 0.048 | 0.028 |
| Original with emulsifier (a) (Pa s) | 0.034 | 0.034 | 0.043 | 0.028 |
| Purified only (b) (Pa s) | 0.031 | 0.030 | 0.045 | 0.029 |
| Purified with emulsifier (Pa s) | 0.031 | 0.030 | 0.044 | 0.029 |
| difference with emulsifier | + 0.001 | + 0.003 | - 0.005 | 0 |
| difference with purification | - 0.002 | - 0.001 | - 0.003 | + 0.001 |
| difference with purification & emulsifier | - 0.002 | - 0.001 | - 0.004 | + 0.001 |

*Notes:* (a) Addition of 1 mmol $L^{-1}$ of phosphatidylcholine (Epikuron 200); (b) Purification via activated charcoal

In this table we can observe that cocoa butter generates the highest viscosity and palm kernel oil the lowest viscosity. In the case of sunflower oil and soybean oil, we noted intermediate viscosities. The presence of surfactant or the purification of the oils has shown no great effect on either reducing or increasing the viscosity. The maximum difference in viscosity exhibited is about 5 mPa s. This difference is negligible as conventionally 10 % errors are accredited to rheological measurements. Due to the little effects of the oil purification observed on the viscosity of the oils and with or without emulsifier, it was decided to further study the rheological behaviour of the oils in the presence of sugar particles.

After studying the effects of oil purification *via* activated charcoal on oils, it became interesting to compare the viscosity of three different oils at high sugar content without the presence of emulsifier. We investigated two types of purification agents: activated charcoal and magnesium silicate, also known as florisil.

### *Effect of purification onto concentrated sugar/oil systems*

In this study, each sample's viscosity refers to the value obtained at the steady state plateau when plotting the graph of viscosity versus shear-rate. Figure 5.7 shows the viscosity of the sunflower oil, soybean oil and cocoa butter at different sugar concentrations under charcoal or florisil purifications.

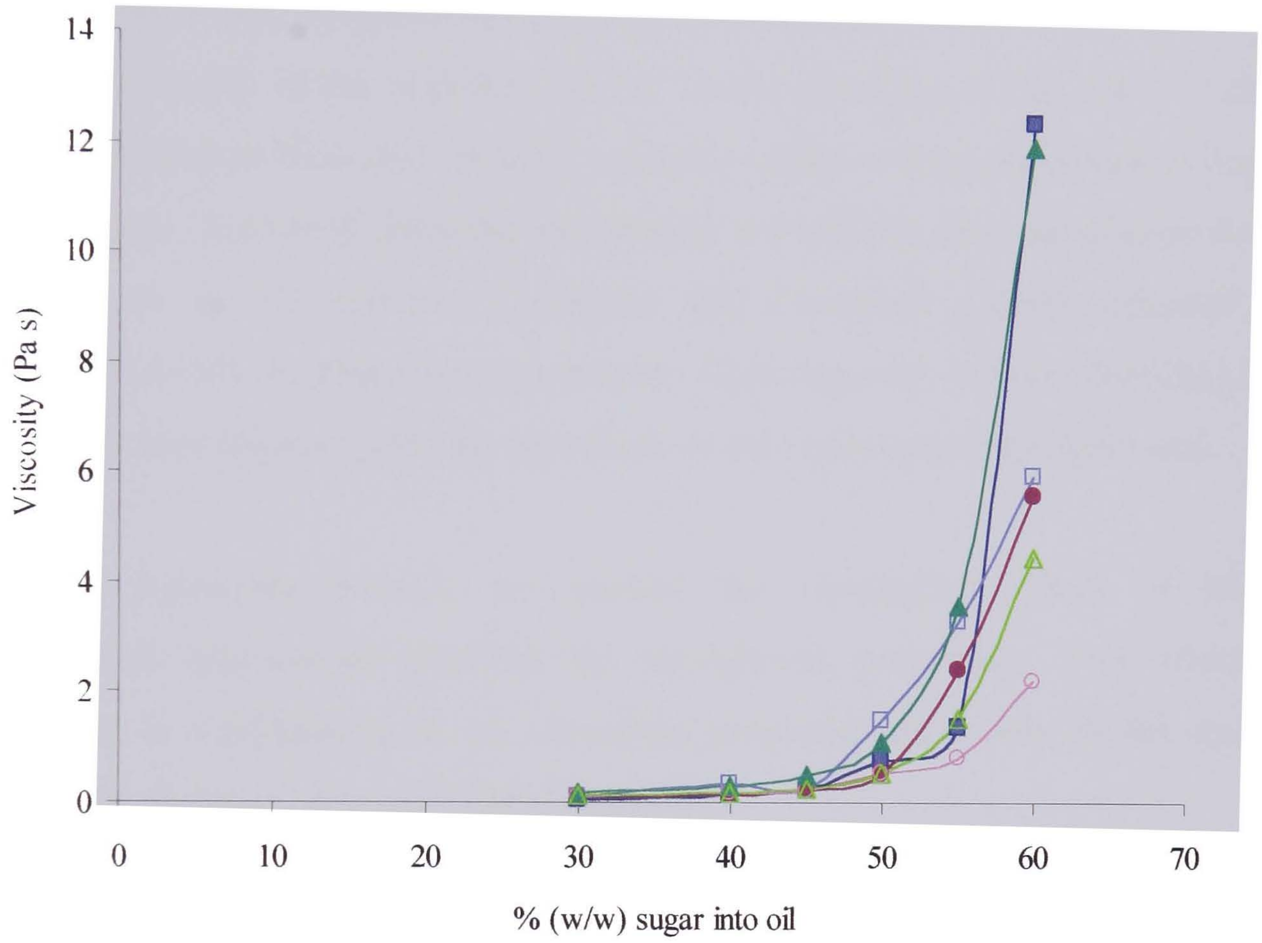


**Figure 5.7:** Viscosity of oils containing high sugar content and purified either by activated charcoal or florisil. (■) and ( ) represent the sunflower oil samples purified respectively by charcoal and florisil; (●) and ( ) represent the soybean oil samples purified respectively by charcoal and florisil; (▲) and ( ) represent the cocoa butter samples purified respectively by charcoal and florisil. Experiments made with the "refined mixing method".

In Figure 5.7, no difference exists between the two filtrations until the sugar concentration reaches 45-50 % (w/w) sugar. Above this concentration, all the samples purified by florisil give lower viscosities than the samples purified *via* activated charcoal. As the concentration in sugar increases, the viscosity of the samples that are purified with activated charcoal increases drastically too. It may be possible to say that the poor efficiency of charcoal as an adsorbent of minor components in sugar oil systems results in a stronger viscosity at each sugar concentration. It has been observed that different types of adsorbent may give beneficial effects to oils up to certain extents. All the oils present the same pattern as the sugar concentration increase in the sample. The samples purified *via* activated charcoal exhibit stronger viscosity than samples purified with florisil. Consequently it may be possible to say that the more efficient the adsorbent, the lower the viscosity of the samples will be. The comparison of the effect of these two adsorbents has been also reported with the sedimentation technique (Chapter IV). However, activated charcoal and florisil are not the sole adsorbents which can be used in oil systems. Dedinaite and Campbell (2000) reported the purification of oil *via* phosphorus pentoxide. Consequently, the purification effect was further investigated with this adsorbent on two concentrated dispersions.

In the following section, we studied the rheological effect of highly concentrated suspensions purified by phosphorus pentoxide. This chemical component is well-known for its adsorbing properties especially in the case of water removal (Dedinaite and Campbell, 2000).

### *Purification via Phosphorus Pentoxide on highly concentrated suspensions*

In this section, only cocoa butter and palm kernel oil have been studied. The level of sugar content used in this study was constant at 70 wt % sugar. To realise these experiments, the samples were prepared following the "*refined mixing method*". Then each sample (~ 100 g of sample) was placed in a vacuum oven at 40 °C, in which a crucible full of phosphorus pentoxide was already deposited. The amount of phosphorus pentoxide was added in excess, approximately 10 g, in order to remove most of the water contained in the blend of sugar and oil. The samples were left for 24 hours in the oven and were rapidly analysed on the

Malvern rheometers. Figure 5.8 shows the effect of the purification via phosphorus pentoxide on the viscosity of cocoa butter and palm kernel oil samples.

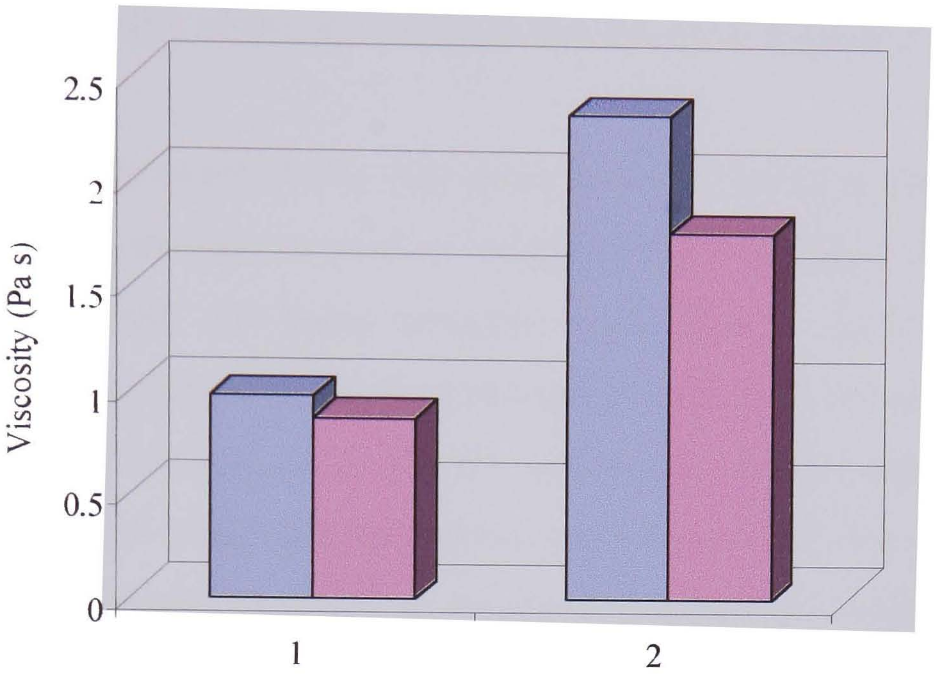


**Figure 5.8:** Effect of the purification via phosphorus pentoxide on the viscosity of cocoa butter and palm kernel oil samples containing 70 wt % sugar (precision < 10 % errors). The sample numbers refer to the different oils, 1: cocoa butter; 2: palm kernel oil. The colour (🟦) characterises the samples without purification and the colour (🟪) represents the samples purified by phosphorus pentoxide.

Figure 5.8 shows that the addition of phosphorus pentoxide has an effect on the reduction of the viscosity of both samples. We noted that the viscosity diminished by almost 10 % for cocoa butter samples and by 24 % for palm kernel oil samples in presence of phosphorus pentoxide. From previous experiences we have shown that the viscosity of oils purified by other adsorbents, activated charcoal and florisil is not significantly affected by the purification stage. The previous experiment has shown that in presence of sugar, florisil and activated charcoal reduce the viscosity of certain oils to different extents.

With this new experiment, we have shown that the water content which is mainly present at the sugar surface plays an important role on the viscosity measurement. The removal of water molecules greatly affects the viscosity of the systems by reducing it. By removing counteracting surfactants such as water molecules, more stability is gained towards sugar particles and oils. The percentage difference in viscosity between the two oils is largely scattered. It mainly results in the ability of the oil to bind in specific areas of sugar particles.

As a result, this experiment shows that cocoa butter is highly preponderant in binding sugar particles, and the removal of water significantly affects the samples made at 70 %. However, the sugar particles aggregation in palm kernel oil samples is much more subjective in the presence of water. In reference to the works published by Claesson *et al.* (1997) and by Dedanaite and Campbell (2000), the presence of water greatly affects the adhesion of sugar particles. Therefore this feature should been seen in rheology, as a method which quantifies the strength of the particle interactions. As a confirmation of this statement, our results have shown that the presence of water has a substantial effect on the viscosity of the samples which should not be neglected.

### V.3.1.2. Rheological results of dilute sugar in oil dispersions

In the following sections we describe the rheology behaviour of the oils in the presence of sugar particles at different concentrations. This investigation was done on samples applicable to industry.

In these experiments, non-purified oils and sugar were carefully weighed out separately on a sensitive balance (precision: $1\times10^{-4}$ g). The liquefied oil, at 40 °C, was placed in a beaker and was put on a hot magnetic stirrer. The sugar particles were gradually added to the system until the sugar was fully coated by the oil. It was observed that the sugar was completely dispersed after 2 hours of mixing in the oil. The viscosity of the mixture was measured straight after mixing as shown in Figure 5.9.

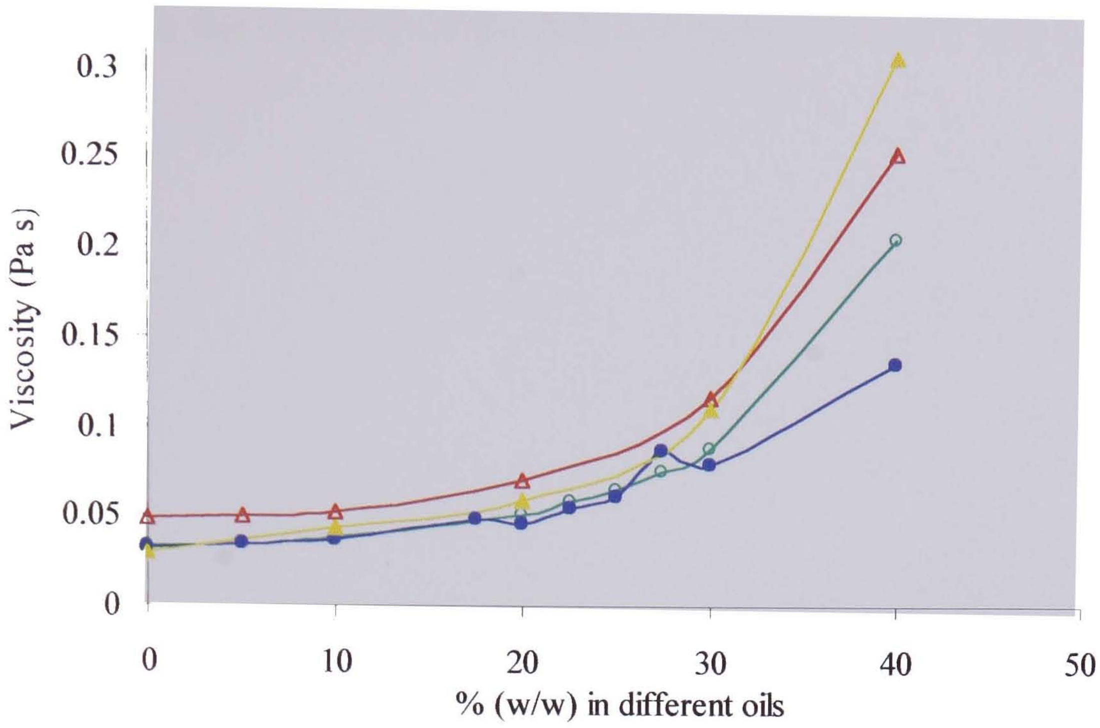


**Figure 5.9:** Viscosity against percentage sugar in different non-purified oil suspensions at 40 °C (experimental errors < 10 %). (•) represents the soybean oil dispersions; (○) is the sunflower oil dispersions; (△) represents the cocoa butter dispersions and ( ) is the palm kernel oil dispersions.

It can be noted from Figure 5.9 that at low sugar concentration the viscosities of the sunflower oil and soybean oil dispersions are almost the same up to 30 % (w/w) sugar. The viscosity of the samples containing cocoa butter is the highest at the different sugar concentrations. Palm kernel oil blend has the lowest viscosity of the oils without sugar, but it can be observed that the addition of 10 % (w/w) sugar has the biggest effect on the viscosity amongst all the different oils. At 40 % (w/w) sugar it can be observed that palm kernel oil has the largest viscosity of all the studied samples. In order to quantify the effect due to the presence of sole sugar particles, relative viscosity was calculated from (Dickinson, 1992):

$$\mu(relative) = \frac{\mu(mix : sugar / oil)}{\mu(oil)} \tag{5.1}$$

Using equation (5.1), it has been possible to quantify the relative effect of sugar particles on the viscosity of the different systems. This is shown in Figure 5.10.

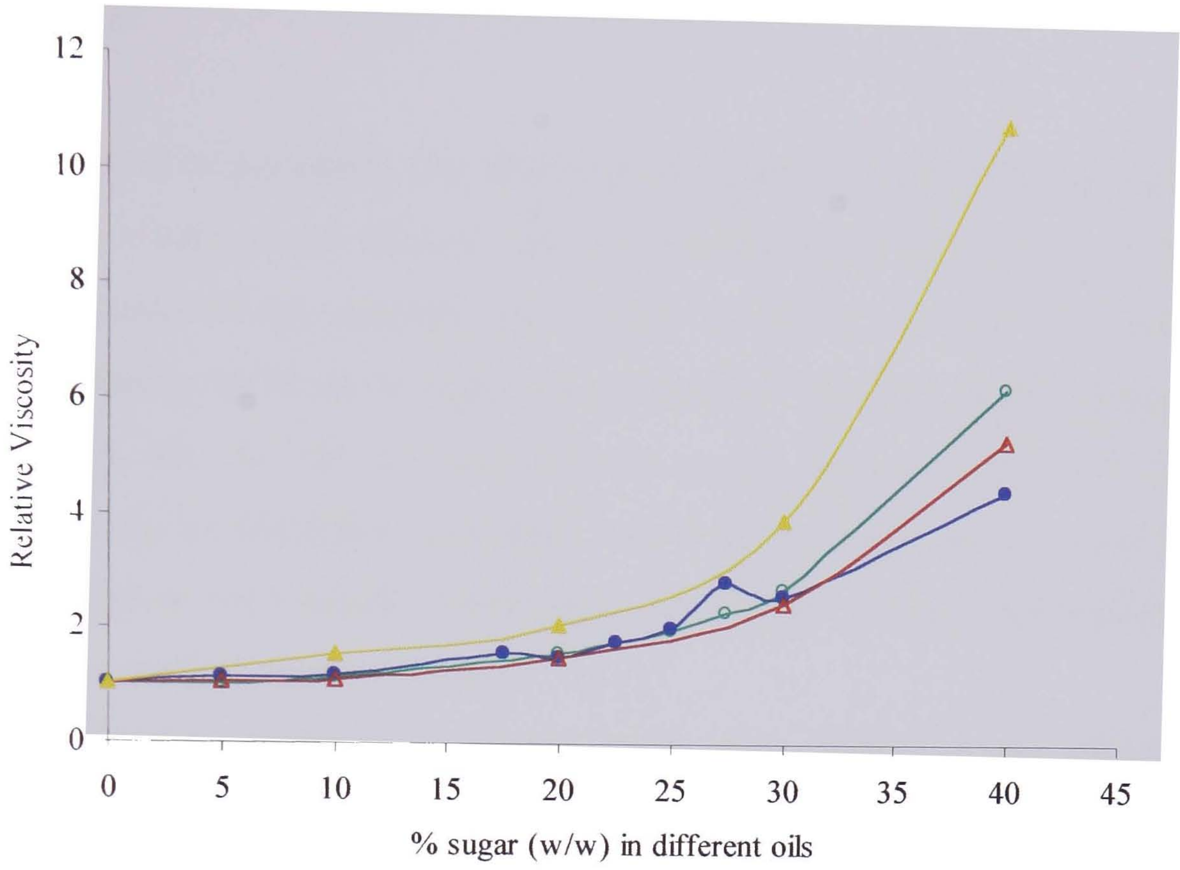


**Figure 5.10:** Relative viscosity against percentage sugar for different oil suspensions at 40 °C (experimental errors < 10 %). (●) represents the soybean oil dispersions; (○) is the sunflower oil dispersions; (△) represents the cocoa butter dispersions and (▲) is the palm kernel oil dispersions.

It can be observed from Figure 5.10 that the palm kernel oil curve shows the greatest effect with sugar particles. Until 30 % (w/w) sugar, the viscosity is only affected a little by the sugar concentration, and it only increases slowly. We observed that sunflower oil, soybean oil and cocoa butter seem to show an increase in viscosity which is equal to the increase of the sugar concentration. This general pattern is observed up to 30 % (w/w) sugar. From 30 % (w/w) up to 40 % (w/w) sugar, it may be possible to differentiate the interactions of each oil. Therefore we noted that the palm kernel oil has the highest viscosity compared to sunflower oil, cocoa butter and soybean oil. Above 30 wt % sugar, these suspensions can be considered as non-dilute ones. In the specific case of palm kernel oil, a considerable increase is noted at a sugar concentration of 40 % (w/w) with this oil in comparison with the other systems. We also noted the strong effect of sugar particles on palm kernel oil. The measured viscosities for these samples are greater than the viscosities of samples made with cocoa butter, sunflower oil or soybean oil.

In this set of experiments, we observed that palm kernel oil samples generate the highest viscosity for diluted and non-diluted samples. By calculating the relative viscosity of the samples, we defined the effect of sugar concentrations. We noted that up to 30 wt % sugar, samples behave similarly at the exception of palm kernel oil. As the concentration of sugar increases, the different oil dispersions can be identified separately. At 40 wt % sugar, palm kernel has the greatest effect on the viscosity when sugar particles are present; whereas soybean has the lowest effect on the viscosity.

The following section discusses about the rheology of concentrated dispersions. Four oil dispersions have been analysed in this set of experiments: sunflower oil, soybean oil, cocoa butter and palm kernel oil.

### V.3.1.3. Rheological results of concentrated sugar in oil dispersions

*Viscosities of samples made with the "basic mixing method"*

With the use of this mixing method, it was only possible to disperse up to 60 % (w/w) sugar into the different oils. The viscosity, which was measured straight after mixing, was determined for different applied shear stresses as a function of the sugar content as shown in Figure 5.11. The range of stresses which were applied to the different systems varied from 5 Pa to 200 Pa.

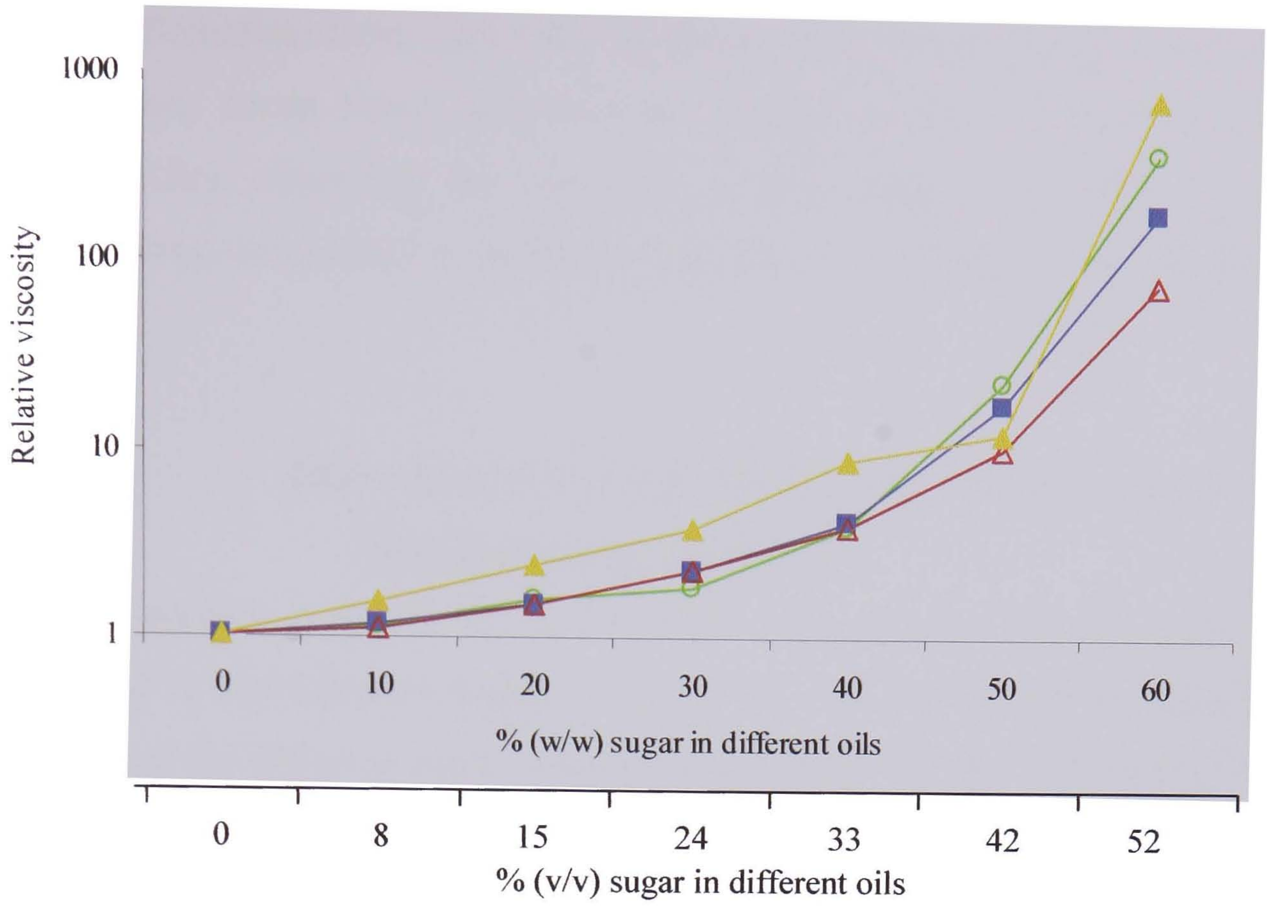


**Figure 5.11:** Relative viscosity versus % (w/w) and % (v/v) sugar content for different oils at 40 °C (experimental errors < 10 %). (●) represents the soybean oil dispersions; (○) is the sunflower oil dispersions; (△) represents the cocoa butter dispersions and ( ) is the palm kernel oil dispersions.

Figure 5.11 shows that sugar particles dispersed into sunflower oil, soybean oil and cocoa butter generated the same effect on the viscosity up to 40 wt % sugar. In the case of palm kernel oil the sugar particles at low volume fractions produced the biggest effect on the viscosity. These observations are in accordance with the results reported by the sedimentation technique (Chapter VI). As the sugar concentration increases in these systems, the viscosity increases and differences between the different oils become evident. It can be noted that, at 60 wt % sugar, the sample made with palm kernel oil has the highest viscosity whereas the cocoa butter sample has the lowest viscosity. Intermediate viscosities were observed for the samples containing sunflower oil and soybean oil.

This experiment confirmed the result obtained with the diluted experiments: palm kernel oil generates the highest viscosity in comparison with the others oil samples. However, the viscosities of sunflower oil, soybean oil and cocoa butter samples differentiate from each other by being above 40 wt % sugar. Above this concentration, cocoa butter generates the lowest viscosity amongst the other samples. After observing the viscosity of the samples at different sugar concentrations, we decided to investigate the behaviour of the viscosity at variable shear rates.

### *Dependence of viscosity on shear-rate and shear stress*

### **Samples without emulsifier**

In this section, only 30 %, 40 %, 50 % and 60 % (w/w) of sugar were represented in the different studied oils. Figure 5.12 represents the rheological behaviour at the different sugar concentrations under variable shear-rate without the presence of Epikuron 200.

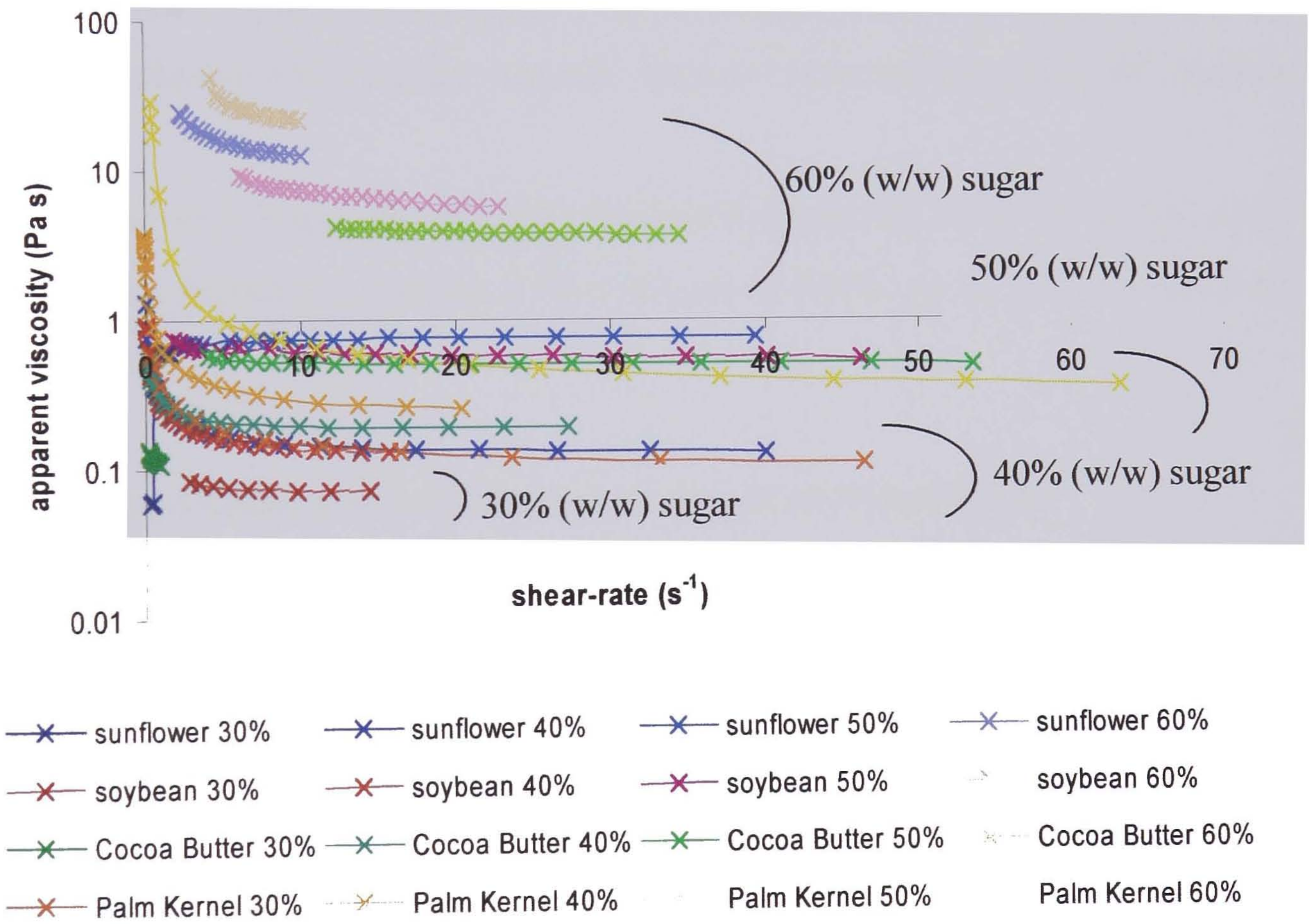


**Figure 5.12:** Viscosity against shear-rate for sugar dispersions in oils at 40 °C (experimental errors < 10 %).

In this figure, it can be observed that the data for each sugar concentration have well defined curves. As the concentration of the sugar increases, three viscosity regions may be identified. The first region corresponds to diluted samples with sugar concentrations below 40 wt %; the second region is for intermediate sugar dispersions with 50 wt % sugar. Finally, the third region corresponds to the viscosities exhibited by samples containing 60 wt % sugar.

The viscosities obtained for sunflower oil, soybean oil and cocoa butter at 30 % (w/w) sugar are about 0.1 Pa s. It can be noted that the palm kernel oil with 30 wt % sugar exhibits a viscosity in the region of the other samples containing 40% sugar. Its viscosity is slightly above 0.1 Pa s. As the concentration increases up to 40% (w/w) sugar, all the samples have a viscosity just above 0.1 Pa s and palm kernel oil shows the strongest effect on the viscosity of the suspension.

Above these concentrations, another plateau is observed for a viscosity range of 10 Pa s which includes the concentration of 50 % (w/w) of sugar. The viscosity of this plateau is slightly lower than 1 Pa s. At this concentration, sunflower oil shows the greatest effect on the viscosity whereas palm kernel shows the smallest effect.

For 60% (w/w) sugar, the viscosity increases drastically to $10^1$ Pa s. The range of viscosities increases, ranging 5 Pa s for cocoa butter up to 20 Pa s for palm kernel oil.

The same experiment was carried out in the presence of emulsifier.

## **Samples with emulsifier**

For this set of experiments, it was possible to disperse up to 60% (w/w) sugar into the oils. Figure 5.13 represents the rheological behaviour of the different sugar concentrations under variable shear-rate. All the samples contained 0.1% Epikuron 200.

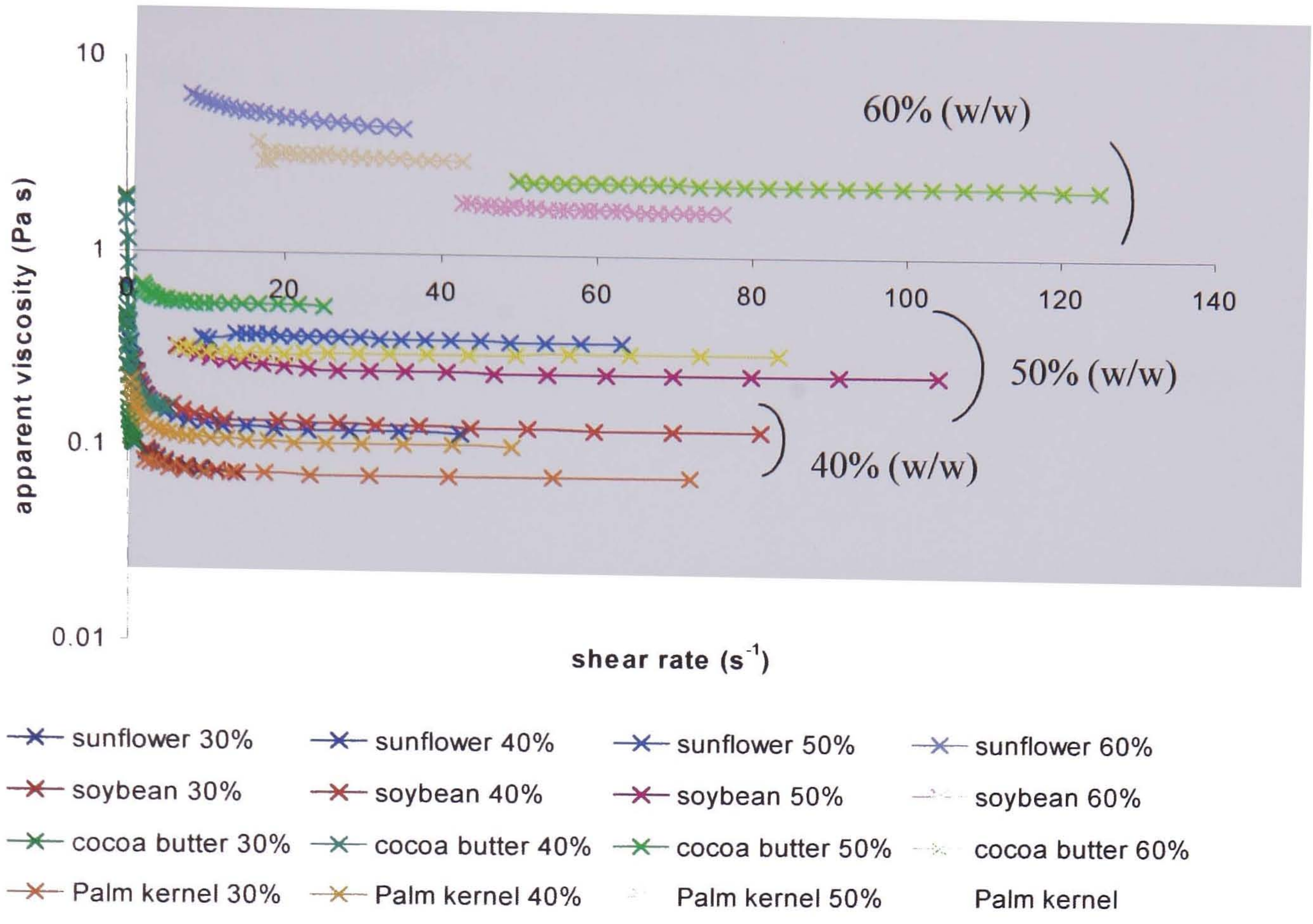


**Figure 5.13:** Viscosity against shear-rate for sugar and emulsifier dispersions in oils at 40 °C (experimental errors < 10 %).

It can be observed that the data for each sugar concentration present three identifiable regions. At 30% and 40% sugar, the samples have almost the same viscosity, around 0.1 Pa s.

As the concentration increases up to 50 % (w/w) sugar, another viscosity region is defined. The range of viscosity is about 0.5 Pa s. At this concentration, it can be observed that cocoa butter has the strongest viscosity of all the oils. Finally it can be observed that at 60 % (w/w) sugar another plateau is observed. The viscosity of this range is more spread and is less than 10 Pa s.

It should also be noted that in the presence of emulsifiers the defined rheological regions still exist but their range of viscosities is lower than the viscosity of the samples containing no emulsifier. As an example the plateau of 50 % (w/w) sugar is just below 1 Pa s for samples without emulsifier but in presence of Epikuron 200, the viscosity is about 0.4 Pa s.

The presence of emulsifier reduces the viscosity of the samples. Most of the concentrations show a viscosity under 1 Pa s, at the exception of 60 % (w/w) sugar.

## **Conclusion**

The rheology experiments allowed the flow properties of dense sugar dispersions to be understood.

The first conclusion that may be drawn is the fact that the addition of 0.1 % emulsifier in the different systems decreases the viscosity. In each oil and for all the different sugar concentrations, a reduction of viscosity has been found. It has been noted that emulsifier has the strongest effect on the viscosity of palm kernel oil. Moreover, the differences in viscosity for each concentration are the largest of all the studied samples. On the other hand, the presence of emulsifier has the least effect on soybean oil.

Another conclusion which may be drawn from this section is the possibility to determine some rheological regions: under 1 Pa s, between 1 to 10 Pa s and above 10 Pa s. It has been observed that samples containing up to 40 % (w/w) sugar have a viscosity under 1 Pa s. Once this concentration is passed, the viscosity is about 1 Pa s. At this stage, the sugar is still readily dispersed in the oil. Once 60 % (w/w) sugar has been dispersed in the oil, the systems exhibit a very high viscosity (> 10 Pa s). Consequently, it may be possible to classify the results in terms of the produced viscosity:

- *Up to 40 % (w/w) sugar*, the sugar particles and oil are easily mixed and fully dispersed. Sugar crystals are well covered by oil. Such concentrations show a viscosity of 0.1 Pa s.
- *At 50% (w/w) sugar*, this is an intermediate concentration, with particles fully dispersed but less oil surrounding the sugar crystals and perhaps some friction between the crystals. The viscosity, which is generated by this amount of sugar, is about 1Pa s.
- *Above 50 % (w/w) sugar*, there is difficulty in mixing such samples,

with sugar particle becoming fully dispersed only after long mixing times. The level of oil on the crystals is even lower, and this increases the friction even more between the sugar crystals. The viscosity fluctuates around 10 Pa s.

This study carried out on the dependence of viscosity on shear rate and shear stress has shown that sugar particles dispersed in oils have three domains of aggregation as the sugar concentration increases: under 40 % (w/w) sugar, up to 50 % and above 50 % (w/w) sugar. Moreover, the addition of emulsifier at a constant ratio of sugar / emulsifier led to a decrease in the viscosity of all the samples. We compared the rheological values obtained at different sugar concentrations with the rheological values of the different steady state plateau. We observed that each sample viscosity had the same viscosity as the one obtained in the steady state plateau. This feature confirms the fact that the viscosities which are determined at variable stresses are the optimum viscosities value as being on the steady state plateau.

Wildemuth and Williams (1985) suggested that graphs of relative viscosity against shear-rate can be useful for expressing the flow behaviour in dense slurries. By using their rheological model, they were able to correlate the viscosity with high volume fraction in non-Newtonian dispersions.

After defining the steady state plateau for each sugar dispersion, the possible theoretical relationship between the dispersion viscosity and the sugar concentration will be examined. We will investigate our dispersions systems *via* two different rheological models: Einstein and Casson equations as described below.

### *V.3.2. Theoretical Approach*

In this section we investigate whether the results from the diluted dispersions can be described using the Einstein equation. Also we see whether the concentrated dispersion results fit the Casson equation. Finally the approach to the interaction energy model defined by Quemada and Berli (2002) is investigated

### V.3.2.1. Validity of the Einstein relationship for dilute dispersions

Systems of sugar particles dispersed into oil can be considered as suspensions of large particles in a liquid. Dickinson (1992) refers to the following equation, which is applicable for suspensions with Newtonian flow behaviour:

$$\mu_r = 1 + k_1 \phi + k_2 \phi^2 + \dots , \tag{5.2}$$

Here $\mu_r$ is the relative viscosity of a dilute suspension, $\phi$ is the volume fraction of particles, and $k_1$ is the Einstein coefficient, which has a value of 2.5 for hard spheres. The value of $k_2$ can be determined by assimilating the sugar made of large (macroscopic) particles. In this case, it can be reasonably suggested that there is an absence of Brownian motion in the system. Consequently, the value of $k_2$ will be approximately of 5.2.

These calculations have been carried out for all the sugar concentrations up to 40 % (w/w) in sunflower oil, soybean oil and cocoa butter. This section only presents only the example of soybean oil as most of the experiments were based on this oil. For a full description of all the results see appendices **V-i** and **V-ii**.

As an example of the calculations conducted, the sample with 10 % (w/w) sugar has been chosen. The densities of the sugar and soybean oil are 1.23 g mL$^{-1}$ and 0.91 g mL$^{-1}$, respectively. Table 5.3 shows the conversions of weights into volumes and the calculation of volume ratios.

**Table 5.3:** Determination of the volume fraction of sugar into soybean oil

| Mass of sugar (g) | Mass of oil (g) | Total mass (g) | Vol. sugar (mL) | Vol. oil (mL) | $\phi$ (a) | $\phi^2$ (b) |
|---|---|---|---|---|---|---|
| 2.00 | 18.00 | 20.00 | 1.63 | 19.57 | 0.0767 | 0.0059 |

*Notes:* (a): $\phi$ is the volume fraction of sugar particles in soybean oil.

(b): $\phi^2$ is the square root of the volume fraction of sugar particles in soybean oil.

The viscosity of pure soybean oil is 0.0307 Pa s whereas the viscosity of 10 % sugar dispersion is 0.0360 Pa s. For each sugar concentration, the relative viscosity of a diluted suspension is plotted in Figure 5.14. The viscosity is expressed in terms of relative viscosity using equation 5.6.

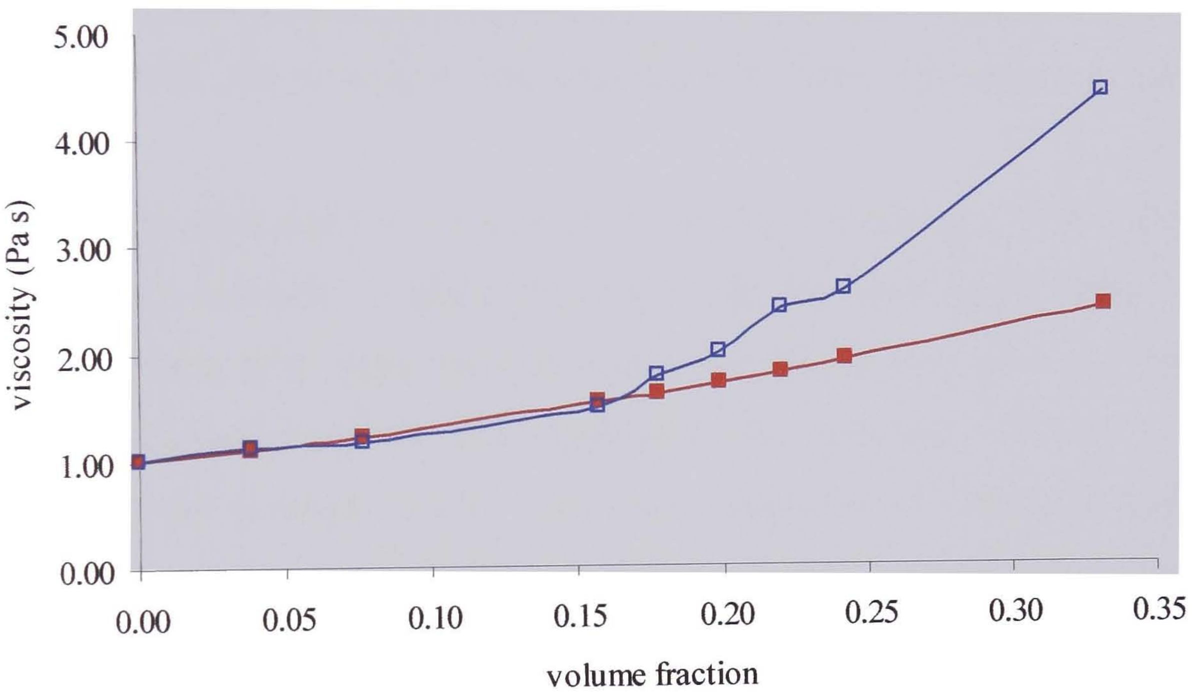


**Figure 5.14:** Comparison of theoretical (■) and experimental (□) relative viscosities of sugar dispersions in soybean oil 40°C.

Figure 5.14 shows that up to a volume fraction of 0.16 the theoretical and experimental values are similar. As the volume fraction increases above 0.16, the experimental results become separated from the theoretical results. The cross-over point represents a sugar concentration of 21 % (w/w). It can be noted that the theoretical and experimental relative viscosities slightly increase until $\phi$ = 0.24

(equivalent to 30 wt % sugar). As the concentration increases to 40% ($\phi \approx 0.33$) the rate of viscosity increase becomes high.

It has been observed that for sunflower oil viscosities and cocoa butter, viscosities were lower than the theoretical predictions at low sugar concentrations. Cocoa butter samples have the biggest viscosity difference between the theoretical and experimental values. At a volume fraction of 0.14 the experimental curve of sunflower oil crosses the theoretical curve; whereas the volume fraction at which the experimental curve of cocoa butter crosses the theoretical curve is of 0.15. Above these points, both experimental curves remain higher than the theoretical ones. Up to 30 % (w/w) sugar, the experimental viscosity curves of sunflower and cocoa butter increase gradually as the concentration of sugar increases. Above this concentration (40 wt % sugar), the experimental rates of viscosity increase drastically.

From a rheological point of view, it can be said that sunflower oil and soybean oil are behaving similarly in dilute dispersion. On the other hand, cocoa butter behaves differently with sugar than the other two oils. It was observed that the Einstein rheological model is only applicable for extremely diluted systems (up to 15 – 20 wt % sugar in oil). This result confirms the statement given by Ferraris (1999), which stipulates that the Einstein equation is mainly applicable for diluted suspensions and assumes that at this stage no interactions between spherical particles are encountered.

The next section focuses on the formation of concentrated suspensions. For each oil the analysis of dense slurries using Einstein rheological model was studied as well as developing a theoretical approach towards the Casson equation and the interpretation of the Casson intercept along with the Casson viscosity coefficient as a function of sugar concentrations.

### V.3.2.2. Validity of the Einstein relationship for dense dispersions

In order to check the statement of Ferraris (1999) regarding the use of the Einstein rheological model for diluted slurries, we tried to validate this equation using our compact slurries. First, we needed to experimentally investigate the correlation between the viscosity and the particle volume.

*Relationship of viscosity to sugar concentration*

From the experiments recorded in section V.3.2.1., it was possible to determine a relationship between viscosity and sugar concentration. Figure 5.15 represents the viscosity of purified oils at high sugar concentrations.

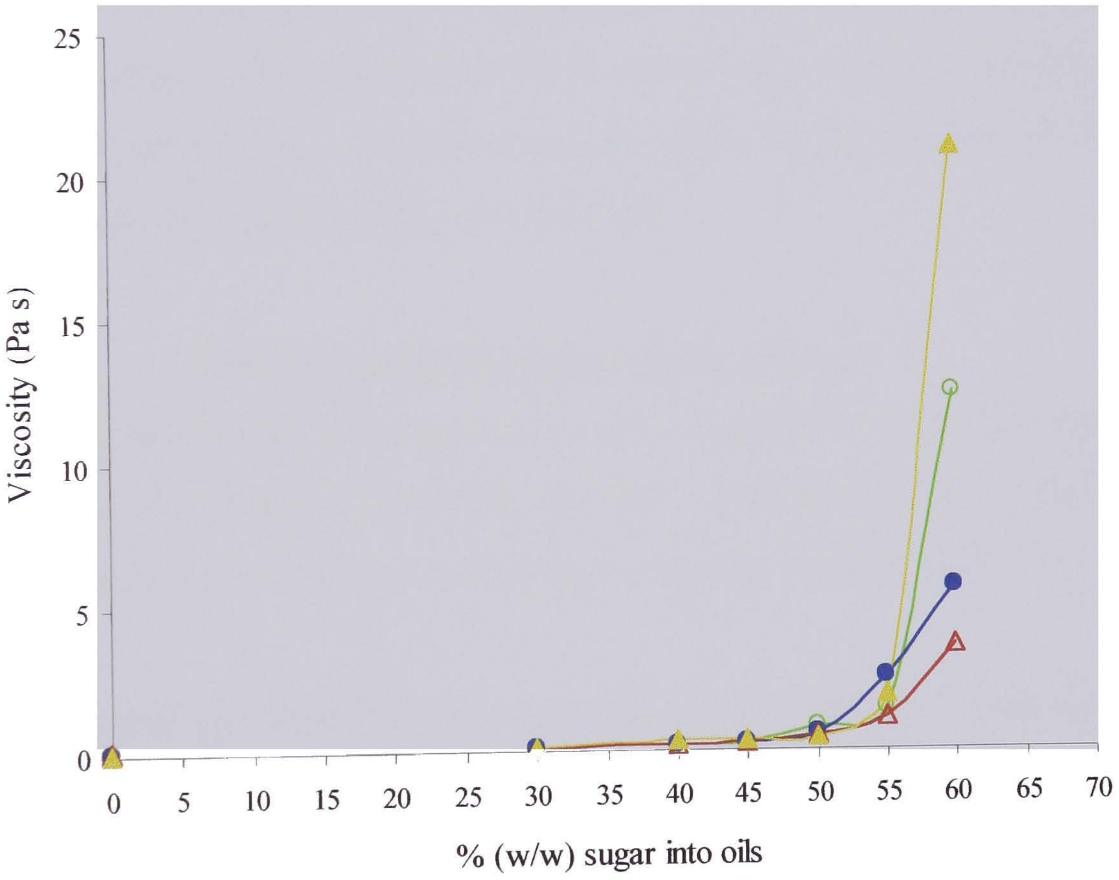


**Figure 5.15:** Apparent viscosity at maximum shear-rate against sugar concentration in purified oils at 40 °C (experimental errors < 10 %). (●) represents the soybean oil dispersions; (○) is the sunflower oil dispersions; (△) represents the cocoa butter dispersions and ( ) is the palm kernel oil dispersions.

The viscosity value was determined once the steady state plateau was reached, but selected at different shear rates. In this graph it can be noted that for up to 45% (w/w) sugar the viscosity does not increase significantly and is almost nil. This phenomenon is present with all the oils.

As the sugar concentration increases up to 55 % (w/w), all the samples' viscosities increase slightly and remain contained in the same range of values. It can be noted that the soybean oil shows the strongest effect on change of viscosity within this range of sugar.

Above this concentration, the oils behave differently. Palm kernel oil was the strongest viscosity (20 Pa s), followed by sunflower oil (~ 12 Pa s), soybean oil (5 Pa s) and cocoa butter (2 Pa s).

It has been observed that most of the dispersions do not produce a significant viscosity until they reach a content of 50 % (w/w) sugar. At this concentration it has been observed that the viscosity increases either linearly below 50 % (w/w) sugar or logarithmically above this concentration.

### *Einstein's relationship for dense slurries*

It should be noted that in this section it was not possible to analyse the sample in the presence of emulsifier with the Einstein's relationship due to the difficult interpretation of volume fraction caused by the presence of emulsifier.

The theoretical and experimental viscosities were composed with the help of the Einstein relationship defined in section V.3.2.1 (equation 5.7). Figure 5.16 shows the comparison between the two relative viscosities of charcoal purified oils.

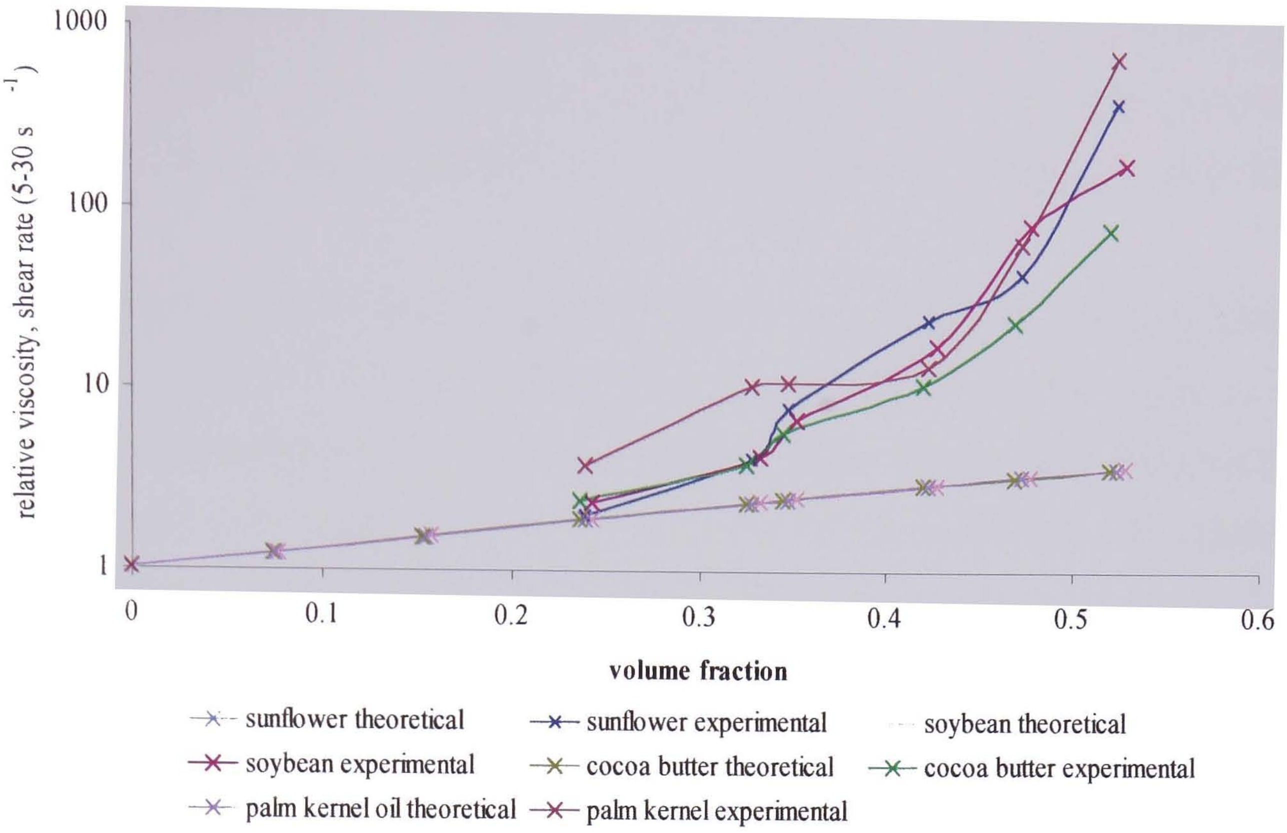


**Figure 5.16:** Comparison of theoretical Einstein relation viscosity and experimental relative viscosities of purified oils and sugar dispersions as a function of volume fraction

It can be seen that palm kernel oil shows the largest effect on viscosity with approximately 24 vol. % sugar whereas the remaining oils exhibit the same range of viscosity. The viscosity increases drastically up to 42 vol. % sugar for the oils. From 42 % to 52 % (v/v) sugar, the viscosities of the oils increase slightly. Above 42 % (v/v) sugar, the relative experimental viscosity increases drastically in each oil. The strongest effect can be observed for palm kernel oil, followed by sunflower oil and soybean oil and the least effect is produced by cocoa butter.

From this graph two main rheological regions can be observed: below 42 % (v/v) and above 42 % (v/v) sugar. Moreover the main feature of this analysis is the fact that the viscosity of the experimental results is no longer equivalent to the viscosity of the theoretical values obtained with the Einstein equation.

## *Conclusion*

From these two experiments, it can be observed that the rheological behaviour of these oils have similarities. Consequently it can be suggested that there are two behavioural regions as the concentration in sugar increases. It can be noted from Figure 5.16 that the four oils behave differently from each other but generally tend to have the same rheological behaviour of a logarithmic increase after 50% (w/w).

From Figure 5.16, it can be observed that above 24 % (v/v) sugar the Einstein theoretical curve is no longer representative of the viscosity for sugar in oil dispersions. Consequently, it can be said that the model defined by Einstein is not applicable for such concentrated sugar particles in oil dispersions. Therefore a different model system will have to be developed.

In section V.2.3, we noted that rheological models such as Roscoe (1952) or Krieger Dougherty (1959) equations can be used for the analysis of dense slurries. Nevertheless these models are not completely applicable for these compact systems and it is commonly reported in the confectionery literature (Chevalley, 1975, Nelson and Beckett, 1999) that the semi-empirical Casson equation gives a good interpretation of the flow behaviour of molten chocolate. Therefore we decided to apply this model to our results.

### V.3.2.3. Comparison with the Casson equation

*Casson equation*

Chevalley (1975) and Nelson and Beckett (1999) have previously stated that the Casson equation is used in the confectionery industry for estimating the quality of melted chocolate by interpreting its rheology. This equation gives some information about the initial stress required for breaking down the chocolate structure. It has been observed that chocolate rheology data almost perfectly match the equation for high shear rates and they cross the shear stress axis to give the Casson yield stress value. The equation is re-presented by:

$$\sqrt{\tau} = \sqrt{\tau_{CA}} + \sqrt{\mu_{CA}} \times \sqrt{\dot{\gamma}}, \tag{5.3}$$

where $\tau_{CA}$ is the Casson yield stress value and $\mu_{CA}$ is the viscosity exhibited at high stress.

In order to define a new correlation between the viscosity and the quantity of sugar in the samples, calculations based on this equation have been carried out. These involve possible dependence of the slope of the Casson curve and the Casson yield stress value ( $\tau_{CA}$ ) on the mass fraction of sugar in the dispersions. Each set of oils, with or without emulsifier, has been examined using the Casson equation.

### *Experimental results with the Casson Equation*

In this section, the Casson equation analysis was carried out from 40 % (w/w) sugar up to the experimental limit of sugar being dispersed in oil. Figure 5.17 represents the Casson equations for samples without Epikuron 200. The graph representing the Casson equations for the samples containing emulsifier is in the appendices **V-iii**.

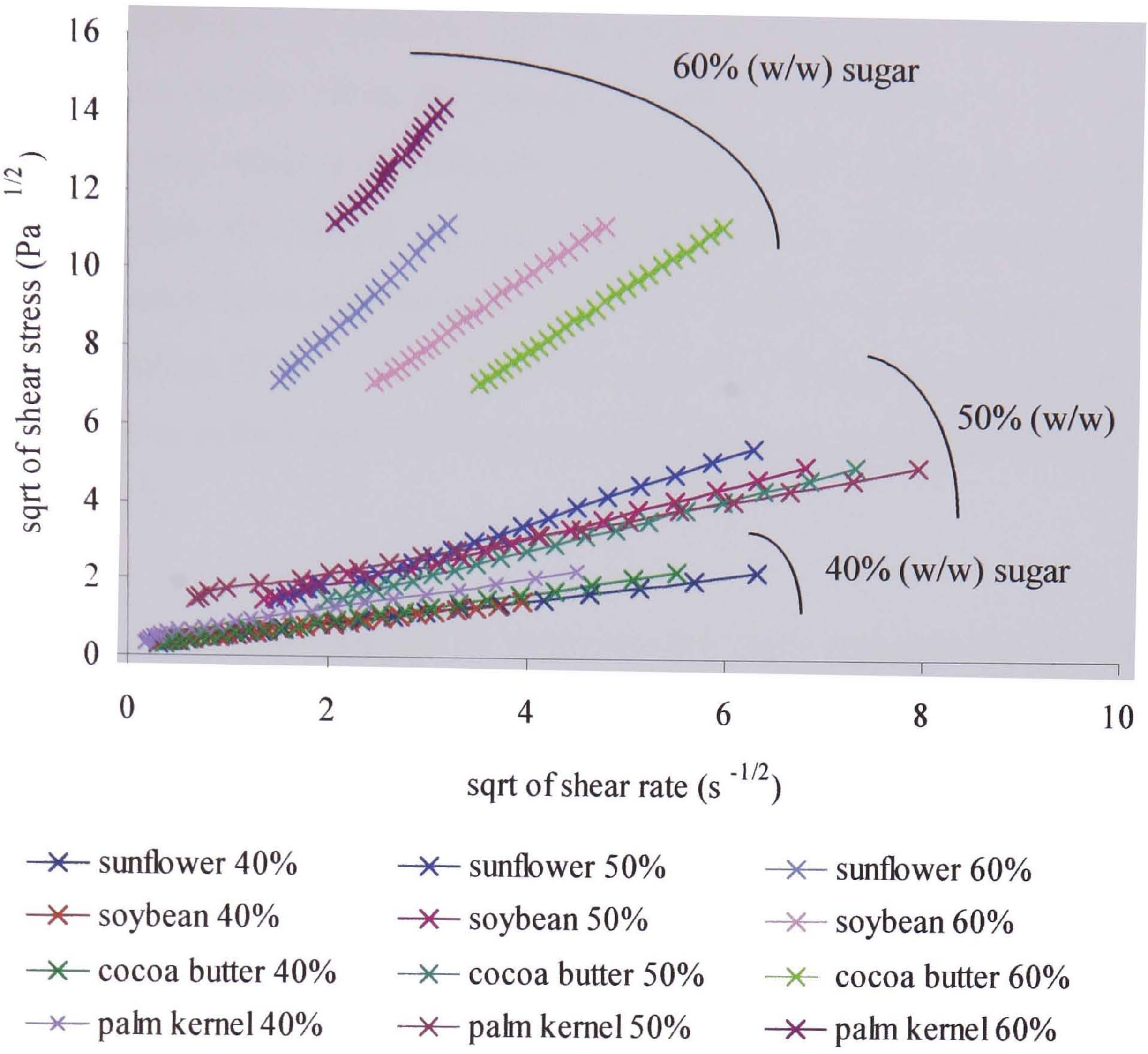


**Figure 5.17:** Casson plots for sugar dispersions in purified oils at 40 °C

It can be observed from Figure 5.17 that, as the sugar concentration increases, the slope of each curves increases. It can also be noted that up to 50 % (w/w) sugar, the oils behave similarly. All the oils of lower concentration are grouped together within a similar range of values. As the concentration increases up to 60 % (w/w) sugar, each samples behaves differently. We can note that palm kernel oil has the highest values for the square root of shear stress (from 11 to 14 Pa$^{1/2}$), whereas sunflower oil, soybean oil and cocoa butter have the same range of shear stress. On the other hand, all the oils have different shear-rate ranges. Cocoa butter has the strongest values upon the square root of shear-rate (3.5 to 6 s$^{-1/2}$).

The graph showing the Casson plots for the emulsifier containing samples (see appendices **V-iii**) shows a similar type of behaviour as in Figure 5.17: for the samples containing 40 % and 50 % (w/w) sugar, no difference can be made between the different oil systems. We can note that at 50 % (w/w) sugar, the soybean differs totally from the others samples. This line is in an unusual position; this may relate to experimental error and it will need to be repeated in order to confirm this result. As the concentration in sugar increases up to 60 % (w/w), each curves differs from each other. Sunflower oil has the strongest shear stress values (7 to 12 Pa $^{1/2}$) but this difference is less significant than the one compared to palm kernel oil. Soybean oil has the highest values for the shear-rate (5 to 9 s$^{-1/2}$).

In this study it has been possible to subdivide the chart, representing the Casson equation analysis, into three main regions. These regions may account for the different ranges of sugar concentrations. Figure 5.18 is a schematic of the chart representing these three main regions:

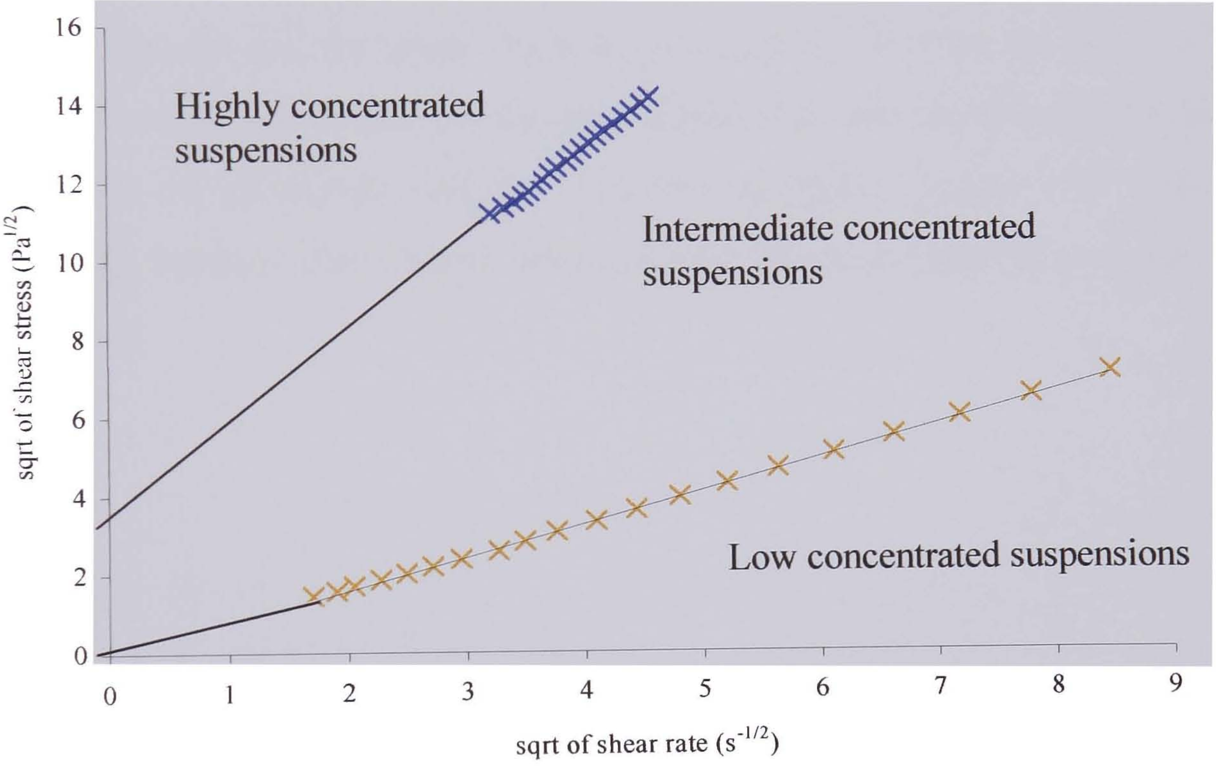


**Figure 5.18:** Representation of the different regions by Casson equation analysis of suspensions of sugar particles in oil at 40 °C

From Figure 5.18, the different regions may be identifiable as:

- Low concentrated dispersions correspond to the concentrations of 40 % to 50 % (w/w) sugar. The intercept value of this region is characteristically close to or below zero

- Above this region, the zone represents mainly a transition state between two types of dispersions. The percentage sugar at this stage varies between 50 and 55 % (w/w).
- Highly concentrated dispersions are mainly represented by the 60 ± % (w/w) concentrations of sugar. It has been observed that generally the rise in their value increases linearly or logarithmically, respectively, depending on the studied oil.

More analysis has been performed on this set of results by further exploiting the Casson intercept and the Casson slope values at different sugar concentrations and in the presence of different oils.

### *Casson intercept for samples in presence and absence of emulsifier*

From these results, a graph has been plotted showing the relation between the Casson intercept and the mass fraction of samples. In order to allow a direct comparison between samples, it was considered that both samples in absence and in presence of emulsifier will be presented together. Figure 5.19 shows the relationship between the Casson intercept and the mass fraction of sugar in the dispersions.

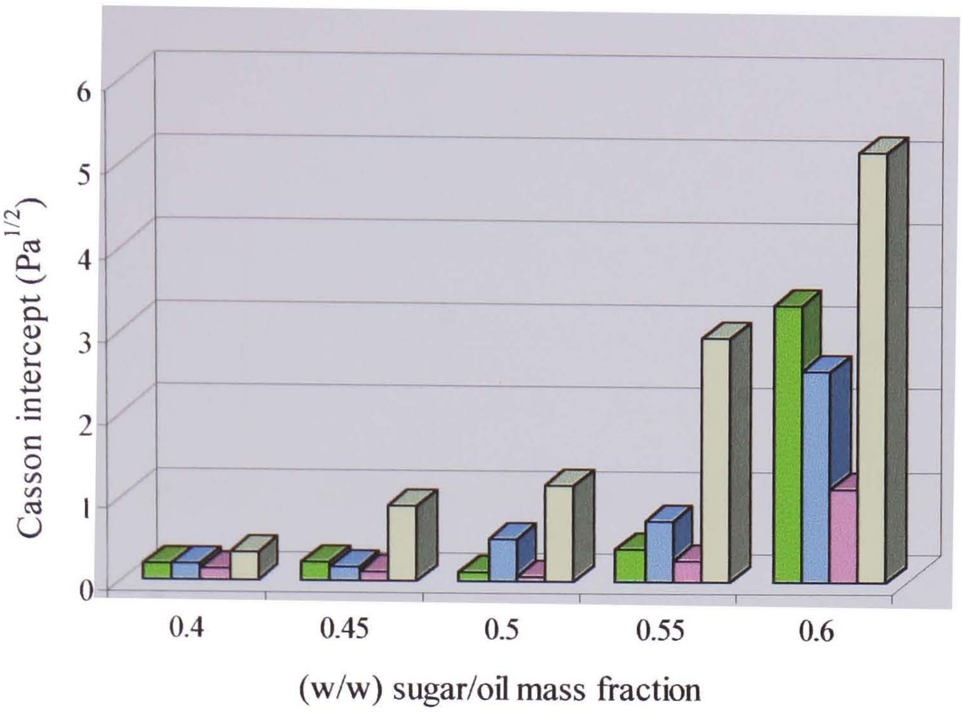


**Figure 5.19:** Casson intercept against mass fraction of sugar in purified oils at 40 °C (experimental errors < 10 %). (▨) represents the soybean oil dispersions; (▨) is the sunflower oil dispersions; (▨) represents the cocoa butter dispersions and ( ) is the palm kernel oil dispersions

It can be observed that apart from the palm kernel oil samples, the intercept value is both small and constant up to a volume fraction of 0.55. This constant value is less than 1 $Pa^{1/2}$. For 50 % (w/w) sugar, the palm kernel oil samples already have a value above 1 $Pa^{1/2}$. As the mass fraction increases, the intercept value increases too. It can be noted that until 55 % (w/w) sugar, there is no significant difference between the samples. Above 55 % (w/w) sugar, all the oils exhibit different values for the intercept. Sunflower oil and soybean oil have the same range of value for the intercept (2.5 to 3.5 Pa ½) whereas palm kernel oil has the highest value of approximately 5 $Pa^{1/2}$ and cocoa butter has its value around 1 $Pa^{1/2}$.

Figure 5.20 shows the relationship between the Casson intercept and the mass fraction of sugar and emulsifier in the dispersions.

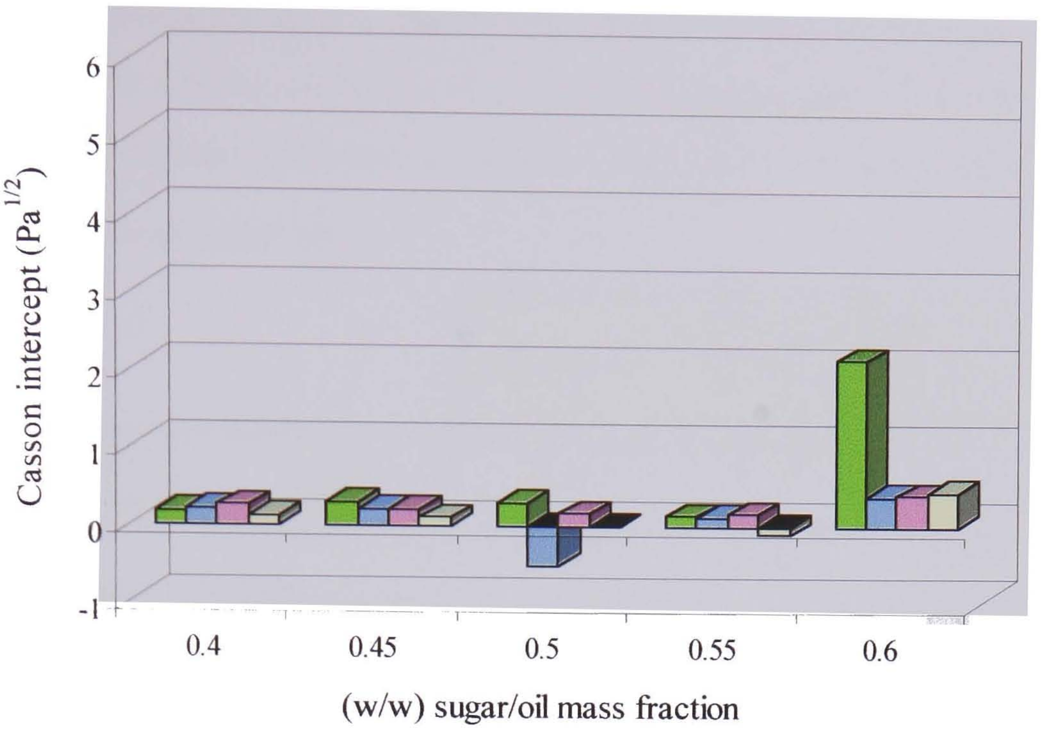


**Figure 5.20:** Casson intercept against mass fraction of sugar and emulsifier in purified oils at 40 °C (experimental errors < 10 %). ( ) represents the soybean oil dispersions; (🟩) is the sunflower oil dispersions; ( ) represents the cocoa butter dispersions and ( ) is the palm kernel oil dispersions.

It can be observed that in almost all the oils, the value of the intercept is ≤ 1 $Pa^{1/2}$. Soybean oil has negative values, which may correspond to an experimental error. As the sugar concentration reaches 60 % (w/w) sugar, the intercept value of sunflower oil rapidly increases up to 2.3 $Pa^{1/2}$. This observation may relate to experimental error and this value will need to be re-confirmed.

As a conclusion to this section a relationship between the intercept and mass fraction of sugar has been observed. The oils have a constant intercept, ≤ 1 $Pa^{1/2}$, up to 50-55 % (w/w) sugar for samples in absence of emulsifier. In the presence of emulsifier the value of the Casson intercept remains almost constant up to 60 % (w/w) sugar with a value of 0.5 $Pa^{1/2}$. Sunflower oil exhibits a high Casson intercept value at 60 % (w/w) sugar and this may relate to experimental error.

Table 5.4 summarises the different intercept values due to the presence of emulsifier at a volume fraction of 0.6.

**Table 5.4:** Casson parameters calculated from non-Newtonian rheology data for sugar dispersions (60 % w/w) in sunflower oil, soybean oil, cocoa butter and palm kernel oil in the presence or absence of lecithin emulsifier (Epikuron 200). The tabulated values are the intercepts from plots of $\sqrt{\tau}$ .

| | Casson parameter values | | | |
|---|---|---|---|---|
| | Sunflower oil | Soybean oil | Cocoa butter | Palm kernel oil |
| $\sqrt{\tau_{CA}}$ (Pa$^{1/2}$) [without emulsifier] | 3.355 | 2.55 | 2.95 | 5.19 |
| $\sqrt{\tau_{CA}}$ (Pa$^{1/2}$) [with emulsifier] | 2.153 | 0.39 | 0.40 | 0.45 |

*Notes:* Table from H.Babin *et al.*, (2005), *Food Hycrocolloids*, **19**, 513.

From this table, it can be deduced that the presence of emulsifier reduces the value of intercept compared to the value of the sample without emulsifier. We can note that without the presence of emulsifier, the intercept has a range of values which vary from 2.5 to 5 Pa$^{1/2}$. On the other hand, the presence of emulsifier reduces the value to zero with the exception of sunflower oil which has a value of 2.1 Pa$^{1/2}$.

This experiment has shown that in the samples without emulsifier the Casson intercept increases proportionally with the sugar concentration. However, when emulsifier is added to the mixtures, the Casson intercept remains constant to a value below 1 Pa$^{1/2}$ in most of the oils.

### *Casson slope for samples in presence and absence of emulsifier(Phosphatidylcholine)*

From the results in section V.3.2.3, it is also possible to plot a graph showing the relation between the Casson slope and the mass fraction of samples. Direct comparisons between samples, containing or not containing emulsifier, were analysed. Figure 5.21 exhibits the relationship between the Casson slope and the mass fraction of sugar in the dispersions.

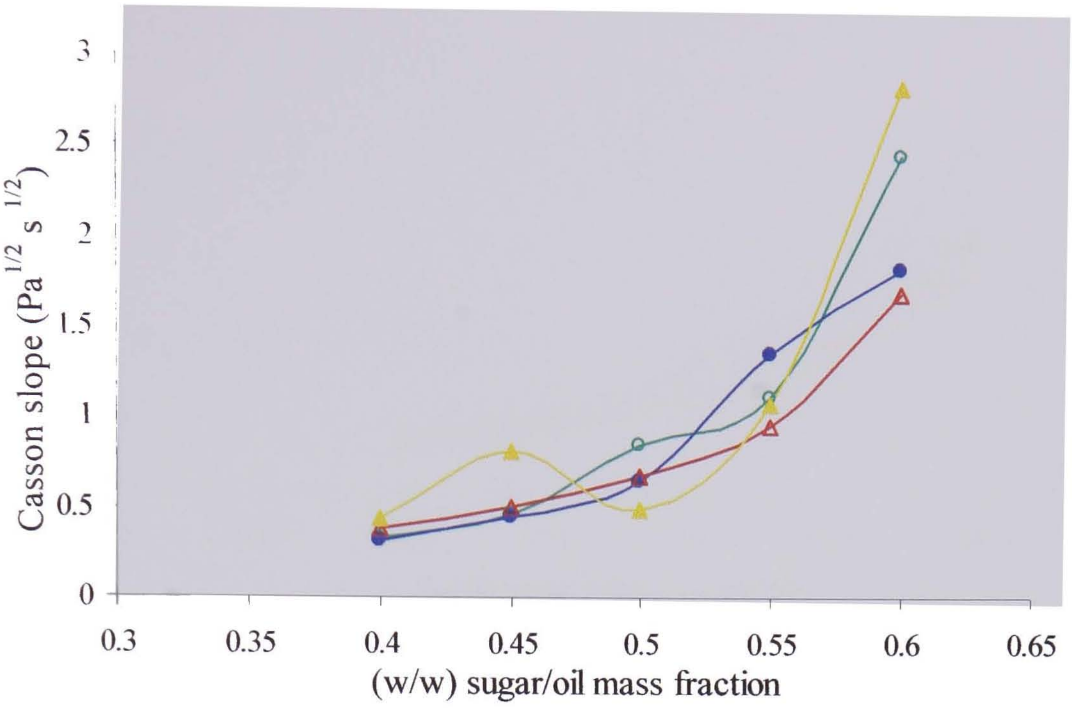


**Figure 5.21:** Casson slope versus mass fraction of sugar in purified oils at 40 °C (experimental errors < 10 %). (●) represents the soybean oil dispersions; (○) is the sunflower oil dispersions; (△) represents the cocoa butter dispersions and (▲) is the palm kernel oil dispersions.

It can be observed that the slope value is increasing constantly up to a mass fraction of 0.55 for all the systems with the exception of palm kernel oil, which has variable slope values. As the mass fraction increases, the slope value increases rapidly. It can be noted that until 55 % (w/w) sugar there is no significant difference between the samples. At 60 % (w/w) sugar, the systems start to differentiate from each other and we can note that palm kernel has the strongest slope value (2.7 Pa$^{1/2}$ s$^{1/2}$) and cocoa butter the lowest one (~ 1.5 Pa$^{1/2}$ s$^{1/2}$).

The same experiment was carried out with the presence of Epikuron 200 and results are shown in Figure 5.22.

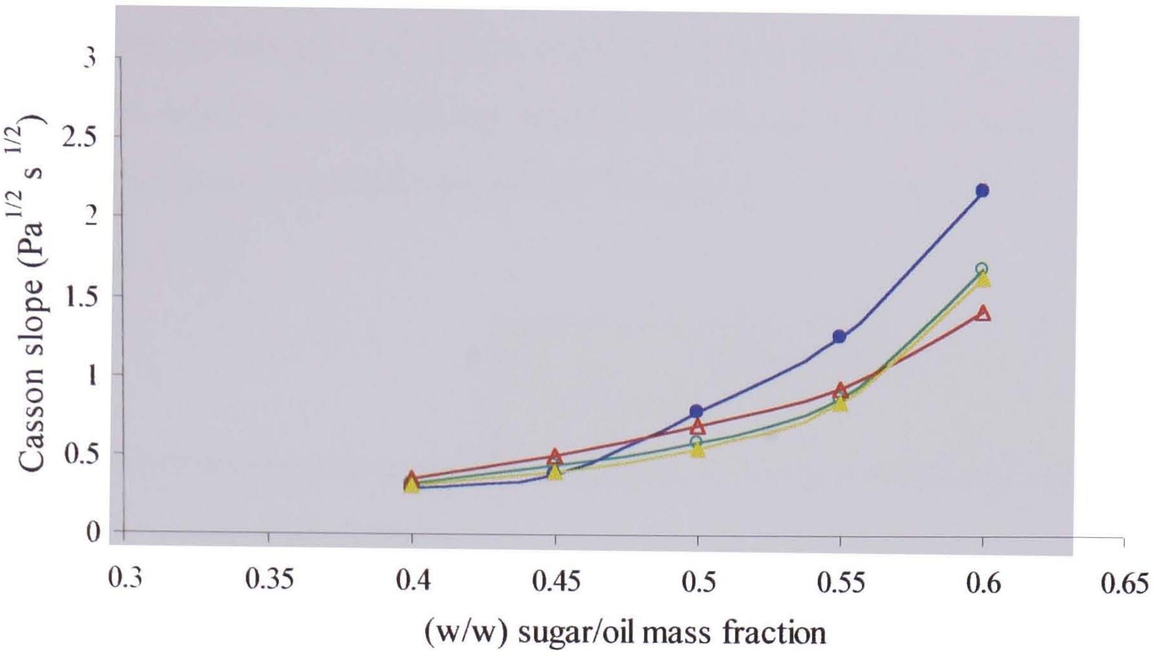


**Figure 5.22:** Casson slope versus mass fraction of sugar and emulsifier in purified oils at 40 °C (experimental errors < 10 %). (●) represents the soybean oil dispersions; (○) is the sunflower oil dispersions; (△) represents the cocoa butter dispersions and (▲) is the palm kernel oil dispersions.

In Figure 5.22, it can be observed that almost all the systems exhibit the same trend with the exception of the soybean oil samples. Sunflower oil and palm kernel oil have pretty similar characteristics whereas cocoa butter tends to exhibit a lower slope at high sugar concentration. Soybean oil curve behaves differently as its slope increases linearly from 45 % (w/w) sugar up to 55 % (w/w) sugar. Above this latest point, the slope increases significantly up to 2.2 $Pa^{1/2}$ $s^{1/2}$. We compared the effect of the emulsifier on the Casson slope values for a volume fraction of 0.6. These results are summarised in Table 5.5.

**Table 5.5:** Casson parameters calculated from non-Newtonian rheology data for sugar dispersions (60 % w/w) in sunflower oil, soybean oil, cocoa butter and palm kernel oil in the presence or absence of lecithin emulsifier (Epikuron 200). The tabulated values are slopes from plots of $\sqrt{\dot{\gamma}}$.

| | Casson parameter values | | | |
|---|---|---|---|---|
| | Sunflower oil | Soybean oil | Cocoa butter | Palm kernel oil |
| $\sqrt{\mu_{CA}}$ (Pa$^{1/2}$ s$^{1/2}$) [without emulsifier] | 2.45 | 1.82 | 1.69 | 2.83 |
| $\sqrt{\mu_{CA}}$ (Pa$^{1/2}$ s$^{1/2}$) [with emulsifier] | 1.70 | 2.20 | 1.44 | 1.65 |

*Notes:* Table from H. Babin *et al.*, (2005), *Food Hycrocolloids*, **19**, 513.

From Table 5.6, it can be observed that the addition of emulsifier further reduces the value of the Casson's slope to different extend. These values are generally lower than the values obtained without emulsifier. We can note that the presence of emulsifier increases the value of the Casson's slope for soybean oil. From the Figure 5.21, the value of Casson's slope does not seem to progress exponentially and this may be interpreted as an experimental error.

### *Conclusion*

In conclusion it can be said that a relationship exists between the Casson slope and the mass fraction of sugar in the different oil systems. We can note that the slope increases slowly up to a mass fraction of 55 % (w/w) for the sample without emulsifier. Then the slope value increases drastically for all the samples. In comparison with the samples containing emulsifier, it can be seen that their slope values are lower compared to the samples without emulsifier. Moreover it can be observed that all the systems containing emulsifier follow the same pattern. The slope of the Casson equation plot gives the effective viscosity of the samples. It has been observed that the different oils produce different results and these are not readily generalised.

From all these results, two different types of aggregation involving the sugar and the oils are defined. The first type may be represented by the formation of small aggregates, which are easily disrupted and may have a globular shape. Once the sugar concentration is at its maximum, another distribution of sugar particles occurs. This packing has a higher density of interactions between the particles and with the oil. At this stage, the aggregates may have an ordered rearrangement, which does not allow for much flow movement. Consequently, the Einstein rheological model is no longer applicable to our systems, due to the numerous interactions between the particles. As the sugar concentration increases, the rearrangement of the particles may occur until the dispersion of these particles is no longer possible in a simple mixing design. This behaviour is illustrated schematically in Figure 5.23:

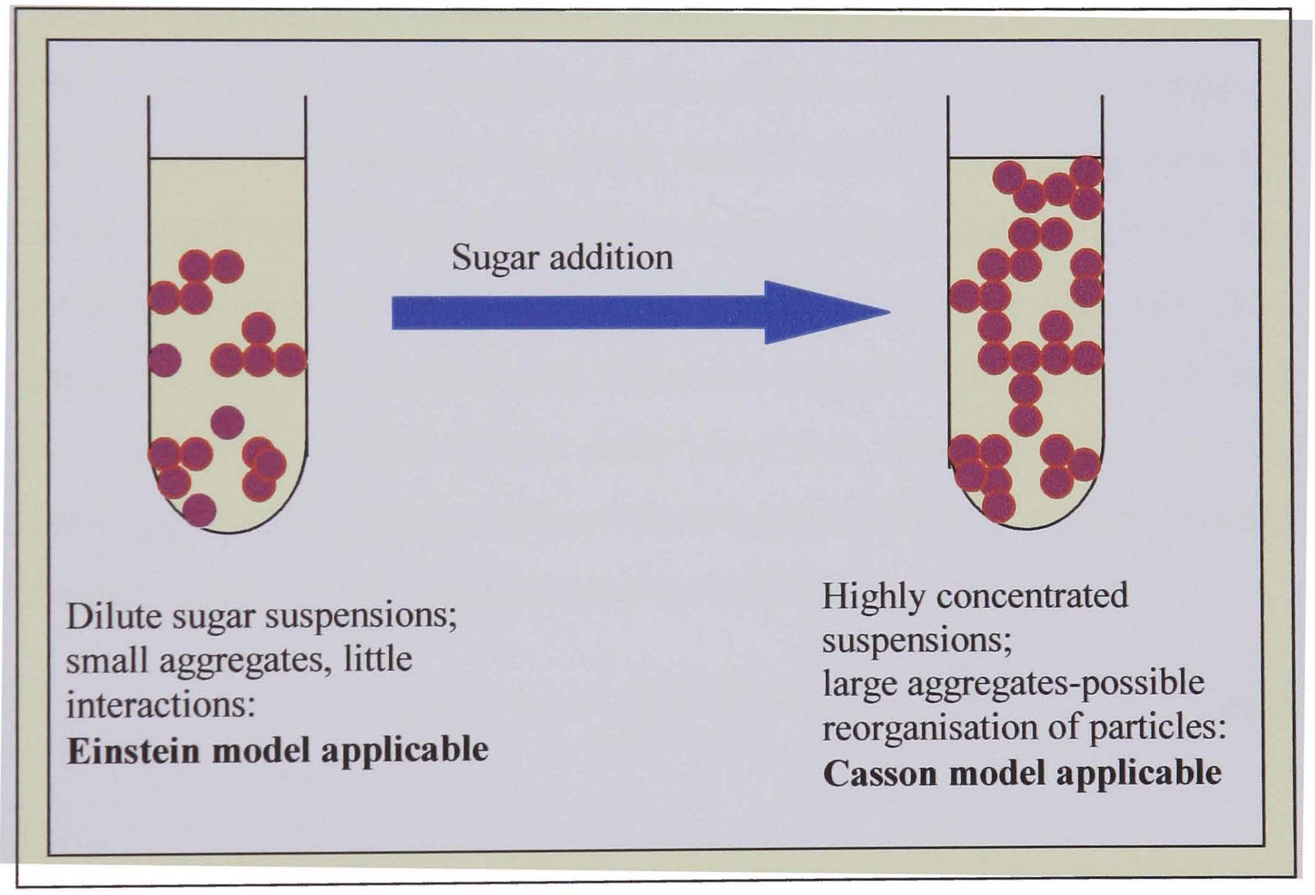


Figure 5.23: Schematic diagram of two suggested states of sugar particle aggregation in oil at 40 °C. ( ● ) represents lone sugar particle and ( ● ) represents an aggregated sugar particle.

The applicability of the semi-empirical Casson equation has been previously reported in the confectionery literature (Chevalley, 1975, Nelson and Beckett, 1999). It allows a good interpretation of the flow behaviour of molten chocolate specifically. However, recent research has been carried out which studies and models the rheological behaviour of dense slurries. One equation, Quemada and Berli model, has been selected in these recent works and is further developed in the following section.

### V.3.2.4. Recent advances on rheological modelling

*Quemada and Berli equation*

Quemada and Berli (2002), previously cited in Chapter II, explain that appropriate modelling of rheological behaviour depends on numerous variables which allow a quantification of the flow. However, the effects of these parameters on the rheology of fluids have not been fully understood (Goodwin and Hughes, 1992). They state that most of the empirical models which are currently used, for instance the equations of Roscoe (1952) or Krieger and Dougherty (1959), are limited to one parameter: the maximum close packing $(\phi)_{\max}$. In their approach, Quemada and Berli (2002) explain that interaction potentials should be integrated into viscosity models of dense slurries under shear flow. Their model is based on the assumptions that the structure follows the relaxation kinetics of hydrodynamic and Brownian forces. Their equation is represented by

$$\eta(\sigma) = \eta_\infty \times \left( \frac{1 + \sigma/\sigma_C}{\Re + \sigma/\sigma_C} \right)^2 , \qquad \textbf{(5.4)}$$

where $\eta(\sigma)$ is the suspension viscosity as function of shear stress, $\eta_\infty$ is the effective maximum viscosity corresponding to $\sigma \rightarrow \infty$, $\sigma$ is the shear stress, $\sigma_C$ is the critical shear stress defined $\sigma_C = k_B T/a^3$; and $\Re$ is the rheological index of the equation defined by the following ratio:

$$\Re = \left( \frac{1 - \Phi/\Phi_0}{1 - \Phi/\Phi_\infty} \right) = \left( \eta_\infty / \eta_0 \right)^{1/2} \qquad \textbf{(5.5)}$$

here $\Phi$ is the effective volume fraction, $\Phi_\infty$ and $\Phi_0$ are the effective maximum packing fractions corresponding to $\sigma \rightarrow \infty$, $\sigma \rightarrow 0$, and $\eta_0$ is the effective maximum viscosity corresponding to $\sigma \rightarrow 0$ for $\Phi_\infty < \Phi_0$. The critical shear stress is defined by $\sigma_C = k_B T/a^3$ with a being the mean radius of the particles.

The equation 5.9 shows that the viscosity model obeys a rheological equation as it is function of the volume fraction ($\Phi$), the hydrodynamic interaction energy ($\tau_{hy} = \sigma a^3$) as well as the Brownian thermal energy ($\tau_{Br} = k_B T$).

Consequently Quemada and Berli's equation can be applied to study the flow properties of several suspensions, including weakly attractive, repulsive, soft repulsive potentials. However, the authors explain that by varying the particle concentration, the colloid is modified and the physicochemical variables, effective radius and volume, cannot be easily extrapolated. Another approach which may be interesting to consider would be to extrapolate these parameters and make them vary. The effects of these parameter changes on the flow behaviours of concentrated suspensions could be interpreted by using computer simulations. This tool would allow a complete interpretation of the flow behaviour of sugar/oil by varying different parameter values.

Further work in the rheology section was carried out which were relevant to industry. Interest lay in the comparisons between laboratory scale experiments and suspensions developed industrially. Therefore, we developed a dispersing technique, which would mimic the chocolate factory dispersions and thus would allow for the comparison of the results from original dispersing process and "refined mixing preparation".

### *V.3.3. Developments in rheological experiments*

This section reports the work carried out on the dispersions of sugar particles in oils at highly concentrated suspensions (above 60 wt % sugar). Our aim was to develop a process which would mimic the chocolate factory dispersions and to analyse the different dispersions by rheology. The process which has been created is known as the "*refined mixing preparation*". As the project developed, emphasis was given towards ingredients used in the chocolate confectionery. Therefore, use of sunflower oil and soybean oil tended to be minimised, whereas results regarding the uses of milk fat or fat mixture (blend of milk fat and cocoa butter) were favoured. In this section, we tried to correlate the results obtained from the sedimentation technique with the rheology technique.

#### V.3.3.1. Effect of emulsifier on the viscosities of different amounts of sugar samples

In order to make even more concentrated sugar suspensions (up to 70 wt %), a new mixing method was explored. This method was based more closely on the process of chocolate making and was developed in our laboratory. The procedure has been fully described in the section V 2.2.1. Figure 5.24 shows the effect of emulsifier, phosphatidylcholine, on the viscosities of cocoa butter, palm kernel oil and fat mixture.

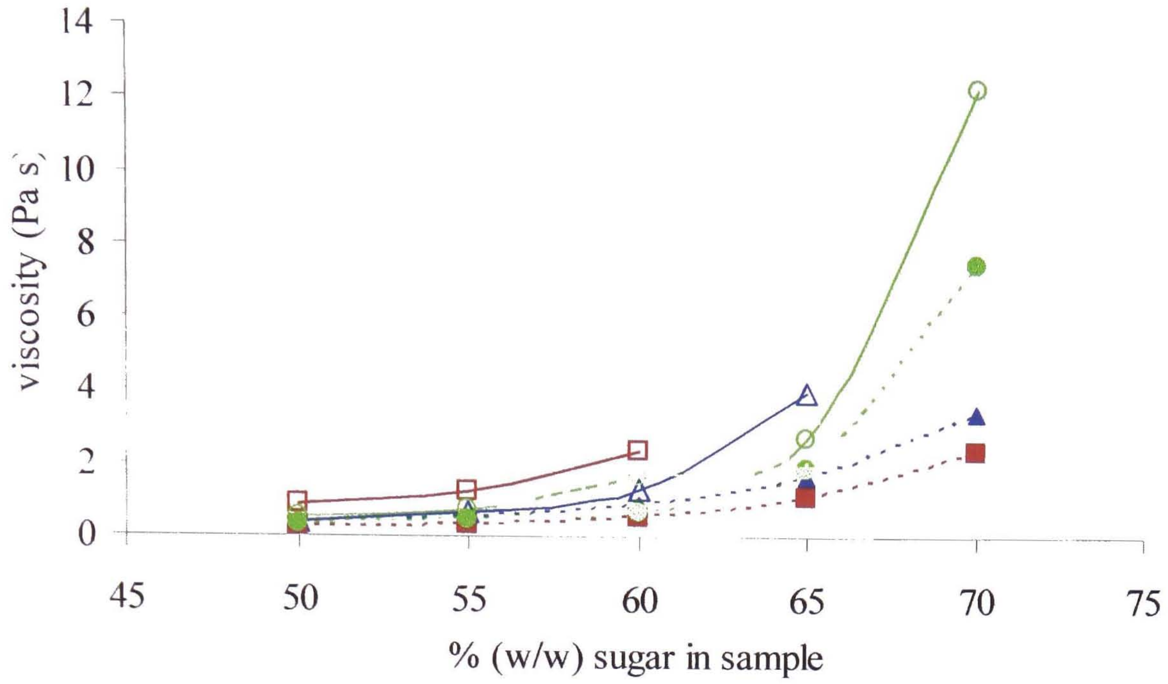


**Figure 5.24:** Comparison of the viscosity of samples with and without emulsifier added at 40 °C (experimental errors < 10 %). (■) and (□) represent the cocoa butter dispersions with and without emulsifier; (▲) and (△) represent the palm kernel oil dispersions with and without emulsifier; (●) and (○) represent the fat mixture dispersions with and without emulsifier.

From Figure 5.24, it can be observed that the presence of emulsifier in the different systems has significantly reduced the viscosity. For samples not containing emulsifier, we noted that palm kernel oil has the highest viscosity over the range of concentrations studied compared with the other oil samples. Dispersions made with palm kernel oil could not be investigated over the sugar content of 60 wt % as the sample was too "thick". The same trend was observable for cocoa butter, the samples made with more than 65 wt % sugar giving a really high viscosity. On the other hand, the viscosity of samples made with fat mixture (83.5 % cocoa butter and 16.5 % milk fat, w/w) could be measured for sugar contents up to 70 wt %.

For dispersions containing emulsifier, it can be observed that the presence of surfactant was most significant for the palm kernel oil samples in reducing the viscosity. At 70 wt % sugar content, the sample made with palm kernel oil had the lowest viscosity amongst the studied systems. In the case of cocoa butter, it can be noted that the presence of emulsifier had a strong effect in reducing the viscosity of the samples. But this effect, despite being important, was still lower than for palm kernel oil. Samples based on fat mixture show a reduction of viscosity due to the presence of surfactant, but the effect was more limited than for samples based on cocoa butter and palm kernel oil.

From these results it may be suggested that there is interference between the fat mixture and the emulsifier, which reduces the effectiveness of the emulsifier. The fat mixture is made from cocoa butter and milk fat, and it seems likely that the milk fat mostly infers with the action of the surfactant. In order to further investigate this effect, we performed a side experiment which involved the addition of different amounts of surfactant to the different oils.

### V.3.3.2. Effect of different amounts of emulsifier on the viscosities of 70 wt % sugar samples

Each sample was made of 70 wt % sugar with a range of emulsifier (phosphatidylcholine only) from 0.15 % to 0.5 %. Figure 5.25 shows the effect of different surfactant concentrations on the viscosity of different samples. Samples were analysed at variable shear stresses, from 100 to 200 Pa, depending on the viscosity of the material.

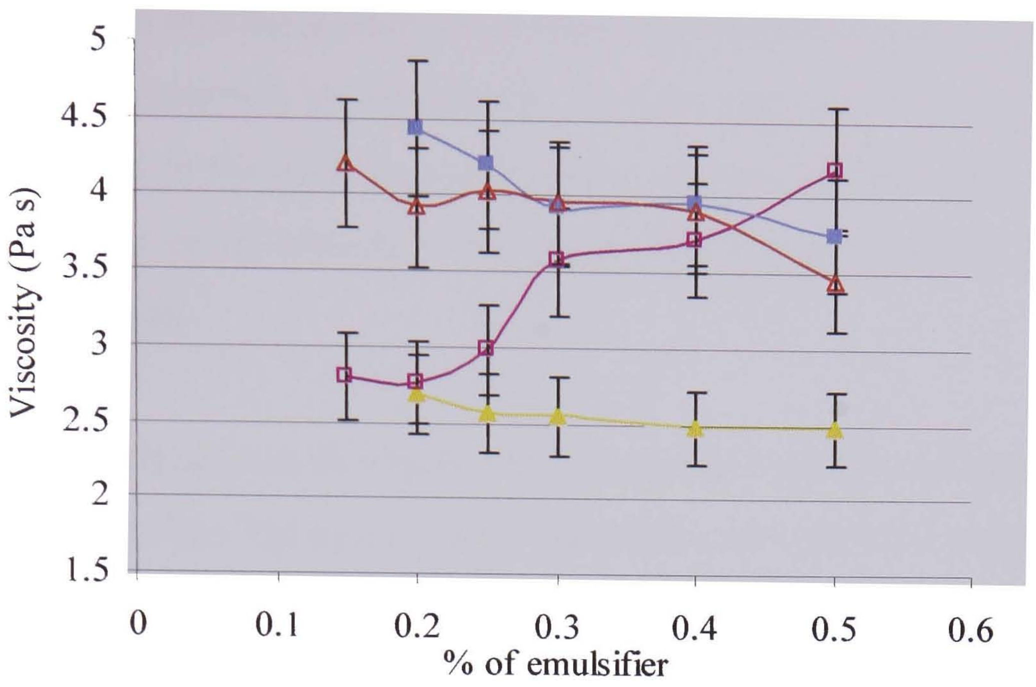


**Figure 5.25:** Effect of different amounts of emulsifier on the viscosity of 70 % sugar dispersed in oils at 40 °C (experimental errors < 10 %). (△) represents the cocoa butter dispersion; ( ) represents the palm kernel oil dispersion; (▨) represents the fat mixture oil dispersion and ( ) represents the milk fat dispersion.

From Figure 5.25, we could note that each oil behaves differently. In the presence of emulsifier, the viscosity of the samples made with palm kernel oil decreases from 2.7 to 2.45 Pa s. In the case of cocoa butter and fat mixture, we noted that their viscosities are the highest amongst the different samples. It seems that the addition of small quantities of emulsifier, 0.15 % up to 0.3 %, has a predominant effect on the viscosity of the samples. On the other hand, we can define a rheological plateau for both samples between 0.3 and 0.4 % emulsifier. At these emulsifier concentrations, viscosity of the cocoa butter stabilised at 4.0 Pa s, whereas fat mixture stabilised at 3.9 Pa s. As the concentration of emulsifier increases, the viscosity of the samples decreases down to 3.45 Pa s for cocoa butter samples and 3.7 Pa s for fat mixture samples respectively. Samples made of milk fat exhibit a total different behaviour than the previous systems. As the emulsifier concentration increases, the viscosity of the samples increases from 2.8 to 4.2 Pa s.

The results from Figure 5.25 show that all the oils with the exception of milk fat show a reduction in the viscosity of the samples. In the case of milk fat, it may be suggested that the presence of minor oil identities counteract the action of the emulsifier contained in the system. The fact that the action of the emulsifier is counteracted in the milk fat samples may explain why the effect of emulsifier on the viscosity of fat mixture is less predominant than with the viscosity of cocoa butter samples.

As a conclusion to this experiment, it can be noted that the percentage increase in emulsifier into the sample has a beneficial effect on reducing the viscosity of concentrated systems. A disparate result was observed for the sample made with milk fat.

#### V.3.3.3. Comparison of the rheological effects between three different types of emulsifiers addition

The addition of several kinds of emulsifier to concentrated systems has been developed with rheological analysis (Schantz & Rohm, 2005; Johansson and Bergenstahl, 1992b). In this section, the effect of emulsifier on the specific oil samples via rheology was investigated. Figure 5.26 represents the effect of three surfactants, phosphatidylcholine, monoolein and polyglycerol polyricinoleate.

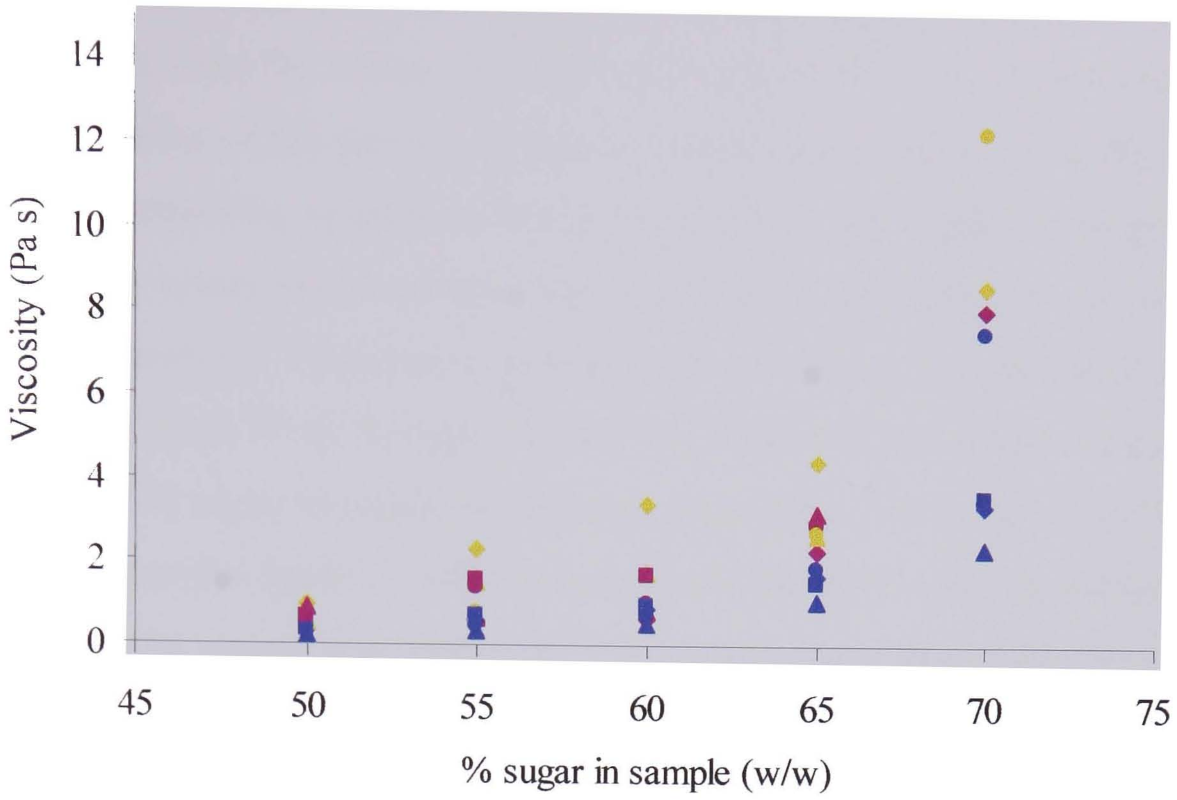


**Figure 5.26:** Effect of three different surfactants on four different oil samples at 40 °C (experimental errors < 10 %). The blue colour represents the samples with an addition of PC; the pink colour represents the samples with an addition of monoolein; the yellow colour represents the samples with an addition of PGPR. The different symbols (♦), (▲), (●), (■), represent cocoa butter, palm kernel oil, fat mixture and milk fat samples. The addition of emulsifier was proportional to the amount of sugar contained in the sample.

From Figure 5.26, we can denote three separate ranges of interactions produced by the different addition of surfactants to the oils systems. We noted that most of the oils behave similarly with the presence of a specific emulsifier. Samples made with phosphatidylcholine generally present the lowest viscosity of the samples. As the sugar concentration increases gradually, the viscosities of the different samples increase proportionally. At 70 wt % sugar, each oil samples behave independently and the range of viscosity is more scattered.

The viscosity of the samples made with monoolein, is slightly higher than the viscosity of the phosphatidylcholine samples. With the addition of monoolein it seems that each oil behaves differently from the other creating a large scattering of viscosity response. In the specific example of milk fat, samples generate the highest viscosity with this emulsifier; however in presence of monoolein, cocoa butter samples show the lowest viscosities amongst the different studied oils.

The viscosities of the samples containing PGPR have viscosities pretty similar to samples containing monoolein at the exception of cocoa butter samples. We observed that cocoa butter samples had the highest viscosities at the different sugar concentrations. We noted a proportional increase in the viscosity with the sugar content up to 70 wt % sugar. In the case of fat mixture samples we can see that at 70 wt % sugar its viscosity increase drastically. The same behaviour was notified for samples made of palm kernel oil. No analysis could be performed at this stage as the sample was extremely viscous and had a thick paste texture. No viscosity analysis was performed with the milk fat samples containing PGPR until this stage.

From the results obtained in Figure 5.26, the phosphatidylcholine allows dispersions of the all the studied oils up to 70 wt % sugar due to its specific surface active properties. In the case of monoolein and PGPR, we have seen moderate and similar viscosities exhibited with these two surfactants. However, it seems that oils behave differently from each others. Their viscosity varies depending on the nature of the surfactant added to the system (e.g. cocoa butter with monoolein and with PGPR). Presumably, the difference of viscosity in presence of the three emulsifiers may reside in the chemistry of the surfactant and their ability to bind the sugar particles with the oil. The molecular representation of these surfactants has been described in Chapter III. The main differences between phosphatidylcholine, monoolein and PGPR at a molecular level, relies on the prevalence of hydrophilic and hydrophobic bonding sites in the molecule. We noted that phosphatidylcholine possesses two different binding sites, the ionic part for hydrophilic interactions and the organic part for hydrophobic interactions.

However, monoolein molecule does not have an ionic part but is essentially an organic molecule. Therefore monoolein has already one binding site less that phosphatidylcholine molecule. This means that the level of interactions is less favoured with this emulsifier and thus will affect the viscosity. In presence of this emulsifier the viscosity may be reduced to a lesser extent in comparison with the viscosity of samples containing phosphatidylcholine.

In the specific case of PGPR, the molecular differences which exist between phosphatidylcholine are based on the fact that PGPR is a polymer and organic molecule which is almost similar to monoolein molecule. But it differs from monoolein as PGPR is a polymer whereas monoolein is monomer. This means that PGPR molecule may rearrange itself depending of the environment to which this emulsifier has been added. Due to the size of the polymer (~ 3000 g $mol^{-1}$) the molecule may be restricted to move freely and to easily bind with the sugar particles with the oil phase. The steric effect added to the fact that this emulsifier does not possess ionic part, reduces the level of interactions in the systems. Consequently, the samples containing PGPR are highly viscous dispersions in comparison with the phosphatidylcholine samples.

As a conclusion, we observed the rheological effect of adding three different types of emulsifier to four highly concentrated sugar suspensions. At this concentration, there was a specific level of interactions depending of each surfactant. In this case, we noted that samples containing phosphatidylcholine would generate low viscosity and thus independently of the oil phase. However the addition of monoolein and PGPR to the dispersions showed higher viscosities and disparate results amongst the oils. This result may be due to the chemistry of the surfactants depending of the prevalence of their binding sites and the steric blocking effect of polymer molecules. However, we discovered that the oil properties may influence greatly the level of interactions between surfactant and sugar particles (Chapters III and IV). The specific example of these interactions between fat mixture and phosphatidylcholine has been reported in the section V.3.3.1. We decided to further investigate these interactions by adding the emulsifier at different stages of the process.

### V.3.3.4. Effect of sequence of addition of emulsifier to fat mixture system

Assuming that there is interference between the milk fat and the surfactant, the time of adding the surfactant (Epikuron 200) could be important. Therefore we investigate the effect of addition of surfactant at different stages of the mixing procedure depending on the presence or not of milk fat. The different stages of addition of emulsifier are schematised in Figure 5.27.

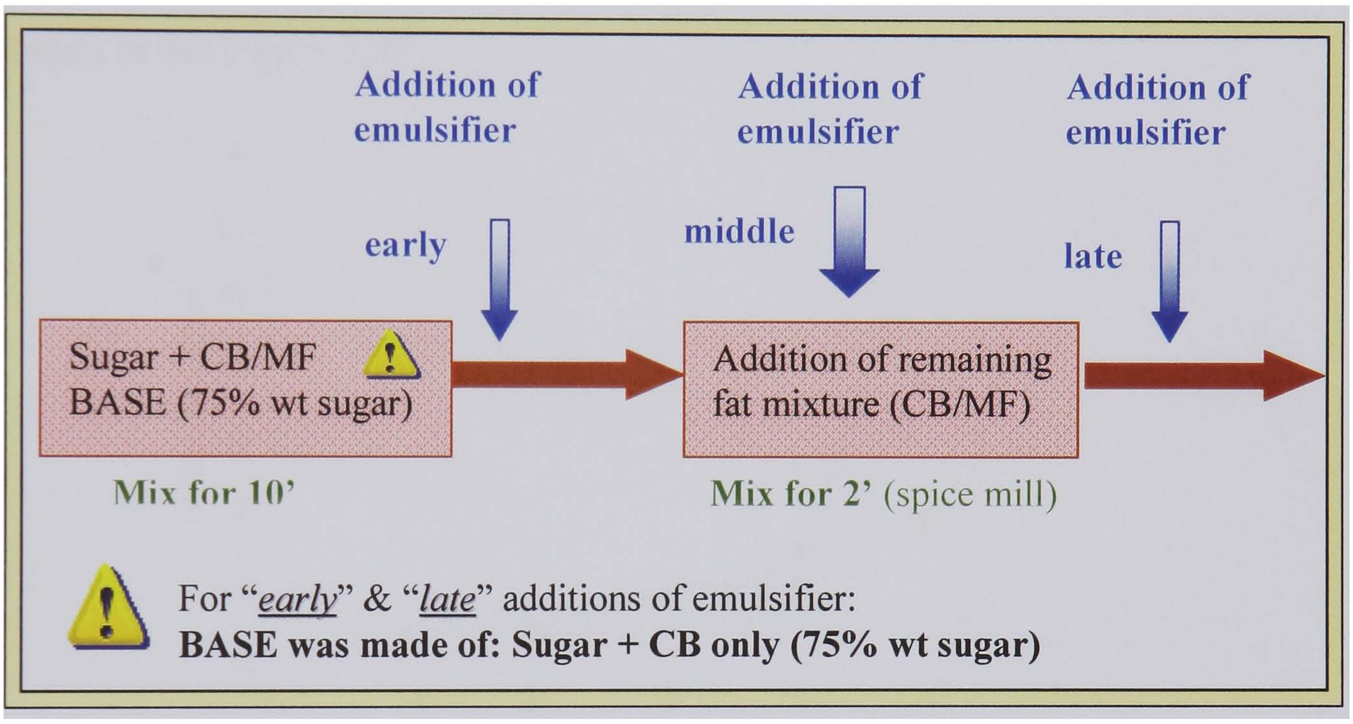


**Figure 5.27:** Addition of emulsifier at different stages of the mixing process

Three different addition sequences were made, which are classified as: "*early*", "*middle*" and "*late*" additions. "*Early addition of emulsifier*" involved a sugar base made with cocoa butter. The base contained 75 wt % sugar and was mixed for 10 minutes. A 100 g aliquot of this mixture was then taken and a known amount of emulsifier was added (see Chapter V section 3.2.1). This sample was then mixed for 1 minute on the spice mill. Finally the remaining fat mixture was added to the sample in order to dilute down to 70 wt % sugar. The sample was then mixed for another minute with both emulsifier and remaining fat.

The same procedure was almost used for the "*late addition of emulsifier*". The same base was made as in the "*early addition of emulsifier*", however in this case the addition of emulsifier was done after mixing 1 minute the dilute dispersion.

For the "*middle addition of emulsifier*", the base was prepared with the whole fat mixture (cocoa butter and milk fat added together). This mixture was blended for 10 minutes. Then 100 g of this mix was diluted down to 70 wt % sugar and simultaneously emulsifier was added. The sample was further blended all together for 2 minutes. The results of these different stage additions have been analysed and plotted in the Figure 5.28.

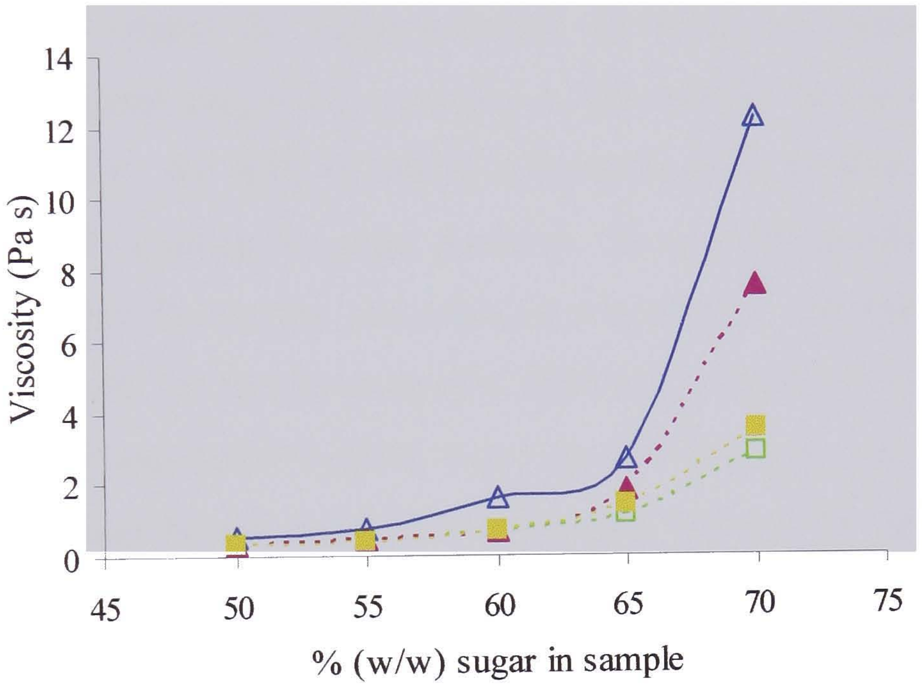


**Figure 5.28:** Viscosity effects of the addition of emulsifier at different stage of the mixing process at 40 °C (experimental errors < 10 %). (△) represents the fat mixture dispersion; (▲) represents the fat mixture dispersion with the "*middle addition of emulsifier*"; ( ) represents the fat mixture dispersion with the "*early addition of emulsifier*" and ( ) represents the fat mixture dispersion with the "*late addition of emulsifier*". For emulsifier concentrations please refer to table 5.1.

In Figure 5.28, the samples without emulsifier generate a higher range of viscosities (> 10 Pa s) in comparison with samples containing emulsifier (< 10 Pa s). It can also be noted that the addition of emulsifier at different stages of the process affects the viscosity of the samples. The "*early addition of* *emulsifier*" generates the lowest viscosity amongst the different samples. Similarly the "*late addition of emulsifier*" also exhibits low viscosities within the samples. However, the "*middle addition of emulsifier*" produces a high viscosity.

These results may be explained by the fact that competition at the sugar surface may exist between milk fat components and added emulsifier. Figure 5.28 shows that, when emulsifier is added on its own (not simultaneously with milk fat), the viscosity of the sample is greatly reduced.

Consequently, it may be said that, for "*early addition of emulsifier*", the surfactant has well coated the sugar particles, allowing the suspension to be fluidised and thus generating a low viscosity to the sample. In the case of "*late addition of emulsifier*", the milk fat which contains specific surface-active lipids may be preferentially coating the sugar particles. As a result, the addition of this single ingredient may favour the reduction of viscosity of the sample. On the other hand, the results for the simultaneous additions of milk fat and emulsifier ("*middle addition of emulsifier*") shows that high viscosity amongst the samples is generated. This may be the result of interferences between the emulsifier and the surfactants already present in the milk fat. This competition may occur due to preferential adsorption at specific regions of the surface of the sugar crystals for the different surface-active species present.

From the results obtained on the adsorption field (see Chapter III) it has been shown that interferences may be present between emulsifier and surface-active components present in the oils. The presence of both surfactants may involve competition between them two at a specific biding area of the sugar surface. Based on the hypothesis, it was decided to give evidence of these laboratory results by running some experiments on full chocolate recipes at a pilot plant scale (see Chapter VII).

## *V.4. Conclusion*

In rheology, two kinds of analysis of the experiments conducted have been carried out. One involves the viscosity of low concentration sugar/oil dispersions involving the "basic mixing method". The other analysis was done on highly concentrated samples involving the "refined mixing method". The results of these two techniques were compared to theoretical rheological models with the Einstein's and Casson's relationship.

It has been observed that, for low sugar/oil dispersions, the effect of purification of the oils reduces the viscosity. When emulsifier was added to the oils and purification was undergone, it could be seen that the effect of emulsifier with no sugar content had no significant effect on the viscosity of the dispersions.

When sugar was added to the different oils, the relative viscosities of sunflower, soybean oils and cocoa butter were similar up to 30 % (w/w) sugar. Above this concentration, differences occurred. In the case of palm kernel oil, there is apparently a strong affinity between the dispersed sugar crystals, therefore considerably enhancing the viscosity of the samples. When applying the Einstein's relationship to the data for the sugar/soybean oil dispersions, it was noted that up to a certain volume fraction of sugar ($\phi = 0.16$) the experimental viscosity curve matches the theoretical viscosity curve. Above this point, the experimental viscosity of each concentration is rapidly increasing and the curves no longer match each other. This shows the limitation of Einstein's relationship for describing sugar particles dispersed into oils.

The second experiment which was carried out was based on high sugar particle concentrations. It has been observed, from graphs of viscosity against shear-rate, that the presence of emulsifier considerably reduces the viscosity of the samples.

Palm kernel oil was found to have the strongest rheological effect (up to 20 Pa s), followed by soybean oil and cocoa butter having the lowest effect (up to 10 Pa s).

When Einstein's relationship was tested for these concentrated dispersions, it was observed that this equation was not representative for the viscosity of any of the samples. These results confirmed the behaviour for the low concentration sugar/oil dispersions. So, in order to correlate our rheological results with the sedimentation results, the Casson equation was used.

It is possible to subdivide the graph showing the square root of shear stress versus shear-rate into three main regions. The first region, which represents the low concentration suspensions, exhibits a Casson's intercept and a slope value below 1 Pa ½ s ½. The intermediate level of concentration corresponds to 50 % to 55 % (w/w) sugar. Above this concentration range, there is the region of highly concentrated samples corresponding to 65% (w/w) sugar; this is characterised by its high values of the Casson intercept and slope.

From the graphs of the Casson intercept versus the mass fraction of sugar or Casson slope versus the mass fraction of sugar, it has been possible to identify two types of behaviour in the rheology of sugar/oil dispersions. The first one is that sugar particles tend to agglomerate into small "globular" aggregates when present in dilute suspension (up to 50 - 55 % (w/w) sugar). At this stage small aggregates are easily disrupted because the energy required to break them is low, and this statement implies a low Casson intercept value. The second type of behaviour may suggest the formation of "structured" aggregates with a strong affinity between the particles. As a consequence, the fluidity of the samples will be reduced and this will further increase the Casson slope and consequently drastically increase the viscosity of the samples.

For the process of dispersing sugar particles and oil, a new technique of mixing has been developed. The "refined mixing method" is a modification of the process performed during the manufacture of chocolate confectionary. It involves a two-stage mixing procedure, with the making up of a highly concentrated base (75 - 70 wt % sugar) and the creation of a sample under high shear using a spice mill processor. With this technique it has been possible to increase the range of rheological analysis to systems with a high sugar content.

Rheological analysis has involved the use of both concentrated and highly concentrated dispersions. It has been noticed that palm kernel oil samples generate the highest viscosities amongst the different studied oils over the wide range of sugar contents. When applying the "*basic mixing method*", differences of viscosity between the samples were observable at 60 wt %.

When using the "*refining mixing method*", palm kernel oil still has the highest viscosity. Without emulsifier addition, viscosity experiments could only be performed up to 60 wt % with palm kernel oil and 65 wt % for the cocoa butter. On the other hand, it has been possible to measure the viscosity of dispersions made with the fat mixture at up to 70 wt % sugar. In the presence of emulsifier, palm kernel oil gives the greatest reduction in viscosity. Cocoa butter has the second largest effect on viscosity due to the presence of emulsifier. Conversely, the addition of phosphatidylcholine was not so efficient in reducing the viscosity of the fat mixture sample. The viscosity of such dispersion still remained high after adding the emulsifier. Consequently, it was suggested that interference between the emulsifier and surface-active components of the milk fat may occur. This idea has been enhanced by the fact that the viscosity of the samples containing milk fat increased as the concentration of emulsifier increased at a given sugar concentration (70 wt %). On the other hand, we observed that the viscosity of cocoa butter, palm kernel oil and fat mixture samples decreased as the surfactant was added. Therefore, the presence of minor entities present in milk fat which are not present in the others oils, counteracts the action of the emulsifier and do not favour a reduction of the sample's viscosity.

In this chapter, we investigated the dispersion of highly concentrated sugar in oil samples. These were physically analysed by rheology. Analysis involved different research areas such as the purification stage with charcoal and the addition of emulsifier with phosphatidylcholine. We also tried to correlate our systems to rheological models described in the literature. Thus we observed that Einstein's relation was applicable to our samples up to a sugar dispersion of 0.16 %. As the samples got more concentrated, we used another rheological model, Casson's equation. We made some development towards this equation and tried to define the structure of our systems. We developed a mixing process which mimics the industrial chocolate processing at laboratory scale in order to compare the results with the laboratory scale ones. In the next section, we look at the effects of purifications by florisil and phosphorous pentoxide in highly concentrated samples with or without emulsifier. We also investigate the rheological effects of different emulsifier such as PC (Epikuron 200), PGPR (Polyglycerol polyricinoleate) and monoolein.

# *Chapter VI Discussion*

## *VI.1. Introduction*

For the past few years the legislation of chocolate has been improved and allowed the presence of vegetable fat in the composition of the final product. Consequently, a number of opportunities have been given to the food scientist for using these new oils and exploring their properties in comparison of cocoa butter. Our study was raised in the interest of understanding better the colloidal dispersions of sugar particle in common food oils. Past research directed by Cleasson *et al.* (1997) and Johansson and Bergenståhl (1992a) has attempted to characterise the interactions of sugar particles dispersed in oils by different methods. The main techniques which have been used in correlation to our project were based on the adsorption, sedimentation, confocal microscopy and rheology.

Our research is based on the comparison of eight different food oils with different degrees of purity. These oils were subjected to the presence of sugar particles and studied with the same four different techniques (adsorption, sedimentation, rheology and cofoncal microscopy). We investigated the effect of oil purification, surfactant addition, and particles concentrations into the systems. Oils were purified via activated charcoal, florisil or phosphorus pentoxide. In order to adapt the results to the industry the addition of surfactants proceeded. The following emulsifiers were considered: phosphatidylcholine (lecithin), polyglycerol polyricinoleate, pure monoolein, commonly used in the food industry. Furthermore we investigated the addition of free fatty acid (linoleic acid) and observed the physical properties of the sugar/oil dispersions.

## *VI.2. Adsorption*

Our study involved the comparison of the level of adsorption on several oils mixtures in the presence of one emulsifier: phosphatidylcholine. The concentration of emulsifier was proportional to the concentration of sugar present into the mixtures. Three separate concentrations were analysed through this technique: 10, 30 and 70 wt % sugar. In the adsorption field, several publications have related to the measurements of the surface interactions in oils. For example Cleasson *et al* (1997) gave an interpretation of the interactions present at the surface of mica plates in oil. They measured the adsorption by surface force measurements.

However, we measured first the surface activity method used with the De Noüy ring. This technique was limited for determining precisely the level of adsorption of emulsifier onto the sugar particles. We then identified the level of free fatty acids contained into each oil *via* potentiometric method. This analysis has shown that cocoa butter contained the largest amount of emulsifier into the system; whereas sunflower oil contained the least amount of free fatty acids.

However, more information on the oils' fingerprints were required to investigate the level of adsorption of the emulsifier and the presence of the minor impurities such as free fatty acids or diacylglycerols. After the separation of the sugar particles from the oils we analysed the oils via chromatography as described by Johansson and Bergenståhl (1992a). They showed that rate of adsorption can be determined by analytical method such as high pressure thin layer chromatography. However our analysis was based on the work of Beare-Rogers *et al.* (1992) who determined the quantity phospholipids present in oils *via* high pressure liquid chromatography (AOCS Official method Ja 7b-91); whereas Berner and Dieffenbacher (1999) determined the presence of different free fatty acids and diacylglycerols in the oil phase (AOCS Official method Cd 11d-96). With this analysis we observed that 10-20 % of the initial free fatty acid concentration was commonly adsorbed at the surface of the sugar particles. In the presence of emulsifier, we noted that the concentration of free fatty acid was significantly less adsorbed at the sugar surface. This would explain the behaviour of strong adhesion forces between the sugar particles and the emulsifier previously observed from Dedinaite and Campbell (2000). We noted that as the sugar concentration increased, the level of free fatty acid contained in the oil increased as well in most of the studied oils.

When determining the level of adsorption of 1,2 and 1,3 diacylglycerols in the systems, we observed that 1,3 was present in the oils in a larger quantity than the 1,2 diacylglycerols. In the presence of sugar, we noted that both diacylglycerols were slightly adsorbed at the surface of the sugar particles. With the addition of phosphatidylcholine, both diacylglycerols contents increase in the oil phase. This increase may be explained by the fact that diacylglycerols are removed from the sugar surface and replaced by the emulsifier. The result of this analysis can be assimilated to the result obtained with the free fatty acid experiment. However, no work has been previously reported on the specific effect of emulsifier on the replacement of free fatty acids or diacylglycerols at the sugar surface.

We estimated the level of phosphatidylcholine adsorbed at the surface of sugar particles from the AOCS Official method Ja 7b-91. We noted that one third of the emulsifier quantity has been adsorbed at the sugar surface. This remains independent from the type of oil analysed and both samples made at 70 and 30 wt % sugar show the same level of emulsifier adsorption. From this result the surface coverage of the emulsifier is estimated of 1.6 mg $m^{-3}$. We defined our rheological model as a dispersion of hard spheres, in which sugar particles were assimilated as apparent hard spheres. Considering this model, the emulsifier is adsorbed as a monolayer at the sugar surface as previously described on the publication of Johansson and Bergenståhl (1992a). They showed that the emulsifiers adsorb strongly as packed monolayers to the sugar particles. Moreover, Dedinaite *et al* (1998) also studied the forces at the surface of the sugar particles. They noted that the emulsifier which would adsorb in a monolayer form on mica plates adsorbs differently at the surface of the sugar particles. They observed that in the presence of sugar, the emulsifier covers the particles more than a monolayer. They assimilated this behaviour to phase separation due to the irregularity of the sugar surface. This behaviour was not observed in the present case, as the sugar particles were considered as spherical structure. We also noted that the emulsifier quantity was adsorbed at the sugar surface independently from the studied oil.

At a microscopic level, the intermolecular forces of sugar particles can be reduced or further increased by the presence of surfactants in the oils. The level of adsorption of these surfactants can reveal the form of aggregates encountered in specific oils in presence of free fatty acids or diacylglycerols.

Consequently, we noted that the emulsifier was adsorbed as monolayer at the sugar surface as Johansson and Bergenståhl (1992a) described it before. However, it was not possible to differentiate the quantity of emulsifier adsorbed from one oil to the other. In the publication of Dedinaite *et al* (1998), we have seen that emulsifier is adsorbed either more than a monolayer at the sugar surface. Finally our study gives primary information on the adsorption scheme of the emulsifier on sugar particles. However further work in this field would be needed for determining exactly the surface coverage of the emulsifier on our sugar particle and the forces encountered between emulsifier and free fatty acids or diacylglycerols and thus by adding different concentration of surfactants into the dispersions.

## *VI.3. Sedimentation*

Research in sedimentation accounts mainly for the transport of heavy dispersions (Coulson and Richardson, 1991) and the way particles behave in a flow movement. This technique has not been often reported for the analysis of particles' interactions as both intrinsic properties of the particles and the media influence directly the technique (Coulson and Richardson, 1991; Walstra, 1996; Hiemenz, 1986). However, Johansson and Bergenståhl (1992a) characterised the interactions between sugar particles and fat crystals dispersed in three different oils. They also observed the volume of sediment in the presence of different surfactants and combined their results with the adsorption technique *via* high pressure thin layer chromatography (HPTLC). Their results were correlated between the two techniques. Our study further developed this technique by comparing different effects on sediment volume: purification of the oils, addition of emulsifier and free fatty acid. The experiments were performed on eight oils at low sugar concentration (10 wt %).

We showed that the purification of the oils via activated charcoal or florisil did not give the same response in the sediment volume. Florisil reduced significantly the sediment volume present in most of the oils. However, activated charcoal did not have a substantial effect on the sediment volume as Gaonkar (1989) described it. He showed the effect of oil purification *via* measurements of interfacial tension of oil/water systems.

We mainly focused our work on the sedimentation of oils without prior purification. The comparison of the eight systems featured three different types of aggregation: globular, intermediate and networked sediment packing showed in Figure 6.1.

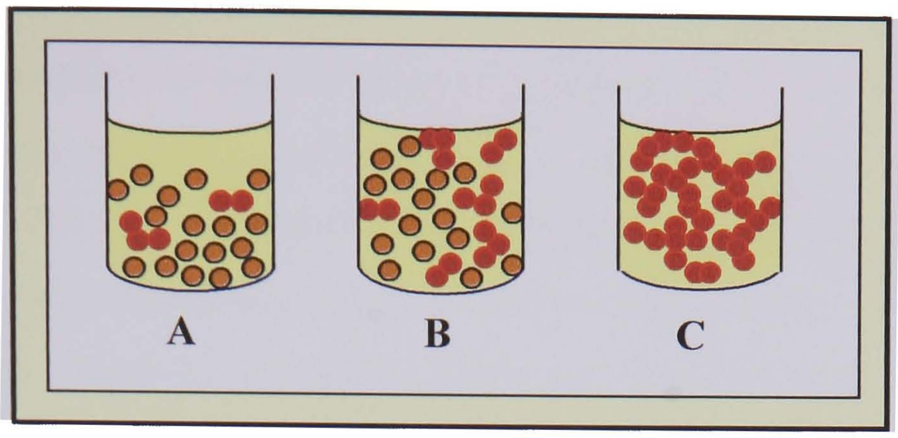


**Figure 6.1:** Representation of the three types of interaction encountered with sedimentation technique (in reference to chapter IV). The aggregation types are represented as globular, **A**; intermediate, **B**; networked, **C**.

The first type represents by the formation of small aggregates, which are easily disrupted and may have a globular shape. This case defined as the **A** diagram was observed for cocoa butter, fat mixture and stearin systems. Intermediate aggregations (**B** diagram) lead to percentage sediment volume contained within a range of 20 to 30 % volume and were encountered with sunflower oil, soybean oil and milk fat. Finally, sugar flocculated systems result in a packing of higher density of interactions (**C** diagram) in which aggregates may have an ordered rearrangement and does not allow much flow movement. Such type of aggregation occurred in the presence of palm kernel oil and olein.

We investigated the effect of different surfactants such as phosphatidylcholine and linoleic acid on the sediment volume. In the presence of both these surfactants, a reduction of the sediment volume was observed for most of the oils as reported in the work of Johansson and Bergenståhl (1992a). They explained that in presence of emulsifier, the sediments are more compact due to a reduction in the adhesion forces. The results have shown a limiting minimum volume of V = 0.9 mL in the samples in the presence of emulsifier. Based on the assumption of our rheological model as a dispersion of hard spheres in a continuous oil phase, we estimated the minimal close packing of particles of 16 % sediment volume which is a characteristic value for a mono-disperse spheres distribution.

We also showed that the addition of linoleic acid has a positive effect in reducing the sediment volume. However the sediments were less compact than the one obtained with the addition of emulsifier. By adding multiple surfactants to our systems. more stability was gained when adding phosphatidylcholine and linoleic acid together. rather than for the addition of a single surfactant, and the sediment was even more compact. Our results suggested that the sugar surface several binding areas which can bind to different surfactants for providing further more stabilised sugar in oil dispersions.

We investigated the effect of different types of emulsifiers with PC, PGPR, (polyglycerol polyricinoleate) and pure monoolein. PGPR and monoolein appeared to be respectively the most and the least effective emulsifier for dispersing sugar particles. These results are in agreement with the results obtained by Johansson and Bergenståhl (1992a). They showed that each emulsifier behaves differently from the other. Monoglycerides exhibit a large adhesion force resulting in a less compact sediment, as the emulsifiers interact in a specific way with the sugar particles. However, they showed that phospholipids lead to the formation of denser sediments and therefore further reducing the sediment volume due to low adhesion forces between the sugar particles. They noted that saturated monoglycerides would form a bulky sediment made of a network of sugar and fat crystal precipitates. Furthermore, we showed that in the presence of linoleic acid, no specific effect was observed on the sediment volume from one emulsifier to the other. Finally, the dependency of the oil nature and oil chemical composition with the sugar particles' interactions strongly influence the sedimentation results.

As a conclusion, we showed that it is possible to compare subjectively the aggregation of sugar particles into different oils with the sedimentation technique. Florisil has a better efficiency in the oil purification in comparison with activated charcoal as shown by Gaonkar (1989). We observed that emulsifier strongly influences the sediment of sugar particles in comparison with free fatty acids as previously described by Johansson and Bergenståhl (1992a). Moreover, adding multiple surfactants to the systems lead to a further stability in the sediment packing. Depending on the type of emulsifier present into the system, we showed that each emulsifier behaved differently from the others,which has already been seen in the work of Johansson and Bergenståhl (1992a). We decided to correlate our results with the rheology, partly developed in the correlation section.

## *VI.4. Rheology*

In this project we attempted to characterise the flow properties of our dilute and concentrated systems to rheological models. The determination of the flow properties of particles dispersed into a media has been well established with the rheological technique (Dickinson, 1992). The theoretical background of our systems has been defined in terms of Einstein and Casson equations (Chevalley, 1991; Dickinson, 1992; Nelson and Beckett, 1999). The results showed that for diluted dispersions the Einstein model fits the viscosity of our samples well up to a volume fraction of $\phi = 0.16$ as explained by Dickinson (1992) who stated that the Einstein model is applicable for diluted suspensions made of rigid, spherical particles dispersed into a liquid phase. However, he stated that this equation is only applicable as long as the large particles do not interact between each other and the Brownian motion is negligible. Our investigations showed that without the presence of sugar particles, the addition of emulsifier has no specific effect on the viscosity of the oils and Newtonian behaviours are observed. Above 16% volume fraction, the experimental viscosities of our samples are all above the theoretical models. These results are in agreement with Dickinson (1992) who explained that Einstein model equation allows a good determination of the viscosity of particles suspensions up to a volume fraction of $\phi = 0.15$. As this equation is limited to diluted dispersions, more rheological models have been developed for highly concentrated dispersions.

For highly concentrated dispersions we assimilated theoretically our systems to the Casson equation as previously done by Chevalley (1975, 1991), Dickinson (1992) and Nelson and Beckett (1999) who refer to the Casson equation for describing the viscosity of molten chocolate. The calculations raised from the Casson equation had exponent value for Casson viscosity and yield stress of 0.5 despite the fact that Chevalley (1991) reported that there are small differences with the viscosity of chocolate and the calculations on the Casson equation. Our calculations used an exponent value of 0.5 whereas his adaptation of this equation to the chocolate viscosity is done by changing the value of the exponent from 0.5 to 0.6. We further identified the influence of sugar concentration individually on the Casson slope and intercept at different sugar concentrations. We observed that both Casson parameters increase as the sugar concentration increases too as shown by Dickinson (1992) who stated that the Casson yield value and the Casson viscosity both increase as the fat content decreases in the mixture. The sugar particles aggregate in two specific ways: "globular" and "structured" aggregates. At low sugar concentration (~ 50 wt %), we assimilated the sugar particles structures as globular by characterising the Casson slope and the Casson intercept with extremely low values. As the concentration in sugar increased, the Casson slope and intercept values were very high and we assimilated this behaviour to a different packing of sugar particles known as the "structured" aggregate. All these results were obtained with the "***basic mixing method***" which allowed dispersions of sugar particles up to 60 wt % into the oils at variable shear stresses and rates. Moreover, Dickinson (1992) reported that this rheological model allows the determination of the viscosity within a large range of shear rates. Furthermore, the presence of emulsifier had substantial effect on the Casson intercept value and a limited effect on the Casson slope.

Recently, we analysed the industrial experiments by measuring the viscosity at two different shear rates in reference to the work of Servais *et al.* (2004) who presented a new method for analysing the viscosity of the chocolate. Our results (see Chapter VII) showed that the experimental viscosity corresponded approximately to the one exhibited at 40 $s^{-1}$ shear rate and no particular difference occured between the viscosity and the development of their technique which measures the viscosity at different shear rates: 5 $s^{-1}$ and 40 $s^{-1}$.

With the "*basic mixing method*", we observed that oils exhibited different ranges of viscosity in the presence of sugar. As the mixing method improved, for a better adaptability of the results to the industry, we noted that the same rheological pattern still existed between the oils. Palm kernel oil dispersions with both mixing methods lead to a high viscosity sample in comparison with all the other oil dispersions.

In the presence of emulsifier, we showed that most of the oils samples have drastic reduction in the viscosity of the system as observed by Chevalley (1975). He showed that different parameters affect the viscosity of chocolate, like the fat and emulsifier contents and the water content. Moreover, he emphasised that lecithin acts specifically on the viscosity of sugar/cocoa butter. However, fat mixture blend, made of cocoa butter and milk fat, did not seem to be affected by the presence of emulsifier. More analysis showed that the viscosity of milk fat samples increases as the emulsifier concentration in the mixture increases. These results disagree with the statement of Chevalley (1975) who said that the viscosity of the chocolate decreases as the fat content increases in the presence of emulsifier. He also specified that lecithin reduces substantially the viscosity of the chocolate and showed that there is an optimum concentration of emulsifier (0.3 %) which gives the lowest viscosity of the chocolate and with the addition of more emulsifier to the system, the chocolate thickens further. From this point, we considered that there was competition between the emulsifier and milk fat on the aggregation of sugar particles within the oil. We developed this further, by determining the effect of different stage addition of the emulsifier onto fat mixture samples. The addition of emulsifier prior to the one of milk fat had a beneficial effect on the viscosity of the blend; whereas the addition of milk fat prior the addition of emulsifier was not as effective. However the conjugative addition of both milk fat and emulsifier did not show any preponderant reduction on the viscosity of the sample. These results have shown that there was competition between the emulsifier and the milk fat.

We compared the influence of three emulsifiers on the dispersions of different sugar/oil systems and noted that phospholipids were the most effective in reducing the viscosity. However, we observed that PGPR was the worst emulsifier and the viscosity of the monoolein samples were contained with the viscosities of the samples made with PGPR and phosphatidylcholine. These results are in agreement with the work of Johansson and Bergenståhl (1992b, c) who observed that phospholipids and saturated monoglycerides caused the largest effects on the viscosity of the sugar/oil blends by reducing it.

We also compared the influence of minor impurities on the viscosity of two oils samples and showed that the water content has great influence on the viscosity of sugar/oil dispersions as Chevalley and Johansson and Bergenståhl (1992b, c) already reported. Chevalley (1975) observed that the water content in chocolate had an effect on the chocolate viscosity, as the viscosity of the sample would increase as the water content increased; whereas Johansson and Bergenståhl (1992b, c) analysed their results by explaining that the interparticle interactions depend on van der Waals forces and water bridges. The set of experiments also showed that viscosity results depends of the oils properties as suggested by Schantz and Rohm (2005) who compared the efficiency of both lecithin and polyglycerol polyricinoleate (PGPR) on the viscosity of the chocolate. Their work reported that the viscosity of chocolate could be modified by adding a blend of emulsifiers rather than a sole ingredient addition or adding limited amount of emulsifiers and the gain of an optimum chocolate viscosity for the blend of 30 % lecithin and 70 % PGPR was detailed.

As a conclusion to this section, our results showed that Einstein and Casson equation can be used for the determination of the viscosity of diluted and concentrated dispersions as previously developed by Chevalley (1975, 1991), Dickinson (1992) and Nelson and Beckett (1999). The Einstein equation was valid up to $\phi = \sim 0.16$ in agreement with the work of Dickinson (1992). Moreover, we defined with the Casson analysis two aggregate states as the sugar concentration increase in the system. This experiment has shown that the Casson viscosity and yield stress increase with the sugar content as Dickinson (1992) notified it. The development which has been done through this equation has also clearly shown the effect of emulsifier on the Casson slope which has not been reported before. The addition of emulsifier has shown a viscosity drop at all the sugar concentrations. This result agrees with the statement made Chevalley (1975) however; we have seen that increasing the emulsifier content at a specific sugar concentration lowered down most of the sugar/oil dispersions in disagreement with Chevalley (1975) who stipulated that there is an optimum emulsifier concentration at 0.3 wt %. In the presence of emulsifier, phospholipids are better viscosity reducer that monoolein in adequacy with the statement made by Johansson and Bergenståhl (1992b). Moreover, our experiments showed that the presence of water in the sugar/oil systems gives higher viscosity than samples purified from water which was already observed in the work done by Johansson and Bergenståhl (1992b), Chevalley (1975) and Dedinaite *et al* (2000).

## *VI.5. Confocal Microscopy*

Finally, a method which has been investigated in this research relates on the confocal laser scanning microscopy technique. The use of this technique has been previously reported by Greespan *et al.* (1985) who investigated the staining properties of oils. It was also noted that microscopy technique has been already used for qualitative interpretations of particles dispersed in oils. However, nothing was reported the strength of interactions between particles with this technique.

The attempts made for characterising the strength of the aggregates interactions in the different oils systems were not significant between the different systems and at various sugar concentrations. Therefore, no further experiments with this technique were performed.

Consequently, confocal laser scanning microscopy does not permit to interpret the level of interaction of sugar particles between each others. This technique mainly accounts for qualitative analysis. Therefore the combination of this technique with other physical methods can may be interpret further the interaction of the sugar particle dispersed in the oil as previously developed by Hickin *et al.* (1985).

## *VI.6. Correlation between the Techniques*

Different results accounted for various effects have been mentioned within in each technique and through this thesis. We have attempted to describe the level of interactions of sugar particles between different oils, under the effects of purification, sugar addition, surfactants (emulsifier and free fatty acid) addition.

### ❧*Effect of purification*

The effect of purification has been mainly reported in the sedimentation and the rheology techniques. The results have shown that the purification via Florisil, which removes impurities in the oils, affects both techniques by reducing either the sediment volume or the viscosity of the samples. The research observations accounted for impurities such as water, free fatty acids and diacylglycerols. We have shown that the purification of oils and sugar can be further improved by exchanging florisil by phosphorous pentoxide.

### ✍*Effect of sugar particles addition*

The effect of sugar particles has been observed in the confocal microscopy, sedimentation and the rheology techniques. We noted that as the sugar concentration increases, the particle packing increases and it has been observed qualitatively with the confocal microscopy. Moreover, results have shown that oils behave differently from each other as the sugar concentration increases. This phenomenon has been seen with sedimentation and rheology technique mainly. However, it was not possible to correlate such results with the confocal microscopy as we could not differentiate the level of interactions between different oils at given sugar concentrations.

### ✍*Effect of emulsifier addition*

The effect of emulsifier has been cited in adsorption, sedimentation and rheology techniques. Results have shown that emulsifier, specifically phosphatidylcholine, adsorb at the surface of the sugar particles. This feature has been observed in the three techniques previously mentioned. The adsorption technique has shown that the level of adsorption is approximately of a monolayer coverage of the sugar particles. From this effect, the sediment volume and viscosity drop substantially in almost all the oils. Moreover, the presence of emulsifier in the sediment samples has shown that sugar particles are mono-dispersed in the oil samples. Furthermore and based on the work of Johansson and Bergenståhl (1992b) we have attempted to correlate that the sedimentation technique with the rheological technique. The rheological samples were made following the "*refined mixing method*" at a sugar concentration of 60 wt % sugar ($\phi = \sim 0.52$). The sugar concentration for the sedimentation technique was 10 wt %, this represents a volume fraction of approximately $\phi = 0.8$. The correlation between the two techniques is represented in Figure 6.2.

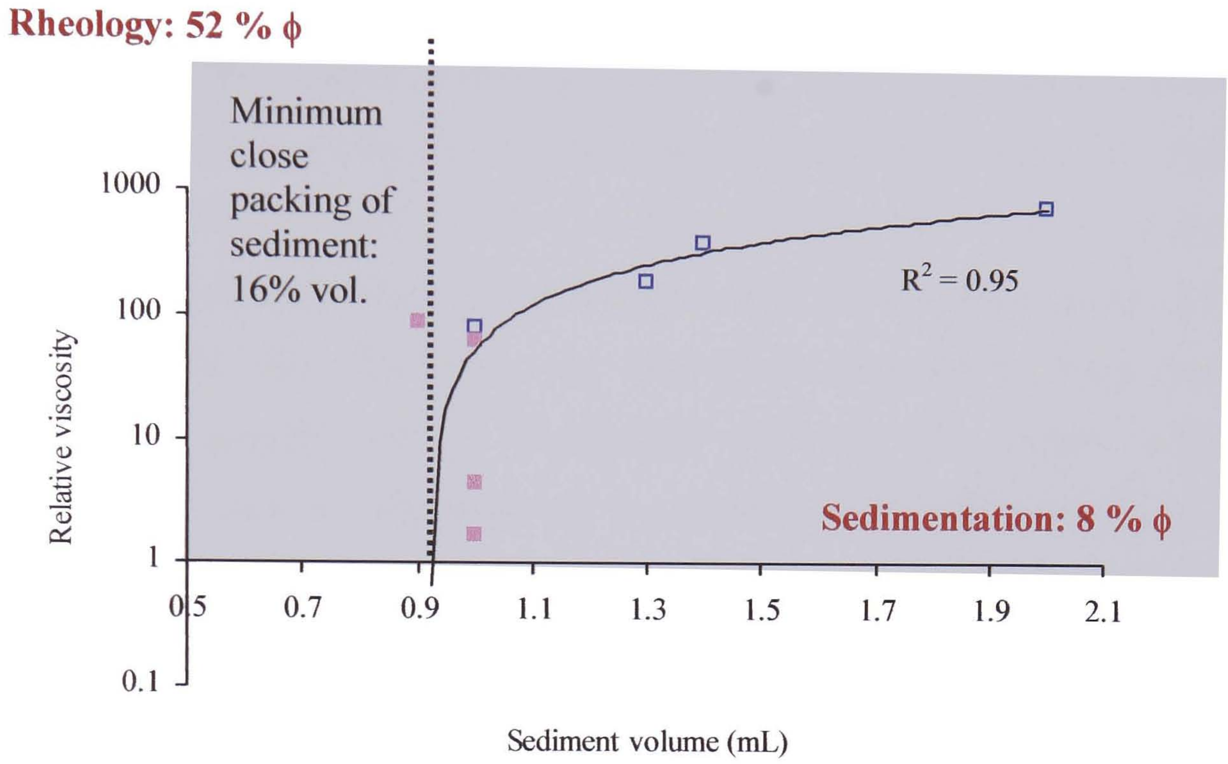


**Figure 6.2:** Correlation of viscosity and sediment volume for samples with or without added emulsifier (□) represents the samples without emulsifier and (▨) represents the samples with emulsifier.

Figure 6.2 shows a correlation coefficient of 0.95 between the two techniques. It can be observed that the minimum sediment volume (~ 0.9 mL) corresponds to a sediment packing volume of approximately 16 %. Assuming that our rheological model is made of hard spheres, this value is characteristic from the closely related volume fraction value (0.64) of the random close packing for mono-disperse spheres previously defined by Ansell and Dickinson (1986). In correlation with the results obtained by the adsorption technique, it may be possible to say that in the presence of emulsifier the packing of sugar particles consists of mono-disperse spheres surrounded by a monolayer of emulsifier.

### ☞*Effect of emulsifier and free fatty acids additions*

The effect of surfactant additions have been previously reported in the adsorption, sedimentation and rheology technique. The adsorption results show phosphatidylcholine tends to compete with free fatty acids already present in the oils and attached at the surface of the sugar particles. This result has also been noted with the rheology technique for the samples made with milk fat. The increasing addition of emulsifier seems to increases further the viscosity of the samples made with milk fat, whereas it generally further reduces the viscosity for all the other studied oils. Therefore, the correlation between rheology and adsorption for this specific subject is coherent with milk fat samples only. Moreover, we have seen in sedimentation experiments that adding linoleic to samples already containing emulsifier reduces further the sediment volume. Consequently this result does not correlate with the results obtained with the adsorption technique. In this specific experiment, we have shown that adsorption, rheology and sedimentation techniques were correlated.

This section has shown the correlation between the different studied techniques under subjects such as the purification of the oils, the addition of sugar and surfactants to the systems. We have shown that most of the techniques are well correlated between each others, especially in the case of sedimentation and rheology with 95 % correlation.

## *VI.7. Conclusion*

As a conclusion, it has been possible to compare the level of interactions of sugar particles dispersed into different oils through adsorption, sedimentation, confocal microscopy and rheology techniques. The results have shown different characteristics due to the oil purification, the sugar addition, the surfactant addition throughout the techniques. However, the observations made on the results of the different techniques have shown similarities and correlations between each other results.

With the adsorption technique, we showed that the emulsifier strongly bind at the sugar surface covering it as a monolayer. This behaviour was observed in all the studied oils. Furthermore, this technique has shown the partial removal of oil entities such as free fatty acids and diacylglycerols by phosphatidylcholine from the sugar surface has been observed. This feature, relating the competition of surfactants at the surface of the sugar particles has been also reported with the rheology technique.

The sedimentation technique gave subjective information on the interactions of sugar particles and this was well correlated with the rheological analysis. The addition of emulsifier favoured the compactness of the sediment in comparison with the addition of free fatty acids. Sugar particles were dispersed as mono-dispersed sphere in presence of phosphatidylcholine and this result was confirmed by the viscosity value corresponding to the viscosity of minimum close packing aggregates. Moreover, the sedimentation technique has shown that the addition of specific surfactants to the systems may enhance further the stability of the sugar/oil dispersions.

Finally, in the rheology section, we reported the close approximation of our results with two rheological models: Einstein and Casson equations for determining the viscosity of diluted and concentrated dispersions. It has been observed that the presence of surfactants into the dispersions lowered down the viscosity of the samples. This result was mainly observed with the presence of emulsifier rather than free fatty acids. However, the addition of designed emulsifier, such as phosphatidylcholine, polyglycerol polyricinoleate or monoolein, on sugar/oil dispersions has shown arbitrary results on the different studied samples. The most effective emulsifier was phosphatidylcholine and the worst one was polyglycerol polyricinoleate. These behaviours are already known as polyglycerol polyricinoleate is more effective in reducing the yield value rather than reducing the viscosity and inversely for phosphatidylcholine. Our study has also shown that the water, as moisture plays an important role in the aggregation and stickiness of sugar particles on the viscosity and the sediment volume of the considered samples. Consequently, the presence of surfactants in the dispersions of sugar/oil systems has a great importance on the stability of the suspensions.

# *Chapter VII Industrial Scale Application*

## *VII.1. Introduction*

In this final section of the project, we have investigated the effect of the sequence of addition of emulsifier into fat mixture samples on an industrial scale. In our laboratory study, because of its potential importance industrially, we compared the effects of the addition of the emulsifier before the milk fat and the addition of the milk fat prior the emulsifier. We found that an increase in the concentration of emulsifier enhanced the viscosity of sugar/milk fat dispersions whereas it reduced the viscosity of all the other dispersions. Furthermore, we discovered an apparent competition between emulsifier and milk fat at the surface of the sugar particles. We noted in particular for the milk fat mixture samples that, if milk fat and emulsifier were added together, the obtained viscosity was very high. However, the addition of emulsifier prior to the addition of milk fat had a substantial effect in reducing the viscosity.

These effects were compared rheologically on the large scale using the new experimental method based on the work of Servais *et al.* (2004). Samples were prepared at two different fat concentrations. The particle size, the fat content and the moisture content were measured and compared, as these factors also have a very significant effect on the molten chocolate viscosity.

## *VII.2. Materials and Methods*

### *VII.2.1. Materials*

Precise materials specifications used for these experiments are not reported here for confidentiality reasons; instead generic names are used. The materials used were sucrose, spray-dried skimmed milk powder, milk fat, cocoa liquor, cocoa butter, vanillin and soya lecithin. The two different recipes were developed for this trial as summarised in Table 7.1.

**Table 7.1:** Percentage composition of the different chocolate recipes

| Ingredients | Recipe n°1 % | Recipe n°2 % |
|---|---|---|
| Sucrose | 53.5 | 53.5 |
| SMP spray dried (a) | 15.3 | 15.3 |
| Milk fat | 0.0 | 5.7 |
| Cocoa liquor | 16.9 | 16.9 |
| Cocoa butter | 7.7 | 7.0 |
| Vanillin | 0.0 | 0.0 |
| Lecithin (soya.) (b) | 0.7 | 0.0 |
| | | |
| Total solids (1st part) | 94.1 | 98.4 |
| Total fat (1st part) | 17.8 | 22.1 |
| Milk fat | 5.7 | 0.0 |
| Cocoa butter | 0.0 | 0.7 |
| Lecithin (soya.) | 0.0 | 0.7 |
| | | |
| Total solids | 100.0 | 100.0 |
| Total Fat | 23.6 | 23.6 |

*Notes:* (a) Spray-dried Skimmed Milk Powder (SMP); (b) lecithin extracted from soybean.

It should be noted that for recipe n°2 the addition of emulsifier was implemented at the beginning of the conching stage. In order to improve the fluidity of these mixtures, 2/3 of the emulsifier quantity was added at the beginning, while the remaining quantity was added at the last stage of conching. The process was developed in this way because the powdery mixtures were extremely dry and the mixing of ingredients into a paste was not possible without the presence of some emulsifier.

### *VII.2.2. Methods*

Each recipe was compared in terms of its final viscosity (after conching), its particle size (before and after conching), and its percentage moisture content. Viscosity was measured on a controlled-stress rheometer (Bohlin-Malvern instrument) and particle size was determined with the Mastersizer (Malvern instrument). The percentages of fat content and moisture content were analysed in a Nestlé UK quality assurance laboratory.

#### **VII.2.2.1. Preparation of samples**

Recipe n°1 involves the addition of lecithin prior to the milk fat. The process is summarised in Figure 7.1.

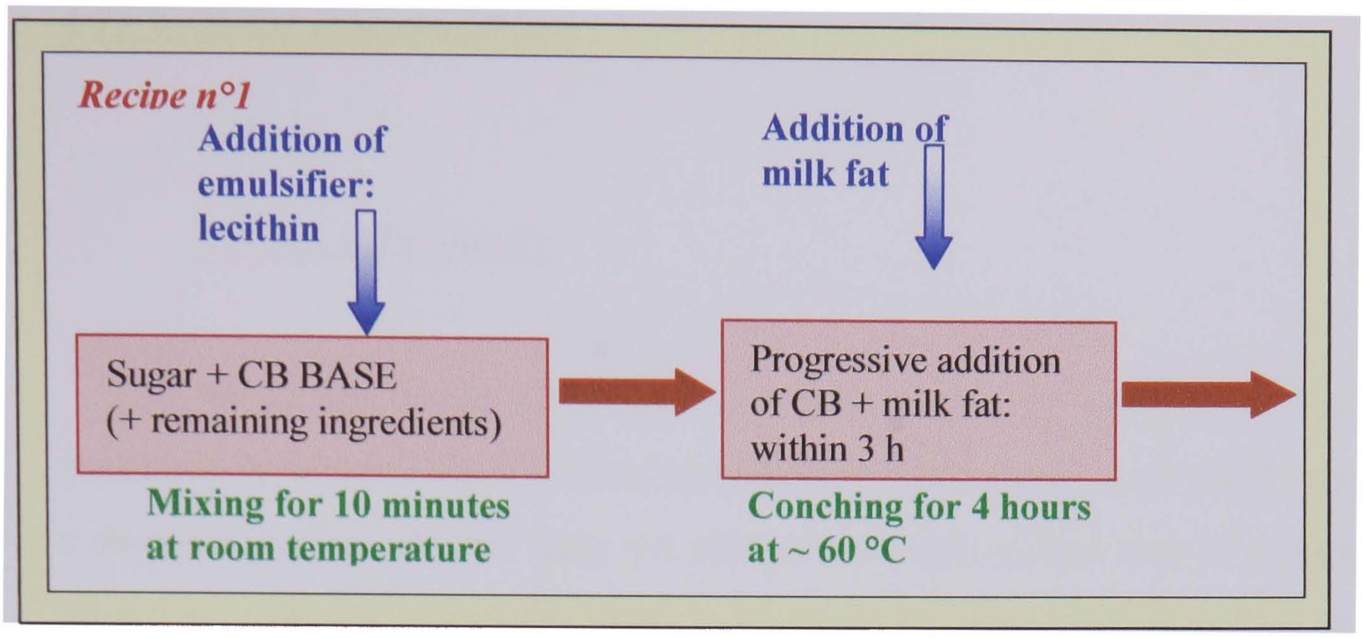


**Figure 7.1:** Summary of the sequence addition of emulsifier for the recipe n°1. CB signifies cocoa butter.

Recipe n°2 involves the addition of milk fat prior to the addition of emulsifier. A representation of this process is shown in Figure 7.2.

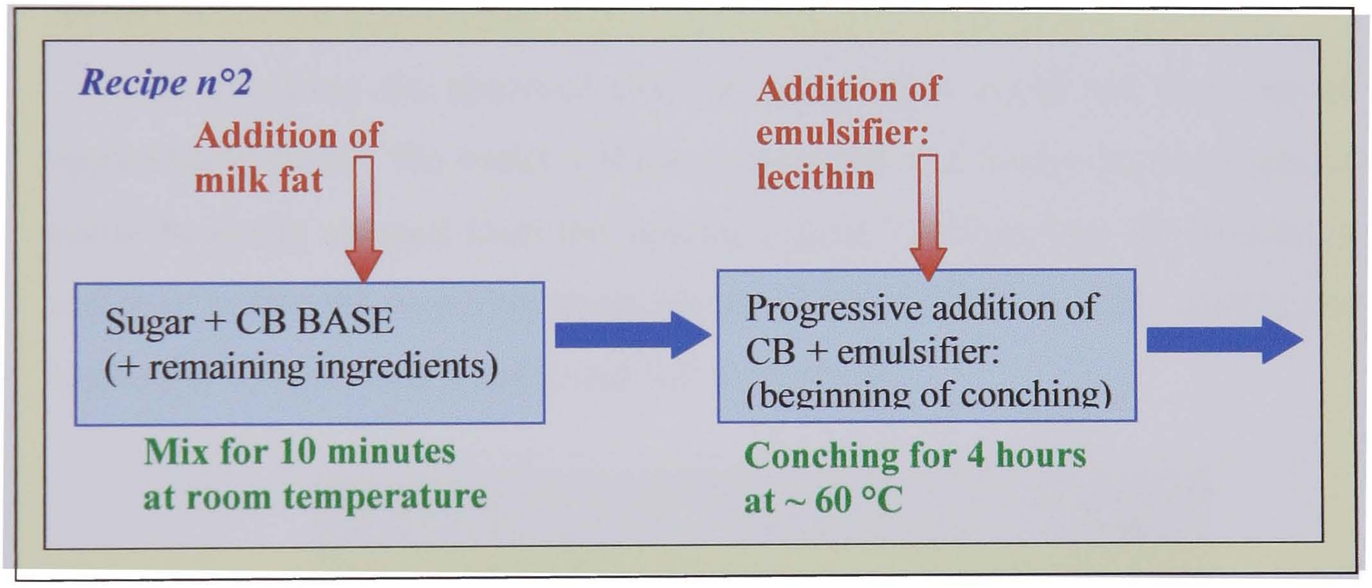


**Figure 7.2:** Summary of the sequence addition of emulsifier for the recipe n°2. CB signifies cocoa butter.

## *VII.3. Trial Observations*

### *VII.3.1. Recipe n°1*

This recipe was mixed with the Crypto (see Figure 1.8a, 1.8b), a peerless-food processor, to obtain a homogenous sample. This mixture was then passed through a three-roll refiner. At this stage we obtained an approximate size of particles of 35 ± 2 µm. The measured size of the particles had a value of D (v, 0.9) = 36 µm. This experimental value corresponds exactly to the set value for the mean size of the particles in this trial. Conching took place in the Lodigë (see Figure 7.3b) at a speed of 60 rpm, and, where milk fat was added after 3 h, there was a similar process to recipe n°1. By the end of the conching process, the speed of the blade rotation was increased up to 100 rpm to reduce the viscosity and degree of dispersion of the ingredients.

After conching we observed that our preparation could not flow out of the apparatus by itself. We noted that the preparation was lumpy but homogenous. It could be easily scraped from the conche. Figure 7.3 illustrates the fluidity of the recipe n°1 after the conching stage: (a) on release from the conche nozzle, and (b) inside the conche once opened and full of product.

**Figure7.3a:** Picture of the sample made according to recipe n°1 released from the conche into a metallic tray. The white arrow shows a lump of the melted chocolate falling out of the conche nozzle.

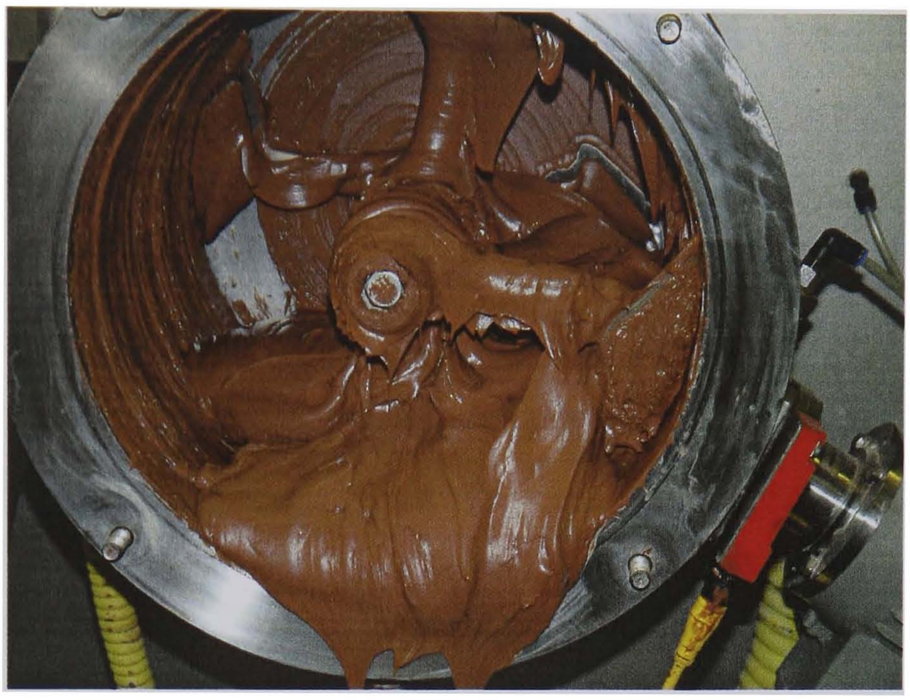


**Figure 7.3b:** Picture of the inner view of the Lodigë conche filled up with chocolate made according to recipe n°1.

In Figure 7.3b, we can see that the chocolate is well spread out in the conche. Moreover, we can observe the glossy state and the thick aspect of this preparation. The analysis of the particle size showed D (v, 0.9) = 38 μm. This value is slightly higher than expected (35 ± 2 μm), but this is mainly attributed to a sampling error, i.e., the initial particle size sample of recipe n°1 was probably not a representative sample of the mean particle size of the whole batch. Viscosity analysis was carried out with the Asci Viscometer. The apparent viscosity of the sample was equivalent to 24.2 Pa s for a shear-rate equivalent to 40 $s^{-1}$. The percentage fat content that we obtained from the analysis performed by the Nestlé UK quality assurance laboratory, 23.3 ± 0.2 %, was within the range fat content expected from the Table 7.1. The percentage moisture was about 0.8 % for our sample.

### *VII.3.2. Recipe n°2*

The ingredients were mixed in the Crypto and passed through a three-roll refiner. After refining the sample had a mean particle size of D (v, 0.9) = 33 μm. This size of particles was slightly smaller than expected, but it still lies within the range of the required particle size set for this trial. Therefore, it was decided to continue further with the procedure. It was decided to add approximately half of the total emulsifier quantity at the beginning of the conching as the mixture was initially extremely viscous. The other half was added one hour before the end of the conching process. The speed of rotation of the conching blades was set to 80 rpm for 3 h, and for the last hour it was increased up to 100 rpm in order to obtain a homogenous sample.

After conching we observed that our preparation could not flow out of the apparatus by itself. We noted that the texture of our sample was similar to the texture of recipe n°1 but even thicker and lumpier. The chocolate sample n°2 was homogenous and no powdery state was observed. It could be easily scraped from the conche. Figure 7.4 is a picture taken of the sample n°2 in the metallic tray.

**Figure 7.4:** Picture of the chocolate formed from recipe n°2 deposited in a metallic tray.

From Figure 7.4, we can observe that our sample had a glossy and shiny texture and contained no powdery regions or coarse lumps. The analysis of the particle size showed D (v, 0.9) = 34 μm after the conching. This value is contained within the range of the expected particle size. Viscosity analysis was carried out with Asci Viscometer. We obtained a viscosity equivalent to 33.8 Pa s for a shear-rate equivalent to 40 $s^{-1}$. For this sample the Nestlé UK quality assurance laboratory determined a percentage of fat content of 23.2 % which is contained within the range of expected value. The sample has a level of moisture equivalent to 0.9 %.

## *VII.4. Results*

### *VII.4.1. Rheology, Particle Sizing, Fat and Moisture Contents*

In these trials, different physical parameters have been analysed: viscosity at 40 $s^{-1}$, yield value at 5 $s^{-1}$, particle size, and the fat and moisture contents of the samples. All these measured parameters are summarised in Table 7.2.

**Table 7.2:** Rheological, particle-size and compositional data for the two different chocolate recipes

| **Parameters** | **Recipe n°1** | **Recipe n°2** |
|---|---|---|
| $\sigma$ (Pa) | 340.1 | 388.3 |
| NYS (Pa) | 34.0 | 38.8 |
| $\eta$ (Pa s) | 24.2 | 33.8 |
| NPV (Pa s) | 17.9 | 25.0 |
| Particle size [D(v,0.9)] (μm) | 38.3 | 34.2 |
| % fat content | 23.3 | 23.2 |
| % moisture | 0.8 | 0.9 |

*Note:* $\sigma$ represents the stress at 5 $s^{-1}$; NYS is the Nestlé Yield Stress (stress/10); $\eta$ represents the viscosity at 40 $s^{-1}$; NPV is the Nestlé Plastic Value (viscosity × 0.74). Results were given by Nestlé PTC York.

From Table 7.2, it can be noted that the NYS value of the recipe n° 1 sample is lower by approximately 4 Pa in comparison with the value for recipe n° 2. In the case of the NPV quantity, we observed that the value for recipe n° 1 lies below the value for recipe n° 2 by 7 Pa s.

From these results, it is reasonable to say that the recipe n°1 shows a reduction in the yield stress and viscosity values in comparison with the values obtained for recipe n° 2. As a conclusion to these results, the addition of surfactant such as lecithin as enhanced the fluidity and the yield stress of the chocolate samples. From these results, another analysis in order to observe the rheological profile of the two recipes at variable shear rate was undergone. The results are described in the following section.

### *VII.4.2. Rheology profile*

Two different types of measurements have been made in order to compare these two samples. One part of the experiment was carried out in the University of Leeds and the other part was done at the Nestlé PTC in York.

The experiments which have been carried out in the University of Leeds refer to a specific method developed and used by Nestlé (Servais *et al.*, 2004) for determining the viscosity of their samples at various shear rates. Figure 7.5 represents the viscosity of the two different recipes at variable shear rates (5-50 $s^{-1}$).

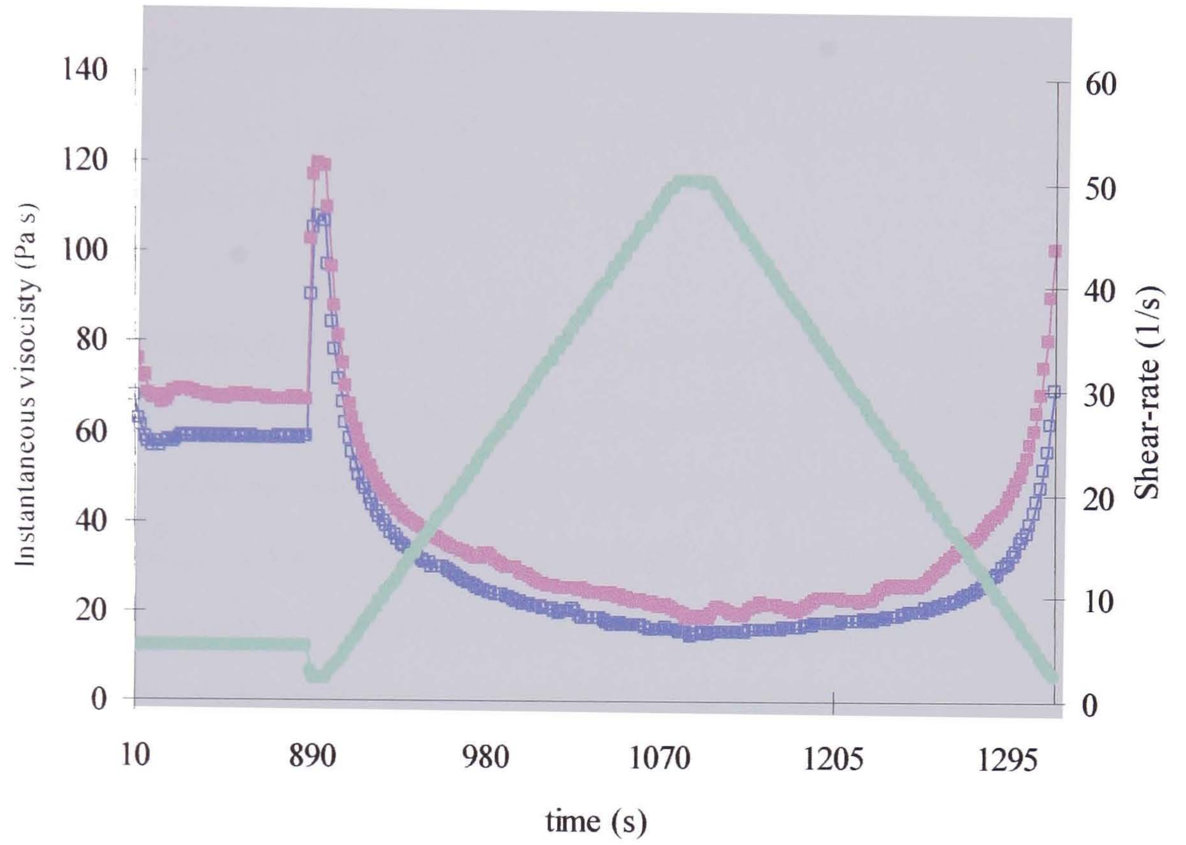


**Figure 7.5:** Viscosity measurements of the two different recipes at variable shear rates: recipe n° 1 (□), recipe n° 2 (■) and shear-rate ( ).

Figure 7.5 shows that recipe n° 1 gives a lower viscosity from the other recipe. By considering these rheological profiles and the information given in Table 7.1, we can observe that the recipe which generates the lowest viscosities is recipe n° 1. In reference to Table 7.1, we noted that chocolate from recipe n° 1 was prepared by adding the surfactant prior to the milk fat, whereas the recipe n° 2 was prepared by adding the milk fat prior to the surfactant.

As a result of this investigation, we have shown that the addition of a specific surfactant, soya lecithin, prior to the addition of milk fat has a positive effect on the subsequent reduction of the viscosity of the sample. The results can be confidently directly compared between chocolate from recipes n° 1 and n° 2 as they both contain exactly the same level of fat (23.2 %) in their samples.

## *VII.5. Conclusion*

This industrial scale trial has clearly showed that the step addition of surfactant into sugar/butter fat mixtures affects both the yield stress and the final viscosity of the samples. The lower viscosities were obtained when the surfactant was adding prior to the milk fat. On the other hand, the higher viscosities were observed when milk fat was added at the preliminary stage.

From a research point of view, these industrial results act as a confirmation of the results obtained in the laboratory-scale work on the sugar/milk fat mixtures involving the addition of surfactant at different stages of the mixing process. This result also adds weight to the importance of the hypothesis of competition between the presence of intrinsic and added surfactants. It is recommended that the effect that such surfactant competition may have on a real product rheology should be carefully considered when making milk chocolate.

# Chapter VIII Concluding Remarks

The aim of this project was to study the partial or total replacement of cocoa butter by a lauric fat. Thus by identifying and comparing the colloidal properties of sugar particles dispersed in food oils in chocolate. Practically, this was achieved by comparing the physical behaviours of eight oils and fats mixed with sugar and surface active entities under four techniques: adsorption, sedimentation, light microscopy and rheology. The main purpose of this thesis was to contribute to a better understanding to food engineers on the colloidal interactions encountered in such complex systems which is chocolate.

The potentiometric analyses carried out on the oils showed that cocoa butter contained the highest amount of free fatty acids amongst the other oils; whereas sunflower had the smallest amount. The adsorption technique has revealed that the presence of emulsifier into a sugar/oil system remove inner oil surfactants form the sugar surface. Three inner surfactants were studied: free fatty acids, 1,2 and 1,3 diacylglycerols. Furthermore, observations showed that a third of the emulsifier quantity added in the mixture was adsorbed at the sugar surface independently from the sugar concentration in the mixtures. The surface coverage of the sugar particle was estimated to 1.6 mg $m^{-2}$. This value refers approximately to the coverage of a monomolecular layer of emulsifier at the sugar surface.

The sedimentation technique allowed visual comparison of the interactions between sugar particles dispersed in the different oils. The purification of the oils was more effective with florisil in comparison with activated charcoal. The purification by sodium pentoxide was also effective in the removal of water at the surface of sugar particles. The addition of combined emulsifier and free fatty acid had the strongest effect in reducing the sediment volume in comparison with singular addition of emulsifier or free fatty acids. Singular additions of surfactant showed that the emulsifier reduces further the sediment volume rather than free fatty acids. However, these results are extremely dependent of the chemical nature of the oils studied and variability of the sediment volume amongst the oils is large. This study showed that sugar particles covered by surfactant was assimilated to mono-disperse sphere suspensions. However, the quantification of the interaction by this technique was not possible. Attempts were made with the light microscopy technique, and our results gave the same observations as the sedimentation technique.

The rheology technique was split in two parts: at low and high sugar concentration. At low sugar concentrations, oil purification experiments showed a reduction in the viscosity of the samples. The addition of emulsifier had a positive effect in reducing the viscosity of the sample with a predominant effect on palm kernel oil and low effect on cocoa butter samples. The Einstein model remained applicable up to a volume fraction of particles of $\phi = 0.16$. Above this volume fraction, Casson development was used to analyse theoretically the experimental results. The theoretical analysis showed three levels of sugar particles interactions in the different oils. Two main rheological behaviour were observed: "globular" interaction model up to 55 % sugar and a "structured" pattern above this sugar concentration. As the concentration of sugar increases, the process of mixing ingredients was improved: from one stage to two stage mixing process. Observations showed that palm kernel oil generates the highest viscosity amongst the other oils. The effect of different emulsifiers had a positive effect in reducing the viscosity with a predominant effect on palm kernel oil. However, the addition of emulsifier at different stage of the process has shown some counter interactions between emulsifier and composed fat influencing greatly the viscosity of the samples. This last point has been testified at a factory scale and experiments have shown the correlation between laboratory experiments and factory trials.

This project has identified the colloidal properties of sugar particles dispersed in oils. The main emphasis was given on oils susceptible to replace cocoa butter in the chocolate application. Comparisons between the oils and the effects of surfactants on the colloidal networks were analysed with four different techniques. However, further work could be done to extend this research field, by estimating and quantifying the interactions of different ranges of sugar particles dispersed in others oils with other techniques.

# *References*

Aquilano D., Sgualdino G., (2001), In: *Crystallization Processes in Fats and Lipid Systems* (N. Garti, Sato K., eds.) Marcel Dekker, New York, p 1.

Aguilar C.A., Ziegler G.R., (1995), *J Food Sci.*, **60**, 120.

Aronhime J. S., Sarig S., Garti N., (1988a), *J. Am. Oil Chem. Soc.*, **65**, 1140.

Aronhime J. S., Sarig S., Garti N., (1988b), *J. Am. Oil Chem. Soc.*, **65**, 1144.

Ayral D., (2001), *A passion for chocolate*, Cassell & Co., London.

Ansell G., Dickinson E., (1986), *J. Chem. Phys.*, **85**, 4079.

Babin H., Dickinson E., Chisholm H., Beckett S., (2005), *Food Hydrocolloids*, **19**, 513.

Barrera R. G., Duke C. B., (1976), *Phys. Review B*, **14**, 3695.

Beare-Rogers, J.L., Bonekamp-Naser, A. and Dieffenbacher A. (1992). *Pure Appl. Chem.*, **64**, 447.

Beckett S. T., (1999), *Industrial Chocolate Manufacture and Use*, 3rd edition, Blackwell Science, Oxford.

Beckett S. T., (2000), *The Science of Chocolate*, The Royal Society of Chemistry, Cambridge.

Bergström L., (1997), *Adv. Colloid Interface Sci.*, **70**, 125.

Berner D., Dieffenbacher A., (1999), *Pure Appl. Chem.*, **71**, 1983.

Bohlin Instruments, (2001), User Manual for Bohlin Rheometers, issue 2.0, Bohlin Instruments Ltd, Cirencester.

Bouzas J., Brown B. D., (1995), In: *Ingredients Interactions* (A. G. Gaonkar ed.) Marcel Dekker, New York, p 451.

Chaplin M., (2002), www.sbu.ac.uk/water/, South Bank Universtiy, London.

Chen J. Y., Fan Z., (2002), *Mater. Sci. Technol.*, **18**, 237.

Chevalley J., (1975), *J. Text. Stud.*, **6**, 177.

Chevalley J., (1991), *J. Text. Stud.*, **22**, 219.

Chevalley J., (1999), In: *Industrial Chocolate Manufacture and Use*, (S. T. Beckett, ed.) 3rd edition, Blackwell Science, Oxford, p 182.

Christie W. W., (1982), *Lipid analysis*, 2nd edition, Pergamon Press Ltd., Oxford.

Claesson, P. M., Dedinaite, A., Bergenståhl, B., Campbell, B. & Christenson, H. (1997) *Langmuir*, **13**, 1682.

Clark A.H., Ross-Murphy S.B., (1987), *Adv. Polym. Sci.*, **83**, 82.

Coe S. D., Coe M. D., (1996), *The true history of chocolate*, Thames & Hudson Ltd., London.

Considine D. M., ed., *Van Nostrand's Scientific Encyclopaedia*, (1983), 6th edition, Van Nostrand's Reinol, London, p 2447.

Coulson J. M., Richardson J. F., Backhurst J. R., Harker J., H., (1999), *Chemical Engineering Volume 1*, 6th edition, Oxford.

Coulson J. M., Richardson J. F., Backhurst J. R., Harker J., H., (1991), *Chemical Engineering Volume 2*, 4th edition, Oxford, p 174.

Dedinaite, A., Claesson, Per M., Campbell, B. & Mays, H. (1998), *Langmuir*, **14**, 5546.

Dedinaite, A., Campbell, B. (2000), *Langmuir*, **16**, 2248.

Dickinson E., (1992), *An Introduction to Food Colloids*, Oxford University Press.

Dickinson E., Stainsby G., (1982), *Colloids in Foods*, Applied Science Publishers, London.

Einstein A., (1906), *Ann. Phys.*, **19**, 289.

Fang T. N., Tiu C., Wu X., Dong S., (1995), *J. Texture Stud.*, **26**, 203.

Fang T. N., Zhang H., Hsieh T. T., Tiu C., (1997), *J. Texture Stud.*, **28**, 11.

Fennema O. R., (1996), *Food Chemistry*, 3rd edition, Marcel Dekker, New York, p17.

Ferraris C. F., (1999), *J. Res. Natl. Inst. Stand. Technol.*, **104**, 461.

Gaonkar A. G., (1989), *J. Am. Oil Chem. Soc.*, **66**, 1090.

Garti N., Sato K., (2001), *Crystallization Processes in Fats and Lipid Systems*, Marcel Dekker, Inc., New York.

Garti N., Yano J., (2001), In: *Crystallization Processes in Fats and Lipid Systems* (N. Garti, Sato K., eds.) Marcel Dekker, Inc., New York, p 211.

Goodwin J.W., Hughes R.W., (1992), *Adv. Colloid Interface Sci.*, **42**, 303.

Greenspan P., Mayer E. P., Fowler S. D., (1985), *J. Cell Biol.*, **100**, 965.

Gunstone F. D., (1997), In: *Lipid Technologies and Applications*, (F. D. Gunstone, F. B. Padley, eds.), Marcel Dekker Inc., New York, p 19.

Heathcock J.F., (1985), *Food Microstruct.*, **4**, 17.

Hemming F. W., Hawthorne J. N., (1996), *Lipid Analysis*, BIOS Scientific Publishers, Oxford.

Hiemenz P.C., (1986), *Principles of Colloid and Surface Chemistry*, 2nd edition, Marcel Dekker, New York.

Hicklin J.D., Jewell G. G., Heathcock J. F., (1985), *Food Microstruct.*, **4**, 241.

Hough D. B., White L. R., (1980), *Adv. Colloid Interface Sci.*, **14**, 3.

Hunter R. J., (2001), *Foundations of Colloid Science*, 2nd edition, Oxford University Press, Oxford.

Israelachvili J. N., (1992), *Intermolecular and Surface Forces*, 2nd edition, Academic Press, London.

Johansson D., (1995), *J. Am. Oil Chem. Soc.*, **72**, 1091.

Johansson D., (1994), *Colloids in Fats – The Fat Crystal as a Functional Particle*, Technology Licentiate Thesis, Department of Food Technology, Lund University and the Institute for Surface Chemistry, Lund.

Johansson D., Bergenståhl B., (1992a), *J. Am. Oil Chem. Soc.*, **69**, 705.

Johansson D., Bergenståhl B., (1992b), *J. Am. Oil Chem. Soc.*, **69**, 718.

Johansson D., Bergenståhl B., (1992c), *J. Am. Oil Chem. Soc.*, **69**, 728.

Kanai H., Navarrete R. C., Macosko C. W., Scriven L. E., (1992), *Rheol Acta*, **31**, 333.

Kaneko F., (2001), In: *Crystallization Processes in Fats and Lipid Systems* (eds. N. Garti and K. Sato), Marcel dekker, New York, p53.

Kawaguchi M., Okuno M., Kato T., (2001), *Langmuir*, **17**, 6041.

Krieger I.M., Dougherty T.J. (1959), *Trans. Soc. Rheol.*, **3**, 137.

Koyano T., Sato K., (2002), In: *Fats in Food Technology*, (K. K. Rajah ed.), Sheffield Academic Press, Sheffield.

de Kruif C. G., van Iersel E. m. F., Vrij A., (1985), *J. Chem. Phys.*, **83**, 4717.

Krüger (1999) in: *Industrial Chocolate Manufacture and Use*, (S. T. Beckett ed.), 3rd edition, Blackwell Science, Oxford.

Kyle D. J., In: *Food Additives* (2002), (eds. Branen, L. A., Davidson P. M., Salminen S., Thorngate III J. H)., Marcel Dekker, Inc., New York p 277

Larsson K., (1976), In: *Food Emulsions* (ed. S. Friberg), Marcel Dekker, New York, p 39.

Lipp M., Anklam E., (1998), Food Chem., **62** (1), 73.

Mackie A. C., Hey M. J., Mitchell J. R., (1989) In: *Food Colloids* (R. D. Bee, P. Richmond, J. Mingins eds), The Royal Society of Chemistry, London, p 172.

Medout-Marère V., (2000), *J. Colloid Interface Sci.*, 228, 434.

Minifie B. W., (1982), *Chocolate, Cocoa & Confectionery: Science & Technology*, The Avi Publishing Company, Westport.

Narine S.S., Marangoni, A.G., (1999), *J. Am. Oil Chem. Soc.*, **76**, 7.

Nawar W. W., (1996), In: *Food Chemistry*, (O. R., Fennema ed.), 3rd edition, Marcel Dekker, New York, p 225.

Nelson R. B., Beckett S. T., (1999), In: *Industrial Chocolate Manufacture and Use*, (S. T. Beckett, ed.) 3rd edition, Blackwell Science, Oxford, p 201.

O'Brien R. D., (2004), *Fats and oils: Formulating and Processing for Applications*, 2nd edition, CRC Press LLC, Boca Raton.

O'Brien R. D., (1998), *Fats and oils: Formulating and Processing for Applications*, Technomic Publishing Company, Lancaster.

Osipow L. I., (1962), Surface Chemistry theory and Industrial Applications, Reinhold Publishing Corporation, London.

Otterstedt J. E., Brandreth D. A., (1998), *Small Particles Technology*, Plenum Publishers, New York.

Padday J. F., (1969), In: *Surface and Colloid Science* (E. Matijević, ed.), Wiley, New York, p 39.

Pal R., (1999), *Ind. Eng. Chem. Res.*, **38**, 5005.

Patton T. C., (1979), *Paint Flow and Pigment Dispersion*, Wiley, New York.

Paul A. A., Southgate D. A. T., Mc Cance & Widdowson's edns, (1978), *Composition of Foods*, 4th edition, Elsevier/North-Holland Biomedical Press, Amsterdam.

Pawley J.B., (1995), *Handbook of Biological Confocal Microscopy*, 2nd edition, Lenum Press, New York.

Ponec V., Knor Z., Černý S. (1974), *Adsorption on solids*, Butterworth & Co, London.

Povey M. J. W., (2001), In: *Crystallization Processes in Fats and Lipid Systems* (N. Garti. Sato K., eds.) Marcel Dekker, New York, p 251.

Prentice J. H., (1983), *Measurements in The Rheology of Foodstuffs*, Elsevier Applied Science, London.

Quemada D., (1998), *Eur. Phys. J. AP*, **1**, 119.

Quemada D., Berli C., (2002), *Adv. Colloid Interface Sci.*, **98**, 51.

Quemada D., Flaud P. Jezequel P.H., (1985), *Chem. Eng. Commun.*, **32**, 61.

Rajah K. K., (2002), *Fats in Food Technology*, Sheffield Academic Press, Sheffield.

Rhodes M., (1998), *Introduction to Particle Technology*, Wiley, Chichester.

Rietveld I. B., Bedeaux D., (2001), *J. Colloid Interface Sci.*, **235**, 89.

Roscoe R., (1952), *British J. Appl. Sci.*, **3**, 267.

Ross-Murphy S.B., (1988), In: *Food Structure – Its Creation and Evaluation*, (Blanshard J.M.V., Michell J.R., ed.), Butterworth, London, p 387.

Sato K., Koyano T., (2001), In: *Crystallization Processes in Fats and Lipid Systems* (N. Garti, Sato K., eds.) Marcel Dekker, New York, p 429.

Sato K., Ueno S., (2001) In: *Crystallization Processes in Fats and Lipid Systems* (N. Garti, Sato K., eds.) Marcel Dekker, New York, p 177.

Schantz B., Rohm H., (2005), *Lebensm.-Wiss. U.-Technol.*, **38**, 41.

Servais C., Ranc H., Roberts I. D., (2004), *J. Texture Stud.*, **34**, 467.

Shaw D. J., (1992), *Introduction to Colloid and Surface Chemistry*, 4th edition, Butterworth-Heinemann, Oxford.

Sherman P., (1983), In: *Encyclopaedia of Emulsion Science*, (P. Becher, ed.), Marcel Dekker, New York, vol. 1, p 405.

Shukla V. K. S., (1995), In: *Developments in Oils and Fats*, (R. J. Hamilton, ed.), Blackie Academic & Professional, Glasgow, p 66.

Snabre P., Mills P., (1999), *Colloids Surfaces A: Physiochem. Eng. Aspects*, **152**, 79.

Stein R.A., Slawson V., (1966), *Prog. Chem. Fats*, **8**, 373.

Talbot G., (1999a), In: *Industrial Chocolate Manufacture and Use*, (S. T. Beckett, ed.) 3rd edition, Blackwell Science, Oxford, p 218.

Talbot G., (1999b), In: *Industrial Chocolate Manufacture and Use*, (S. T. Beckett, ed.) 3rd edition, Blackwell Science, Oxford, p 307.

Taylor R. J., (1965), *The Chemistry of glycerides*, Unilever Information Division, London.

Thomas D. G., (1965), *J. Colloid Sci.*, **20**, 267.

Timms R., (2003), *J. Sci. Food Agric.*, **83**, 1539.

Van der Hoeven Ph. C., Lyklema J., (1992), *Adv. Colloid Interface Sci*, **42**, 205.

Visser J., (1972), *Adv. Colloid Interface Sci.*, **3**, 331.

Walstra P., (1996), In: *Food Chemistry*, (O. R. Fennema ed.), 3rd edition, Marcel Dekker, New York, p 95.

Walstra P., Kloek W., van Vliet T., (2001), In: *Crystallization Processes in Fats and Lipid Systems* (N. Garti, Sato K., eds.) Marcel Dekker, New York, p 289.

Weiss T. J., (1983), *Food Oils and their Uses*, Ellis Horwood, Chichester.

Wildemuth C. R., Williams M. C., (1985), *Rheol. Acta*, **24**, 75.

Wille R. L., Lutton E. S., (1966), *J. Am. Oil Chem. Soc.*, **43**, 491.

Wood G. A. R., (2001), In: *Cocoa*, (G. A. R. Wood, R. A. Lass, eds.) 4th edition, Blackwell Science, Oxford, p 587.

# *Appendices*

**Figure IV i** Confocal pictures of × 500 magnification series for cocoa butter and palm kernel oil.

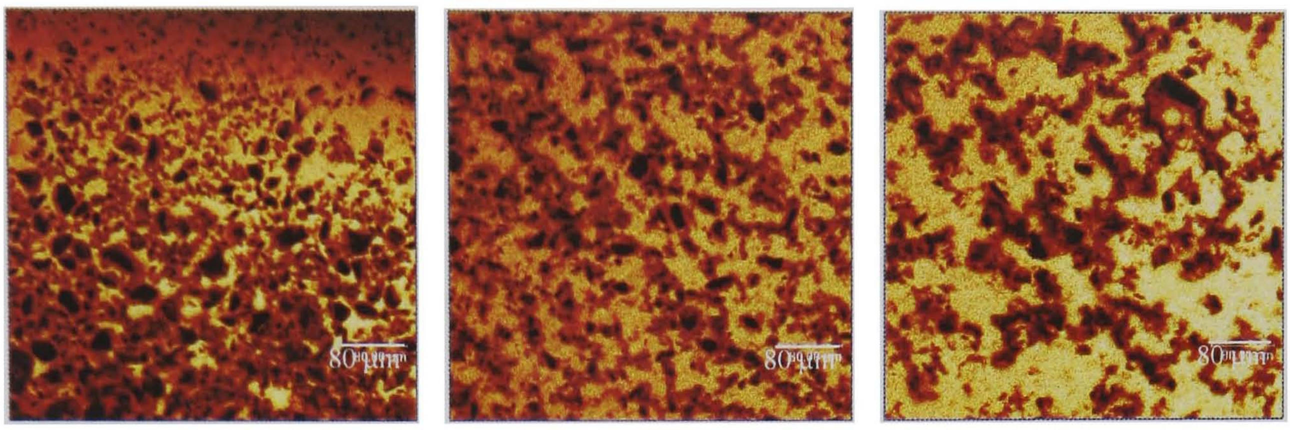
**Figure IV ii** Confocal pictures of × 300 magnification series for all studied samples.

**Figure V i:** Calculations of the volume fraction of 10% (w/w) sugar in sunflower oil, and comparison of the theoretical and experimental relative viscosities

**Figure V ii:** Calculations of the volume fraction of 10% (w/w) sugar in cocoa butter, and comparison of the theoretical and experimental relative viscosities

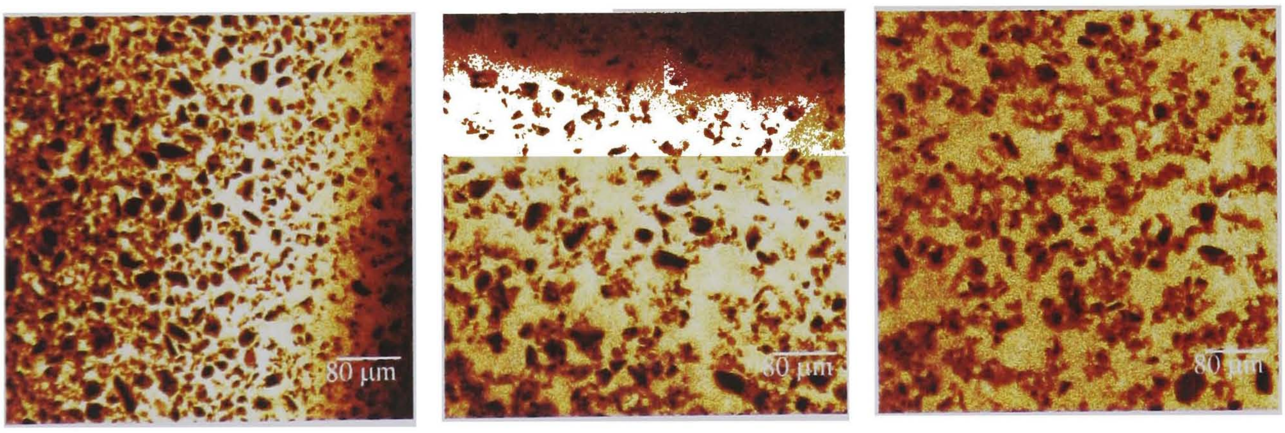
**Figure V iii:** Casson plots for the purified samples containing emulsifier (Epikuron 200)

**Figure IV i** Confocal pictures of × 500 magnification series for cocoa butter and palm kernel oil.



60 wt % 40 wt % 30 wt %

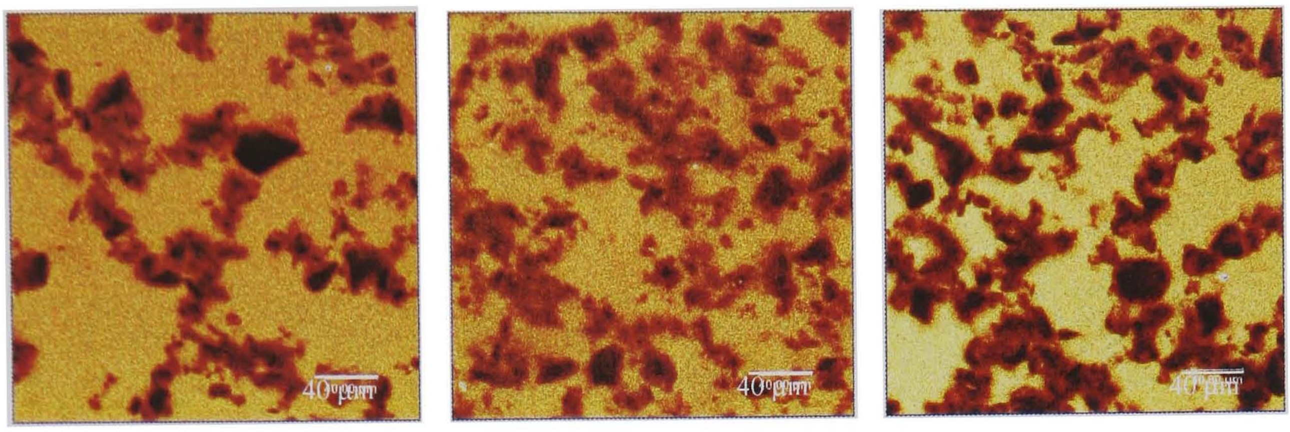
**Figure 4.1a:** Confocal microscopy images for 60, 40 and 30 wt % sugar particles dispersed in cocoa butter.



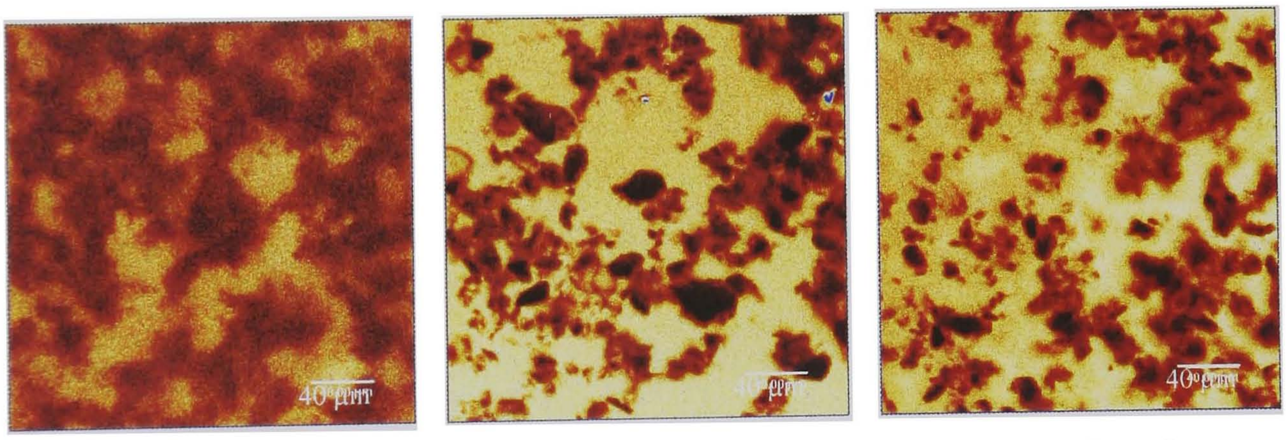
60 wt % 40 wt % 30 wt %

**Figure 4.1b:** Confocal microscopy images for 60, 40 and 30 wt % sugar particles dispersed in palm kernel oil

**Figure IV ii** Confocal pictures of × 300 magnification series for all studied samples containing 30 wt % sugar.



Sunflower Soybean Milk fat



Fat mixture Olein Stearin

**Figure V i:** Calculations of the volume fraction of 10% (w/w) sugar in sunflower oil, and comparison of the theoretical and experimental relative viscosities

**Table 5.1:** Determination of the volume fraction of sugar into sunflower oil

| **MASS OF SUGAR (G)** | Mass of oil (g) | Total mass (g) | Vol. sugar (mL) | Vol. oil (mL) | $\phi$(a) | $\phi^2$(b) |
|---|---|---|---|---|---|---|
| 2.00 | 18.00 | 20.00 | 1.63 | 19.78 | 0.0759 | 0.0058 |

*Notes*: (a) $\phi$ is the volume fraction of sugar particles in sunflower oil. (b) $\phi^2$ is the square root of the volume fraction of sugar particles in sunflower oil.

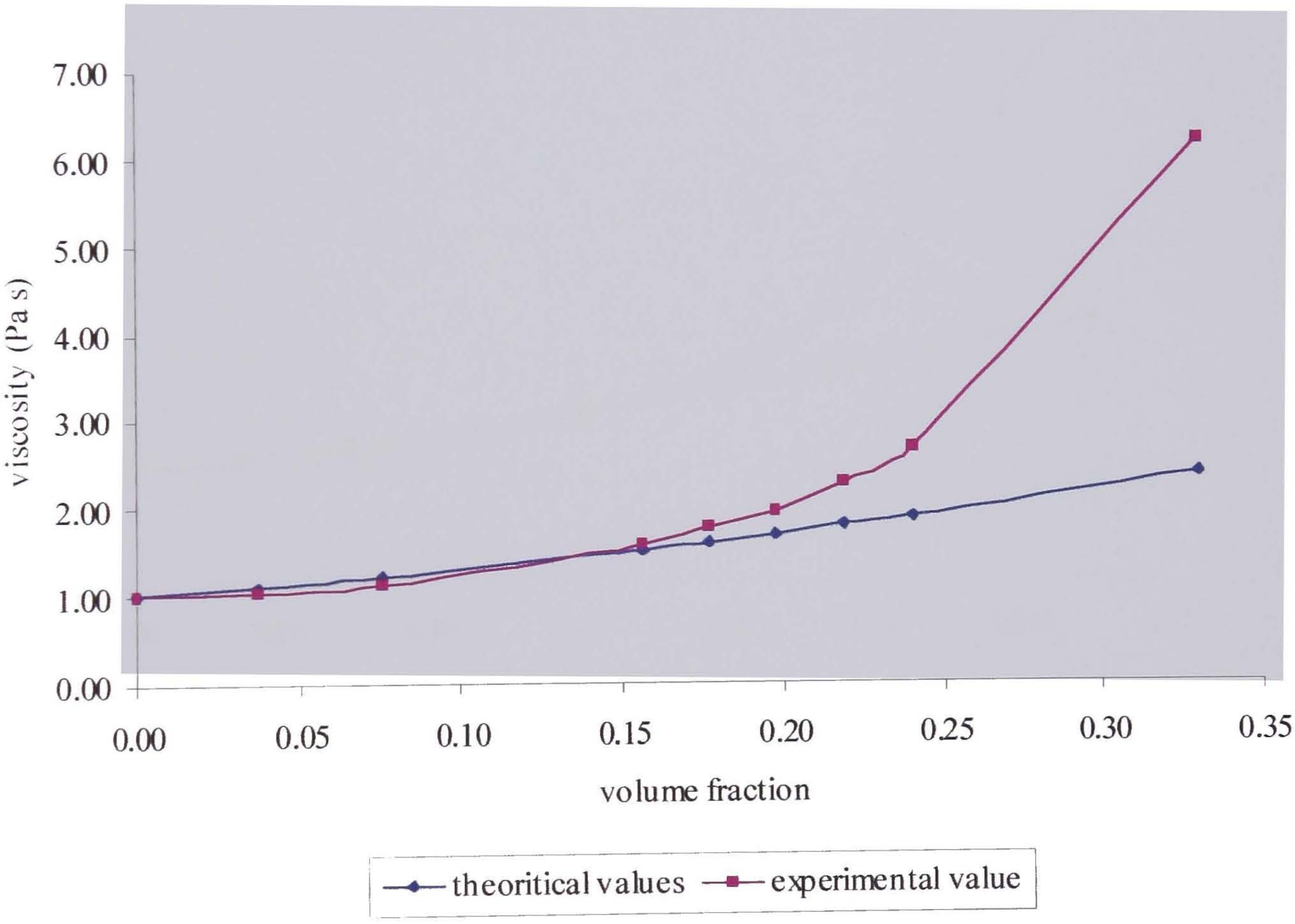


Figure 5.1: Comparison of theoretical (■) and experimental (■) relative viscosities of sugar dispersions in soybean oil 40 °C.

**Figure V ii:** Calculations of the volume fraction of 10% (w/w) sugar in cocoa butter, and comparison of the theoretical and experimental relative viscosities.

**Table 5.2:** Determination of the volume fraction of sugar into cocoa butter

| Mass of sugar (g) | Mass of cocoa butter(g) | Total mass (g) | Vol. sugar (mL) | Vol. cocoa butter (mL) | $\phi$(a) | $\phi^2$(b) |
|---|---|---|---|---|---|---|
| 2.00 | 18.00 | 20.00 | 1.63 | 20.22 | 0.0744 | 0.0055 |

*Notes*: (a): $\phi$ is the volume fraction of sugar particles in cocoa butter. (b) $\phi^2$ is the square root of the volume fraction of sugar particles in cocoa butter.

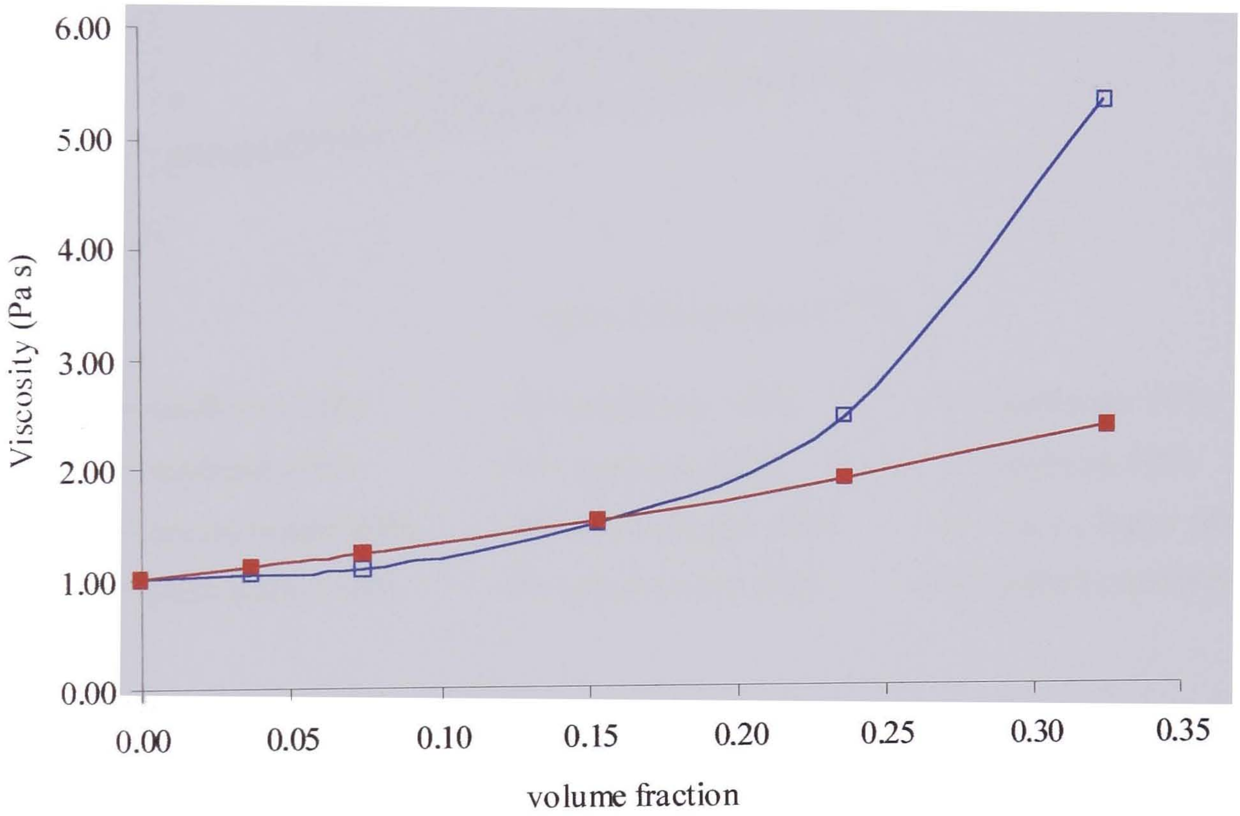


**Figure 5.2:** Comparison of theoretical (■) and experimental (□) relative viscosities of sugar dispersions in cocoa butter 40 °C.

**Figure V iii:** Casson plots for the purified samples containing emulsifier (Epikuron 200)