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Understanding the Effect of Formulation and Processing Parameters on Microstructural and Physical Properties of Ice Cream, Sensory Perception and Appetite

by

Gaia Rizzo

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College of Engineering and Physical Sciences
University of Birmingham
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ABSTRACT

Ice cream is a fatty, low satiating food which may increase obesity levels. This thesis aims to understand if it is possible to develop a more satiating product by manipulating formulation and/or processing.

First, the effect of two emulsifiers (mono and diglycerides, MDGs and Tween 80) on the thermal behaviour of a bulk fat matrix was investigated. MDGs were shown to possibly enhance ice cream fat destabilisation more than tween 80.

This research continued on an ice cream matrix, investigating the effect of different HLB (hydrophilic-lipophilic balance) number emulsifiers (MDGs based). Low and high HLB number emulsifiers (compared with intermediate HLB numbers) led to the formation of a more structured fat network. Moreover, the investigation of different solid fat content (SFC) blends and the aging step showed that 1) the fat network became more structured as the SFC increased (unless this was too high); 2) aging step could be avoided; 3) it is feasible to considerably decrease the SFC without affecting consumers' response; and 4) SFC had a predominant effect on palatability.

Finally, an appetite study allowed answering the research question of this work, demonstrating that formulation manipulation could be a promising way to reduce food intake (i.e. obesity levels).

To my family

"Rare sono le persone che usano la mente, poche coloro che usano il cuore, e uniche coloro che usano entrambi."

Rita Levi Montalcini (1909-2012)

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CHAPTER 1

Introduction

1.1 Background

In the UK, the ice cream market is reasonably robust. In particular, the consumers' expenditure on ice cream was approximately £1.13 billion in 2014, making 1.34% of all food expenditure (£85.78 billion) (Key Note, 2015). Ice cream is a well-established after-dinner treat all year long, but during summer time is consumed also as a snacking food product.

Ice cream is defined by the British Nutrition Foundation (BNF) as a fatty food (www.nutrition.org.uk) and, as all the fat-rich foods, it is energy dense and palatable although it is less satiating than carbohydrates and protein-rich foods. These characteristics may cause overconsumption, which eventually might lead to overweight issues and, in the worst scenario, to a more serious disease such as obesity, greatly influencing all aspects of an individual's life. In fact, obesity is linked to a variety of co-morbidities such as heart disease, stroke, type 2 diabetes and even some cancers. These health problems cannot be underestimated and untreated because they can derive in more serious consequences including death. In addition to this, associated psychological problems (such as discrimination and low self-esteem) to which obese people are subjected cannot be disregarded.

Worldwide, obesity incidence has kept on rising since 1998, and the World Health Organization (WHO) has declared it a problem of epidemic proportions with dramatically increasing social and economic costs for countries (www.who.int/en/). Indeed, recent estimations by WHO shows that by 2030, 74% of men and 64% of women in the UK will be overweight, including 33% of women and 36% of men who will be obese (Breda, 2015). Estimated obesity costs were around £27 billion per year by 2015 and by 2050 it is foreseen

£49.9 billion per year (Butland *et al.*, 2007). Likewise, medical costs to treat obese individuals are approximately 50-80% higher than the ones for normal weight people (Tigbe *et al.*, 2013; Dobbs *et al.*, 2014).

Strategies to fight obesity have been varied; many countries such as Finland, France, Hungary and Mexico have adopted for example fiscal measures applying taxes on unhealthy foods and beverages in order to decrease overconsumption and subsequently obesity. In the UK the government has not opted for this approach yet. Instead, food industries have been encouraged to improve consumer information and to reformulate products or produce "light" food products containing less high caloric ingredients (like fats). However, this is not straightforward because the macronutrients present in food products (i.e. proteins, carbohydrates and fats) and the interactions occurring among these within the food matrix, work as structural agents imparting texture and therefore influencing sensory properties. Removing or reducing one of these components usually leads to a considerable different microstructure and this affects palatability. In turn, a decrease in the palatability affects the food enjoyment and as a consequence the consumers' preferences.

A possible engineering approach may be to maintain the fat content constant while varying the product formulation and the processing parameters to produce tailored products with specific functionalities such as sensory perception and appetite feelings. This could potentially address consumption trends, and therefore obesity levels (Fig. 1.1). Nevertheless, this approach is challenging especially with a product like ice cream. Indeed, this is one of the most complex food products as it is made up of several phases (sol, foam and oil-in-water

emulsion) interacting with each other. The alteration in any of these phases has an impact on the others and consequently on the final product structure.

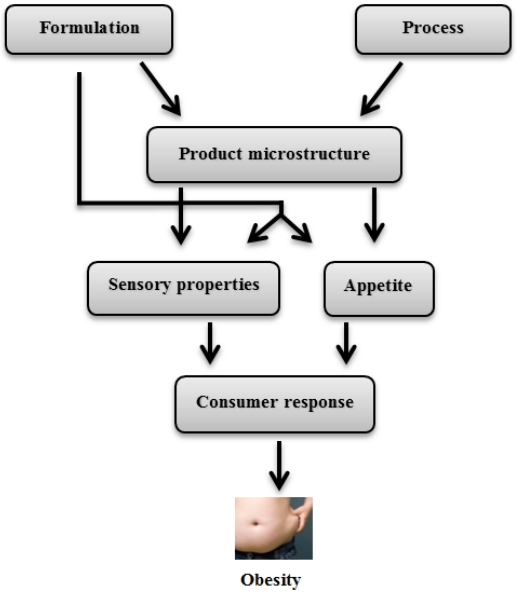

Fig.1.1 Influence of formulation and processing parameters on consumers' response and obesity.

1.2 Research aim and objectives

As partially defined in the previous section, the aim of the present thesis is to understand if it would be possible to develop food products where the amount of fat is kept constant (so that the acceptability would remain unchanged) while the formulation and/or the process are manipulated to produce functional products with enhanced satiety properties.

The specific research objectives of this thesis are:

1. To understand the effect of different formulations (i.e. emulsifiers and fat types) and processing (i.e. aging) on microstructural and physical properties of ice cream (e.g. fat network formation, hardness, overrun and meltdown behaviour);
2. To investigate the influence of these changes on sensory perception (because this can affect eating behaviour as it will be fully explained in section 2.3);
3. To study the effect of different formulation ice creams on appetite and food intake through an appetite study.

This is a novel area of investigation, because 1) factors considered in this study for formulation and processing have not been considered previously and help to understand better the effect of the manipulations made on microstructural and physical properties of the final product; and 2) it is acknowledged that this is the first study investigating the effect of a well-accepted dessert like ice cream on appetite under real conditions. Detailed novelties and any industrial relevance will be presented in each one of the individual result chapters of this thesis.

1.3 Thesis outline

This thesis has been arranged in seven chapters, four of which form the main body covering the experimental work carried out during this PhD. In particular:

- Chapter 1 (the present chapter) is the introduction of this thesis (highlighting the background of this research, the research aim and objectives, the structure of the thesis and the content of each chapter);
- Chapter 2 offers a review of the literature related to this study and allows identifying the areas requiring further research which have been investigated in this thesis;
- Chapters 3, 4, 5 and 6 are experimental chapters. Note that the experimental chapters are written in the form of scientific papers as the thesis is written following a publication style. In particular:
    - In chapter 3, the effect of two surfactants habitually used in the ice cream industry (Tween 80 and mono and di glycerides - MDGs) has been investigated on the thermal behaviour of a fat blend of 75% coconut oil and 25% sunflower oil. This particular fat blend has been chosen because it has been shown to mimic milk fat (in terms of fat crystallisation, partial coalescence development and meltdown behaviour) so that it is likely to be extensively used in the near future as a cheaper alternative to milk for the production of ice cream;
    - Since results in chapter 3 showed that MDGs have the potential to lead to a more structured fat network in comparison to Tween 80, these surfactants have been chosen to continue the research on an ice cream system. Therefore, in chapter 4 the effect of different mono glycerides varying in their hydrophilic–

lipophilic balance (HLB) number on microstructural and physical properties of ice cream was assessed. Also in this case, the chosen fat blend for the production of ice cream was made up of 75% coconut oil and 25% sunflower oil;

- Unlike chapter 4, where the surfactant type was varied while keeping constant the fat blend, in chapter 5 the surfactant was kept constant (DMG) while changing the fat blend used (different coconut oil to sunflower oil ratios). In addition to this, aging of ice cream mix was under scope in order to evaluate its effect on the final product;
- The ice cream produced with different fat blends (chapter 5) contained CO and SO (mainly composed of medium chain triglycerides and long chain triglycerides respectively). These fats may have a different effect on appetite and food intake, and therefore in chapter 6 some of these ice cream samples were employed in an appetite study to evaluate if an effect on food intake and appetite was present. This part of the research was performed in the School of Psychology at the University of Liverpool.

- Chapter 7 summarises the main outcomes of this research and proposes some future research work.

1.4 Research Dissemination

Poster presentation:

Rizzo G, Norton JE, Norton IT. Emulsifier effects on fat crystallization. Food Structure and Functionality conference, Amsterdam (Netherlands), April 2014

Publications:

- Rizzo G, Norton JE, Norton IT (2015). Emulsifier effects on fat crystallization. *Food Structure*; 4: 27–33

- Rizzo G, Masic U, Harrold JA, Norton JE, Halford JCG (2016). Coconut and sunflower oil ratios in ice cream influence subsequent food selection and intake. *Physiology and Behavior*; 164: 40–46

- Rizzo G, Norton JE, Gonzalez Espinosa Y, Masic U, Halford JCG, Norton IT (2016). Effect of solid fat content and aging on microstructural, physical properties of ice cream and sensory perception. *Journal of Food Science;* in preparation

- Rizzo G, Norton JE, Gonzalez Espinosa Y, Norton IT (2016). Effect of formulation on ice cream microstructure and physical properties. *Journal of Food Science;* in preparation

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www.nutrition.org.uk

www.who.int/en/

CHAPTER 2

Literature Review

This chapter aims to review the most relevant literature directly related to the present research. A general overview of ice cream microstructure will be given as well as information about how formulation and manufacture influence microstructure, the effect of microstructural changes and formulation on sensory perception and appetite and how appetite studies are conducted. Some relevant notions of fat digestion and metabolism will be also given at the end of this review to fully understand one of the experimental chapters.

2.1 Ice cream

Ice cream is a frozen food item, typically eaten as a snack or dessert in almost every country of the world. In the UK, the ice cream market is reasonably robust and dominated by small independent companies which produce and sell their ice cream locally, even if few large companies such as Unilever, Nestlé, Mars and Richmond Foods are also present (Clarke, 2004).

Ice cream energy and nutrient content varies with the type and the quantity of the ingredients used but it is certainly a high caloric (~200-300 kilocalories per 100 grams), a high sugar (12-16 wt%) and a high fat (10-16 wt%) food. For this reason, and how it will be fully explained later in section 2.4.3, this food exerts a weak effect on satiety and satiation compared with foods rich in proteins and carbohydrates (Johnstone *et al.,* 1996; Gerstein *et al.*, 2014; Karhunen *et al.,* 2008). Therefore, the consumption of ice cream can contribute to weight gain, overweight and even obesity, linked to a variety of co-morbidities (Lee, 2013). However, every cloud has a silver lining. Ice cream can be a beneficial product for example

for children growth, for increasing the body mass index (when necessary) (Goff & Hartel, 2013), and for elderly or hospital patients (Deosarkar *et al.*, 2016).

2.1.1 Ice cream microstructure

Ice cream is comprised of ice crystals, air bubbles and a network of partially coalesced fat globules. All these components are dispersed within an unfrozen phase of sugars, polysaccharides, proteins, and water (Goff, 1997). It is then clear that ice cream is at the same time an emulsion (fat droplets dispersed in a liquid matrix), a sol (ice crystals in a liquid matrix) and a solid foam (air bubbles dispersed in a solid matrix). These are colloidal dispersions (described in the next sections) whose interaction gives rise to a unique and scientifically fascinating microstructure. Goff and Vegas (2007) attempted to represent schematically this microstructure as depicted in Fig. 2.1 (Clarke, 2004).

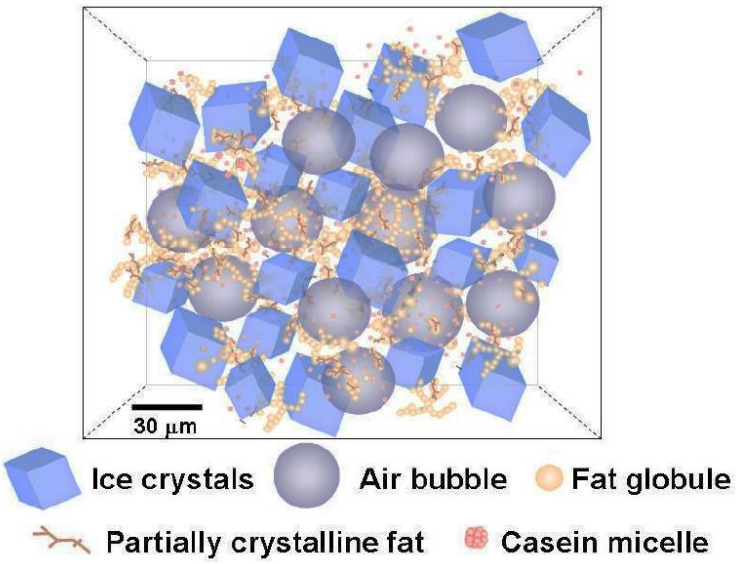

Fig. 2.1 Representation 3D of ice cream microstructure (from Goff & Vega, 2007).

2.1.1.1 Emulsions

Ice cream, such as milk or mayonnaise, is an oil-in-water (O/W) emulsion where oil is dispersed in water (McClements, 2009). Generally, emulsions are not stable systems due to the immiscibility of oil and water which cause a high interfacial tension at their interface (McClements, 2009). In fact, hydrogen bonds are created just among water molecules and not between oil and water molecules (Friberg *et al.*, 2004). Despite their immiscibility, if oil and water are mixed together the oil will disperse into the water in the form of small oil droplets (or fat droplets). When this happens, the oil surface area to volume ratio will increase and so also the surface free energy (as the surface free energy is the product of the surface area and the interfacial tension between oil and water). Therefore, the system will try to lower this energy by separating water and oil again (McClements, 2009).

It is possible to attain stable emulsions by using emulsifying agents, surface-active substances which absorb onto the fat droplets. These molecules reduce the free energy at the surface between the immiscible phases by 1) reducing the interfacial tension and 2) avoiding droplets aggregation (McClements, 2009).

There are a variety of emulsifiers, including small lipid-like molecules and proteins (complex biopolymers). Note that, in the present thesis and to avoid confusion, when different types of emulsifiers are present in the same discussion (such as the present chapter, chapter 4 and 5), the lipid-like emulsifiers are called surfactants. Thanks to the emulsifiers the interfacial tension will decrease and the emulsion will be consequently more stable (McClements, 2009).

In ice cream, the emulsified fat is destabilised during the freezing process (see later in section 2.1.3.5). The principal form of fat destabilisation in ice cream is partial coalescence even if flocculation and coalescence also happen. These destabilisation phenomena will be discussed in the following sections.

Flocculation. Fat droplets within an emulsion are in continuous movement (for instance because of gravitational forces, Brownian motion or employed mechanical forces) so that they collide with one another. After collision, depending on internal and external forces, the globules may separate or remain aggregated. The internal forces can be attractive or repulsive. Principally, the attractive forces are represented by the van der Waals forces, while the repulsive forces are electrostatic forces (because of the equal electric charges associated to the components at the fat droplets interface) or steric repulsion forces (caused also by adsorbed components). External forces are principally due to motion of the liquid (for instance because of mechanical shear applied) and are mainly responsible for the disruption of aggregates (McClements, 2009) (Fig. 2.2).

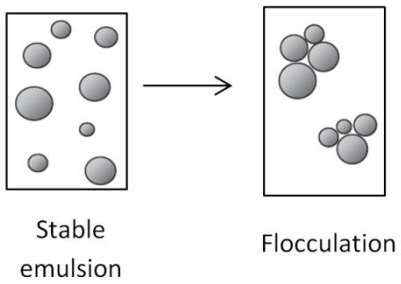

Fig. 2.2 Diagram representing flocculation (adapted from McClements, 2009).

Coalescence. If two droplets are very close for a sufficient time (for example due to flocculation) and they are mostly liquid, they can merge (coalesce) to form a larger globule

(Fig. 2.3). This is due to Laplace pressure: the pressure at the concave side of a rounded interface is higher than the pressure at the convex side and the fat droplets will move towards the lower pressure area (McClements, 2009).

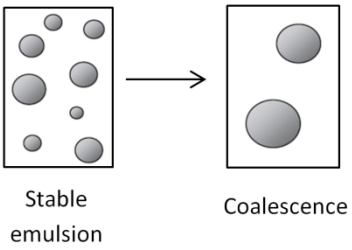

Fig. 2.3 Diagram representing coalescence (adapted from McClements, 2009).

Partial coalescence. Partial coalescence is a particular kind of coalescence occurring when the fat droplets are partially crystalline. In this condition, the fat crystals protruding from the fat droplets pierce other droplets resulting in their connection (Darling, 1982) (Fig. 2.4).

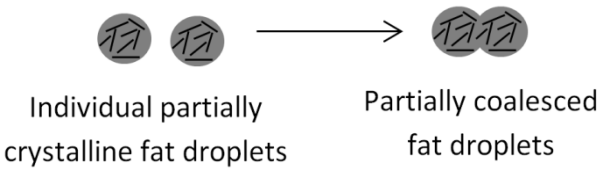

Fig. 2.4 Diagram representing partial coalescence.

Unlike coalescence, where the fat globules merge into a unique larger globule, the name *partial coalescence* comes from the fact that the fat globules maintain their original shape while they are connected by a semi-solid connection which avoids true coalescence. In fact, the protruding fat crystals pierce the surrounding fat droplets in their liquid portion and the

droplets remain aggregated. This happens because it is thermodynamically more favourable for the fat crystals to be in contact with oil than with water (McClements, 2016). It is then clear that, for partial coalescence to occur, the fat droplets need to be just partially crystalline, as both solid and liquid fat is essential (McClements, 2016). Thus, the solid fat content (SFC) is an important factor to consider; if the SFC is too low the droplets would undergo true coalescence, whereas if it is too high they would flocculate (rather than partially coalesce) because the hard droplets will not be able to merge (Walstra, 2003; Boode *et al.*, 1993; Boode & Walstra, 1993; Fredrick *et al.*, 2010).

Partial coalescence does not normally happen at rest, although it may happen if the droplets are packed closely. Instead, partial coalescence is usually initiated by phenomena which lead the fat droplets to collide (Walstra, 2003) such as shear forces and air incorporation. Air incorporation enhances partial coalescence by a surface-mediated mechanism: fat droplets collide during their absorption to the air/water interface (Hotrum, 2004).

Partial coalescence is also influenced by the properties of the fat droplet's interfacial layer. For instance, emulsifying agents that form thick interfacial layers (such as proteins) increase the resistance of the fat crystals to penetrate and so can decrease partial coalescence occurrence (Walstra 2003). In fact, various investigations have reported that when in O/W emulsions the fat droplets are covered by proteins, these are less prone to partially coalesce than those covered by smaller surfactants which form thinner interfacial layers (Palanuwech & Coupland, 2003; Thanasukarn *et al.*, 2004).

In ice cream, partial coalescence arises during the freezing step thanks to the shear applied and the air incorporation. This is an important phenomenon because the resulting fat network gives good stand-up properties to the final product, decreases its meltdown speed and increases creaminess during consumption (Hartel & Goff, 2013). In fact, direct relationships have been observed between the degree of partial coalescence and viscosity (Everett, 2007) and between viscosity and creaminess (Akhtar *et al.*, 2005).

2.1.1.2 Foams

Ice cream, as well as bread, is a solid foam (a dispersion of gas bubbles a solid continuous phase). In these systems the gas bubbles are separated by solid walls which make the structure more stable than liquid foams (where the continuous phase is liquid and the emulsifiers are essential for stabilisation). In ice cream, the gas phase volume is normally relatively low (~ 50%) so that the bubbles are not in contact resulting in spherical bubbles. In ice cream, the air incorporation is usually reported as 'overrun', the ratio between the volume of gas and the volume of continuous phase in percentage.

The most common method to produce a foam structure (especially in foods such as ice cream) is whipping, where air is incorporated in a liquid or semi-liquid thanks to the vigorous mixing. Industrially, air can be incorporated in a more reproducible way, for example by sparging air into the liquid (Clarke, 2004).

There are two factors influencing air incorporation: the presence of emulsifying agents and the viscosity of the matrix. The emulsifying agents stabilise the air bubbles with steric repulsion (i.e. it is more difficult for the air bubbles to coalesce forming larger bubbles); the

viscosity stabilises the air bubbles kinetically (in a viscous matrix, the air bubbles become less prone to migrate and exit the matrix, limiting overrun). Please note that if the viscosity is excessively high the agitation is difficult, resulting also in poor air incorporation (Clarke, 2004).

2.1.2 Ice cream composition

Ice cream composition is normally the following: 10-16 wt% of fat, 9-12 wt% of milk solids-not-fat (MSNF), 12-16 wt% of sweeteners, 0.2-0.5 wt% of stabilisers & surfactants and water (Marshall *et al*., 2003). By regulation in UK the term "ice cream" is applied to frozen products containing not less than 5% fat and not less than 2.5% of milk protein (not necessary in proportions found in milk) (http://www.legislation.gov.uk/).

All the ingredients present in ice cream have an effect on the final microstructure which, in turn, influences the physical properties of ice cream such as texture and meltdown behaviour. A summary of the ingredients used for the production of ice cream in this study as well as their effects on the properties of the final product will be presented in the following sections (Goff & Hartel, 2013).

2.1.2.1 Milk solids not fat (MSNF)

The milk solids not fat (MSNF) are a source of milk proteins. Proteins are important components in ice cream because, being surface-active (they are amphiphilic molecules formed by both hydrophilic and hydrophobic amino acids), they can stabilise the ice cream emulsion during emulsification and the air bubbles during their incorporation (see later in section 2.1.3.5). Moreover, they can improve the texture of the final product through viscosity

enhancement in the unfrozen matrix (even if the viscosity enhancement could have also a detrimental effect reducing air incorporation, as it was explained in the previous section). Last, they contribute to the characteristic dairy flavour of ice cream (Clarke, 2004). In this study skimmed milk powder was used as source MSNF. This is produced by pasteurising the whole milk and separating it into skimmed milk (0.1 % fat) and cream (48-50% fat) by centrifugation. The skimmed milk is finally spray dried to produce skimmed milk powder.

2.1.2.2 Sugar

A variety of sugars can be used in ice cream such as sucrose, honey, fructose, malt syrup, lactose and so on. In this study sucrose was used as source of sugar. Sugars are added in ice cream to enhance the sweetness and to control the softness of ice cream. In fact, as it will be explained later in in section 2.1.3.5, sugars decrease the freezing point of the mix reducing the amount of ice and consequently increasing softness. Sugars increase also the viscosity of the mix which could have both beneficial (increasing creaminess) and detrimental (reducing whippability) effects (Clarke, 2004; Goff & Hartel, 2013).

2.1.2.3 Stabiliser

Stabilisers are a group of biopolymers with incorporated hydroxyl groups which can form hydrogen bonds with water molecules so that, once dissolved, produce a high viscosity solution (Clarke, 2004). The principal function of the stabiliser in ice cream is keeping the water "locked", avoiding the development of bulky ice crystals and so of an icy texture during heat shock events (Marshall *et al.*, 2003). In this study the stabiliser used was Guar gum (E412) which comes from the seeds of *Cyamposis tetragonolobus*, an Indian annual crop. Guar gum has a backbone of mannose units, half of which have galactose side branches. The

free backbone regions side chains are small, resulting in hyper-entanglements which are the cause of the increase of the solution viscosity even at a very low concentration (Clarke, 2004).

2.1.2.4 Fat

The fat used for the production of ice cream can either be from diary sources (e.g. milk or cream) or from vegetable sources (e.g. sunflower oil, coconut oil, palm oil) which are more economical alternatives. The use of vegetable fats is common in the United Kingdom, Latin America and some area of North America. Vegetable fats can be unsaturated or saturated and they are often used as blends (Goff & Hartel, 2013).

In order to form a network of partially coalesced fat globules during freezing it is essential for the fat droplets, as previously mentioned, to contain liquid and solid portions at freezing temperature. In ice cream studies it was shown that using mixtures of 60–80% of solid fat (e.g. palm oil) and 40-20% of liquid fat (e.g. sunflower oil) the resulting ice cream presented the highest rates of partial coalescence. With higher percentages of liquid fat or solid fat there was instead a loss of structural integrity (Goh *et al.*, 2006; Crilly *et al.*, 2008; Sung & Goff, 2010). Moreover, milk fat fractions containing a high percentage of solid fat led to less fat destabilisation than that achieved in presence of fractions with a lower percentage of solid fat (Adleman & Hartel, 2001).

Most of the studies investigating the effect of solid and liquid fat content have employed milk fat fractions (as diary fat) or palm oil (as vegetable fat) as sources of solid fat. However, when adopting a vegetable fat, the use of coconut oil instead of palm oil would be beneficial for its higher concentration of medium chain fatty acids such as caprylic, capric, and lauric acid

(Gunstone, 2013) which have received considerable attention for their health benefits, as they suppress fat deposition enhancing thermogenesis and fat oxidation (Nagao & Yanagita, 2010). In addition to this, the use of palm oil is environmentally harmful as the tropical forests are being cleared to accommodate its plantations. This causes in turn 1) the soil-stored carbon to be released as carbon dioxide (CO_2) leading to global warming, and 2) the extinction of a variety of tropical animals. Moreover, the effect of different fat blends is not fully understood in terms for example of its actual SFC (measured, rather than based on the liquid and solid fat portions), stand-up properties of ice cream during melting, hardness, fat organisation at air bubble surface and most of all sensory perception. Therefore, this is an attractive and novel area to investigate, useful for both the scientific community and the industry.

2.1.2.5 Surfactants

In this thesis a surfactant is intended as a lipid-like emulsifier which comprises of a hydrophilic "head" which has a high affinity for water, connected to a lipophilic hydrocarbon "tail", that has a great attraction for oil (Walstra, 2003; Friberg *et al.*, 2004; Hasenhuettl, 2008a&b; Kralova & Sjoblom 2009). As aforementioned in section 2.1.1.1, this term is used in this thesis when different types of emulsifiers are present in the same discussion to distinguish them from other emulsifying agents (such as proteins). Food-grade surfactants are obtained industrially from different substances (e.g. fats, oils, glycerol, organic acids, sugars) (Hasenhuettl 2008a&b) and they can be found in different forms (e.g. liquids, pastes, solids, powders).

The main function of surfactants is the formation and stability on an emulsion (McClements, 2016). In fact, surfactants adsorb to the oil–water interfaces placing their hydrophilic head in

contact with water and their hydrophobic tail in contact with oil. This absorption decreases the thermodynamically unfavourable contact between the two immiscible phases, and so decreases the interfacial tension. Moreover, surfactants, as they surround the fat droplets, prevent their aggregation. Some surfactants form multilayers, which enhance even more the stability of the emulsion droplets (Friberg *et al*., 2004).

The interfacial membrane provided by some surfactants can undergo phase transitions (liquid–solid) with temperature variation (Walstra, 2003); with high temperatures, the hydrocarbon chains exhibit a great molecular mobility and can be described "fluid-like". On the other hand, with low temperatures the hydrocarbon chains lose their mobility and can be considered more "solid-like" (McClements, 2016).

Various surfactants classification systems exist (Dickinson & McClements, 1996; Salager *et al.*, 2005; Israelachvili, 2011) including the hydrophilic-lipophilic balance (HLB) concept (Pasquali *et al.*, 2008). The HLB is represented by a number (based on the surfactant's molecular properties) which indicates the surfactant's affinity for the oleos and aqueous phases. If a surfactant has a low HLB number (3–6) is mainly hydrophobic, dissolves better in oil and it is employed to stabilise W/O emulsions. If a surfactant has a high HLB number (10–18) is principally hydrophilic, dissolves better in water and it is utilised to stabilise O/W emulsions. If a surfactant has a middle HLB number (7–9) has no inclination for oil or water (McClements, 2016). The HLB number can be estimated knowing of the hydrophilic and lipophilic groups it contains as follows (Davis, 1994):

$$\boldsymbol{HLB = 7 + \sum(hydrophilic\ group\ number) - \sum(lipophilic\ group\ number)} \qquad \textbf{Equation (2.1)}$$

In ice cream, emulsifying agents are required because, being surface-active substances, they absorb onto the fat droplets reducing the free energy at the surface between the immiscible phases (by reducing the interfacial tension) and avoiding droplets aggregation (McClements, 2009). Ice cream formulations contain mainly two emulsifying agents, proteins (complex macromolecules) and surfactants (lipid-like molecules). Surfactants are better emulsifying agents than proteins for a variety of reasons.

It is known that effective emulsifying agents must adsorb onto emulsion droplets quite rapidly (the faster the adsorption rate, the smaller the size of the droplets). The absorption is primarily dictated by the molecular weight of the emulsifying agents. Surfactants, possessing a lower molecular weight, have greater mobility through the bulk in comparison with proteins allowing for a more rapid adsorption.

Once absorbed, it is the interfacial layer formed by the emulsifying agents to stabilise the emulsion lowering the interfacial tension and avoiding coalescence. Proteins, upon absorption, are slower than surfactants to form an interfacial layer because of their re-alignment (they rearrange themselves to the most entropically stable state with the hydrophobic residues in contact with the fat and the hydrophilic residues in contact with the continuous phase). On the other hand, surfactants, having a simpler structure, allow for a more rapid interfacial layer formation. Moreover surfactants, thanks to their structure, can form a better packed interfacial layer than proteins, limiting the contact between the immiscible phases, i.e. lowering more the interfacial tension.

Once the interfacial layer has been formed, the Gibbs-Marangoni effect can cause emulsion destabilisation. In fact, when two droplets approach each other, the continuous phase is forced

out of the gap separating them. When this happens, the liquid drags some of the emulsifiers along the droplet surface, leading to the formation of a region (on the droplet surface) where the emulsifier concentration is low. This causes an increase of the interfacial tension which is thermodynamically unfavourable. For this reason, emulsifier molecules flow toward that region dragging some of the continuous phase along with them. This motion increases the fat droplets stability against coalescence. Surfactants, being more mobile than proteins at the interface can better contrast the Gibbs-Marangoni effect and so better stabilise the emulsion (McClements, 2009).

However, in ice cream there is a protein/surfactant mass ratio of ~12 so that during homogenisation the principal emulsifying agents are proteins, as it has been shown by measuring the absorbed protein at the fat droplets surface in many studies (Courthaudon *et al.*, 1991; Euston *et al.*, 1995; Euston *et al.,* 1996). This happens probably because proteins interact more strongly with the fat in comparison with surfactants during the emulsification process (Wilde, 2009). In fact, emulsification is normally carried out at temperatures above the surfactant melting point (~ 60 ºC in ice cream for instance), so that they are liquid-like at the fat interface as it has been showed by fluorescence (Clark *et al.,* 1989). After homogenisation (during cooling and aging), as the surfactants begin to crystallise and the hydrocarbon chains lose their mobility becoming more "solid-like", they can adsorb more strongly onto the fat droplets displacing the proteins (Clarke, 2004). For example, Goff *et al.* (1987) showed a higher quantity of absorbed proteins at the fat surface in unaged ice creams in comparison with aged ice creams. Also, Barfod *et al*. (1991) showed that the amount of proteins at the fat surface decreased during aging and that, in the presence of surfactants, the interfacial tension decreased proportionally with a decrease in temperature.

The mechanism of protein displacement (which will be explained in details in section 2.1.3.4) lead to a thinner and weaker interfacial layer between fat droplets making the probability of inter-penetration of the fat crystals (once formed during the freezing process) higher, and leading subsequently to partial coalescence and the formation of a fat network (McClements *et al.*, 1993; Goff & Hartel, 2013). Enhancing the fat network formation, surfactants increase the resistance to meltdown and enhance the shape retention. Moreover, surfactants improve whippability of the mix because of their action at the air bubble interface.

Most of the studies focussed on the effect of surfactants with different HLB number on ice cream properties have been addressed to understand their effect on fat aggregation and melting rates (Govin & Leeder, 1971; Lin & Leeder, 1974; Goff & Jordan, 1989). This topic has no longer been investigated even when the effect of the surfactants with different HLB number on other parameters such as hardness, shape retention, and overrun (i.e. air incorporation) is unknown. The focus, in most of the studies has been instead to comprehend the effect of the surfactants according their degree of carbon chain unsaturation (Granger *et al.* 2004, 2005; Méndez-Velasco & Goff, 2012).

2.1.3 Ice cream manufacture

The manufacture of ice cream is comprised of a variety of steps all of which contribute to the development of the product microstructure. In particular, the ingredients are combined together to form an ice cream mix, this is pasteurised, emulsified, aged and finally frozen (Goff & Hartel, 2013) (Fig. 2.5).

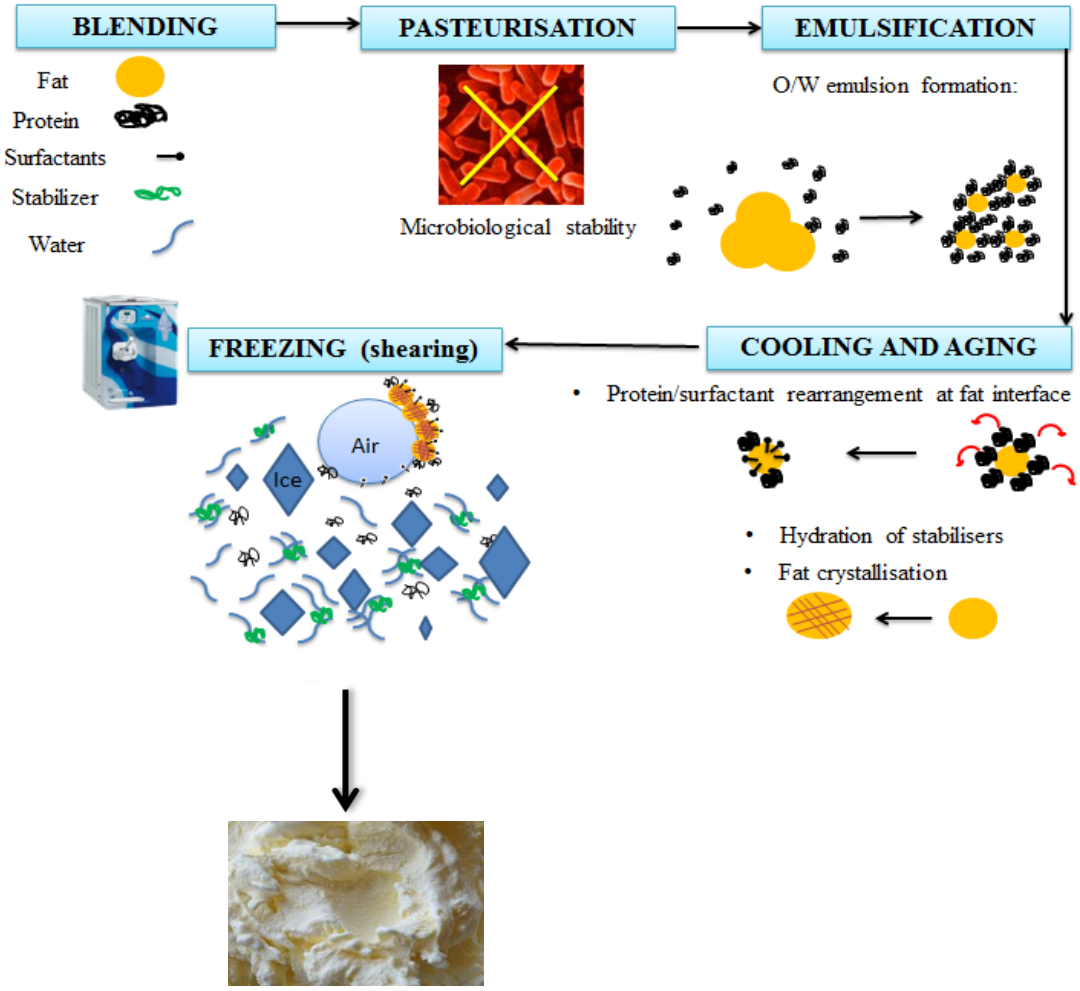

Fig. 2.5 Manufacture of ice cream and microstructure formation.

2.1.3.1 Blending of the ingredients

All the ingredients are mixed together with heat and agitation. Normally solid fats are melted prior the blending, liquid ingredients are incorporated first, followed by dry ingredients added slowly to prevent the formation of lumps. Since milk proteins, such as whey protein, denature at high temperatures, the mix should not be heated over 80 ºC (Goff & Hartel, 2013).

2.1.3.2 Pasteurisation

Once the ice cream mix is formed, this is pasteurised to reduce the number of pathogenic microorganisms to a level safe-for-human-consumption. Typically, two methods of pasteurisation exist: 1) batch pasteurization or low-temperature long-time (LTLT) in which a temperature of 66 ºC or 71 ºC is kept for 30 or 10 minutes respectively, and 2) continuous pasteurisation such as High-Temperature Short-Time (HTST - a temperature of 80 ºC is kept for 15 seconds) (Goff & Hartel, 2013).

2.1.3.3 Emulsification process

The pasteurised mix is then subjected to an emulsification step in order to achieve a stable uniform fat suspension by decreasing the fat globules size. Typically, an emulsified mix displays a fat droplet size distribution ranging between 0.1 and 10 μm. There are a variety of processes that can be employed to carry out the emulsification such as high-shear stator mixer like the Silverson® mixer (used especially on small scales) and homogenisation (used especially on larger scales) (Goff & Hartel, 2013).

Silverson® mixer consists of a perforated work-head containing some rotor blades. Firstly, the rotation of the rotor blades draws the suspension from the bottom of the container into the work-head. Inside the work-head the centrifugal forces pushes the suspension toward the work-head border where it is subjected to a grinding action. The suspension is then forced, at high velocity, out of the work-head through perforations. At the same time, fresh suspension is sucked into the work-head maintaining the circulation (Silverson® website) (Fig. 2.6).

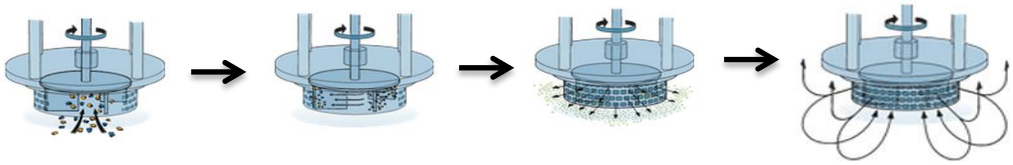

Fig. 2.6 Functioning of Silverson ® mixer (adapted from Silverson® website).

Despite of the differences between the various emulsification processes, all of them will lead to the reduction of the fat droplet size, the increase of the dispersed fat droplets number, and the increase of the fat surface area (Clarke, 2004).

2.1.3.4 Cooling and aging

Following the emulsification, the mix is cooled rapidly to about 5 ºC. During cooling, depending on the type of fat blend used, this can begin to crystallise (Goff & Hartel, 2013). The mix is then kept in the fridge (~5 ºC) about 16 to 24 h. This step is known as *aging*. During aging three phenomena take place; 1) fat crystallisation (depending on the fat blend used) which is essential, as already explained, for the formation of a fat network during freezing, 2) hydration of the stabiliser, and 3) reorganisation of molecules at the fat droplet interface (displacement of the proteins by the surfactants) (Gelin *et al.*, 1994) (Fig. 2.7).

The protein displacement by the surfactant is a multistep process studied in details by Morris and Gunning (2008). In particular, the proteins at the interface interact with each other forming a network whose defects can lead to the absorption of the surfactant. The surfactant domains will grow gradually causing first the compression of the protein network that initially remains connected at the interface but will progressively extend into the aqueous phase until it will be completely discharge into it (Fig. 2.8).

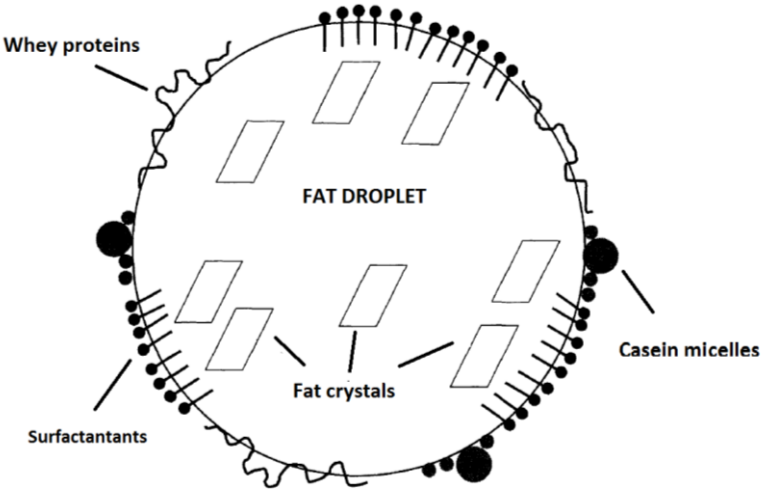

Fig. 2.7 A fat droplet during aging, showing the displacement of milk protein by the surfactant and the fat crystallisation (not drawn to scale) (adapted from Clarke, 2004).

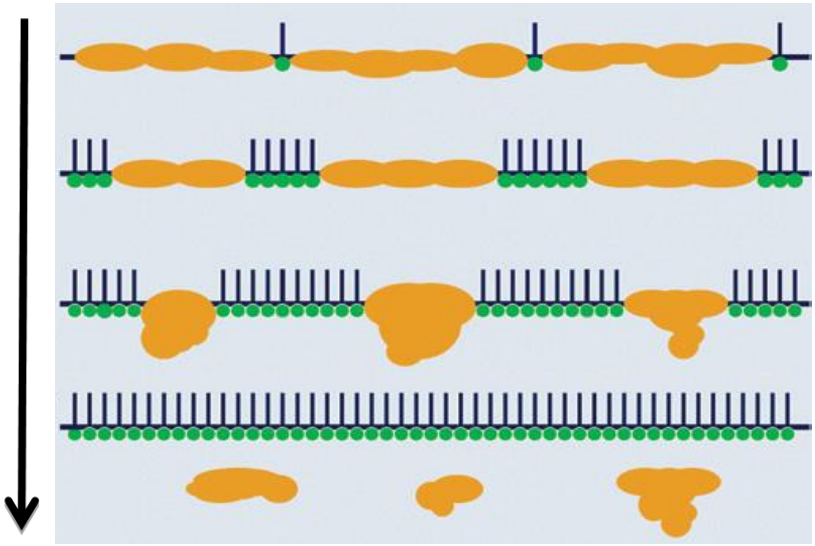

Fig. 2.8 Diagram illustrating the displacement of the milk proteins by the surfactants at the fat globule interface (adapted from Morris & Gunning, 2008).

The displacement of the proteins by the surfactants produces initially a larger but finally a thinner interfacial layer which makes the emulsion unstable under shear and prone to partial coalesce as the fat crystals can more easily pierce the surrounding fat droplets (Goff & Hartel, 2013).

2.1.3.5 Freezing process

Freezing is maybe the most crucial step in the ice cream process because it converts the mix into ice cream by shearing and freezing it. This causes ice crystal formation, air incorporation, and fat destabilisation (Goff & Hartel, 2013).

Ice crystal formation. Ice cream freezers consist of a cylindrical barrel (in which the mix is poured) where a refrigerant, normally a liquefied volatile gas, such as ammonia or Freon, flows through its jacket and cools down the outside of the barrel. In the barrel there is a motorised rotating dasher equipped with scraper blades (Fig. 2.9). When the mix (above its freezing point temperature) is placed in the barrel and comes in contact with the cold wall (~-30 ºC), a film of ice crystals is promptly produced and the dasher, while shearing the mix, rapidly scrapes off this layer. A scrape takes normally a very short amount of time.

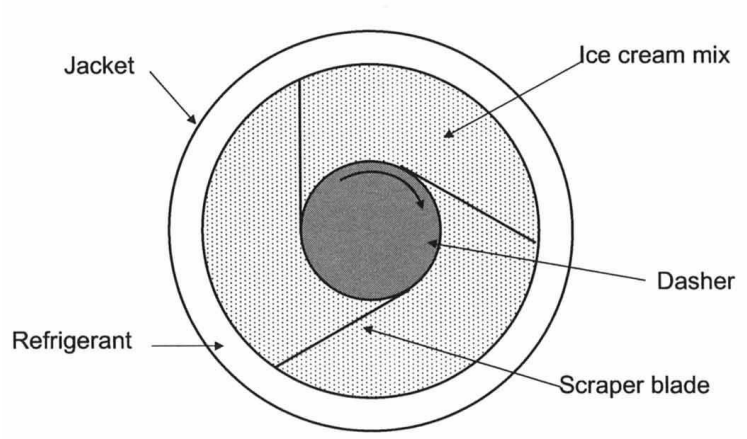

Fig. 2.9 Cross-sections of an ice cream freezer perpendicular to the axis of the barrel (Clarke, 2004).

In consequence, the layer removed by the dasher is typically very thin and not fully crystallised but containing instead a lot of small ice crystals. These ice crystals disperse into the warmer mix in the middle of the barrel and melt (cooling down the mix). The ice crystals keep on melting until the mix is enough cold to allow the survival of the ice crystals and their

growth (Clarke, 2004). As the ice content increases the fresh layer scraped off the wall principally contributes to the growth of the already-formed ice crystals (Fig. 2.10) (Cook & Hartel, 2010).

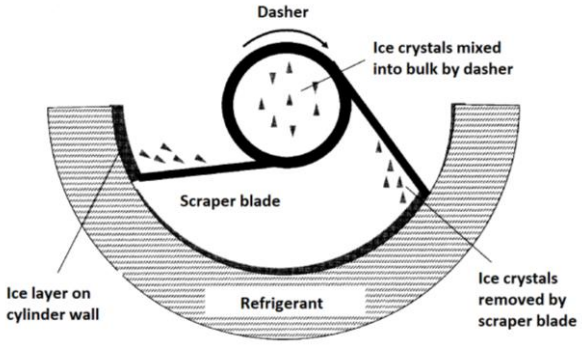

Fig. 2.10 Schematisation of freezing process (adapted from Clarke, 2004).

As the mix is freezed, its temperature decreases, its ice content increases, and its viscosity subsequently increases enhancing its whippability. Nevertheless, if the viscosity increases more energy is required for the dasher to rotate and this energy is dissipated in the mix in the form of heat, eliminated by the refrigerant. The freezing process is then self-limiting because at a certain point the energy supplied by the dasher equals the energy removed by the refrigerant, reason why the lowest achievable temperature at the outlet of a conventional freezer is around - 5 or - 8 ºC (Clarke, 2004).

Ice cream formulation influences ice crystal formation by affecting freezing point depression as follows. Ice cream freezing point is lower than 0 ºC (~-2 or -3 ºC) because of the presence of soluble elements (e.g. sugar and MSNF). During freezing, with the gradual formation of ice crystals, the concentration of the soluble elements in the unfrozen mix increase and, as a result, the freezing point for the remaining solution decreases gradually as the ice cream is

freezed. Even at -30 ºC the percentage of frozen water is not 100 but approximately 90. Different types of ingredients (e.g. sugars) influence differently the freezing point; higher freezing points lead to softer final products due to the low amount of ice crystals (Goff & Hartel, 2013).

Air incorporation. During freezing, thanks to the shear applied, air bubbles are incorporated and continually reduced in size. In the final product, the air cells are in size between few mm to over 100. Milk proteins (especially caseins being more hydrophobic), surfactants and fat globules (both partially coalesced and discrete) have all an important role in air incorporation during freezing (Pelan *et al.*, 1997; Goff *et al.*, 1999). In fact, these adsorb onto the surface of the air bubbles, stabilising them (Turan *et al.*, 1999).

Ice formation is necessary for air incorporation; in fact Chang and Hartel (2002) showed that whipping of the ice cream mix above its freezing point results in poor air incorporation as well as in large air bubbles. This happens because with ice formation lead to an increase of the mix viscosity which stabilises the air bubbles kinetically.

Fat destabilisation. As already mentioned, the shearing and air incorporation (both occurring during freezing) lead to fat destabilisation associated particularly to partial coalescence that converts some of the discrete fat droplets into a three-dimensional fat network which can be found at the air bubble surface (Fig. 2.11) (Goff & Hartel, 2013).

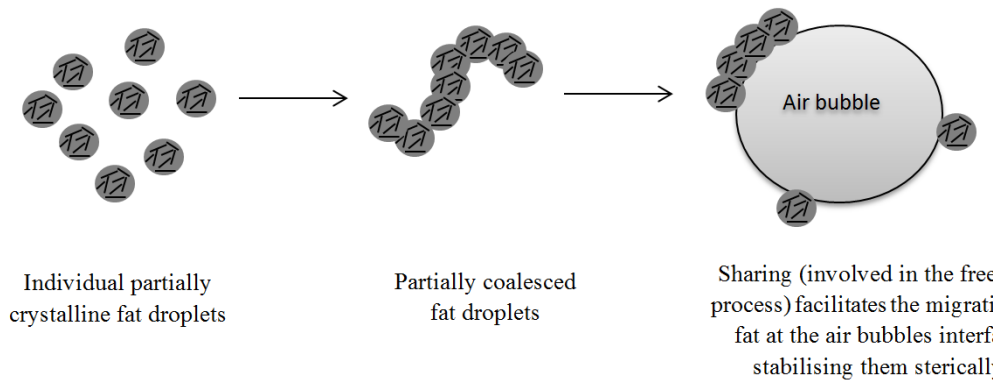

Fig. 2.11 Diagram representing the formation of a network of partially coalesced fat droplets during freezing and the function of the fat in stabilising the air bubbles incorporated.

2.2 Fat crystallisation

From the above discussion it is clear that, in ice cream, crystallised fat is important for the development of a fat network which provides good stand-up properties to the final product, decreases its meltdown rate and increases its creaminess during consumption (Hartel & Goff, 2013). Given the importance of fat crystallisation, relevant notions will be given in this section.

2.2.1 General notions of lipid structure

In food, lipids are normally present in the form of triacylglycerols (TAGs) even if more polar forms such as diacylglycerols (DAGs), monoacylglycerols (MAGs), free fatty acids (FFAs), and phospholipids do exist. TAGs are molecules made up of a glycerol backbone linked with three fatty acid moieties (in three positions: sn-1, sn-2 and sn-3) through an ester bond as shown in Fig. 2.12. MAGs and DAGs differ from TAGs in the fact that the glycerol is linked with one or two fatty acid molecules, respectively (Sato, 2001).

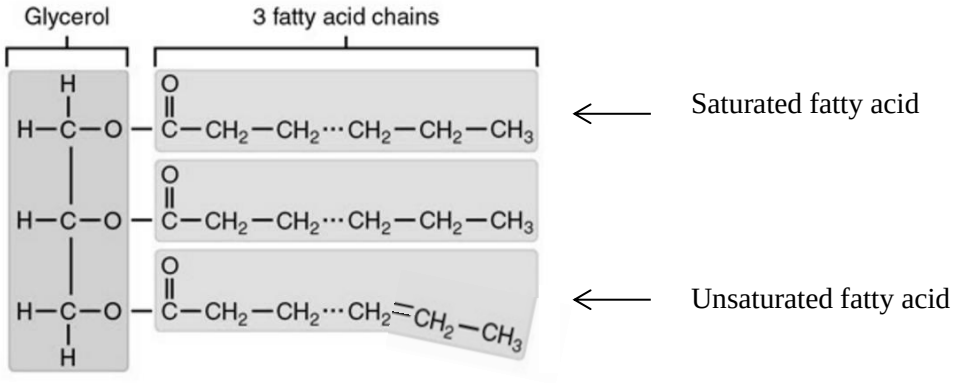

Fig. 2.12 Simplified schematisation of a triglyceride structure showing both saturated and unsaturated fatty acids (adapted from http://study.com/academy).

There are a variety of TAGs (different for instance in the carbon-chain length and the degree of unsaturation of the fatty acids composing them). When the fatty acids are equal within a TAG, this is known as "monoacid", if not "mixed-acid" (Sato, 2001).

2.2.2 General notions of fat crystallisation

Fat crystallisation is the formation of a solid phase within the liquid one. The crystallisation of an element in a solution happens just when the supersaturation of that element is achieved. This occurs when the activity of the crystallising molecules is greater than the one in the solution (Kloek *et al.,* 2000). When this happens, the thermodynamic driving force for crystallisation is the chemical potential difference between the supersaturated liquid and the solid (Himawan *et al.,* 2006). Supersaturation is usually reached cooling the solution below the melting temperature (Tm), a process called "supercooling" (Himawan *et al.,* 2006). The degree of supercooling (ΔT) is the temperature at which the crystallisation is observed and it is defined as follows:

$$\Delta T = T_m - T_{Cr} \quad \text{..... (Equation 2.2)}$$

where, Tm and Tcr are the melting and crystallisation temperatures, respectively. ΔT depends on the lipid phase and the processing parameters (McClements, 2005). Note that a system can remain in a supercooled state for a long time before the start of crystallisation. This happens because TAG molecules need to align and pack into clusters. In fact, there is an energy barrier associated with TAG molecules alignment which is due to their freedom in the solution (Marangoni *et al.,* 2012, McClements, 2012).

Once the conditions for fat crystallisation are achieved, this starts through nucleation and carries on via crystal growth (Sato *et al.*, 2013).

2.2.2.1 Nucleation

Nucleation is the creation of a crystalline "nucleus" from the liquid state. A nucleus is the smallest crystal that can exist at a particular temperature and it requires the association of TAG molecules into clusters which then associate into a nucleus (which, differently from clusters, does not re-dissolve into the liquid solution) (Cook and Hartel, 2010). For nucleation to occur the molecules should remain in contact for quite a long time to form clusters and then a nucleolus, i.e. supersaturation and supercooling are required. Nucleation is classified into primary nucleation (homogeneous or heterogeneous), and secondary nucleation (Metin & Hartel, 2005).

Primary homogeneous nucleation. Primary homogeneous nucleation is based on the accumulation of TAG molecules. Single TAG molecules form clusters (dimers, trimers and so on). There is a critical size which needs to be passed because above it a stable nucleus can be formed and can grow, while below it the TAG molecules disperse again into the liquid state (Hartel, 2001; Timms, 1995; Mullin, 2001). Nucleation requires to overcome an energy barrier (Mullin, 2001). Moreover, it is important to highlight that primary homogeneous nucleation requires a strong crystallisation driving force (e.g. supercooling or supersaturation). For this reason, primary homogeneous nucleation rarely occurs because, before the critical driving force is achieved, foreign surfaces (which reduce the nucleation energy barrier) may induce primary heterogeneous nucleation (Metin & Hartel, 2005).

Primary heterogeneous nucleation. In heterogeneous nucleation, the presence of foreign nucleating points (e.g. dust or impurity particles) reduces the energy barrier to be overcome for the nucleation to occur so it requires a lower crystallisation driving force (supersaturation and supercooling) than that required by homogeneous nucleation (Mullin, 2001; Garside, 1987).

The exact way this nucleation occurs is not clear but it is believed it is a consequence of the interaction between the particles and the fluid which causes a local ordering of crystallising molecules and so decrease of the free energy barrier.

Secondary nucleation. Secondary nucleation is the formation of new nuclei from existing crystals and it can happen under shearing or under static conditions (Metin & Hartel, 2005). Under shearing, the primary cause of secondary nucleation is the interaction and collision of crystals with each other, with the container wall or even with the stirrer. This nucleation can happen in two ways (Hartel, 2001):

- The displacement from the crystals surface of a layer of TAG molecules which will then overcome the critical size and form stable nuclei (Mullin, 2001);
- The release of crystal pieces which are already stable nuclei (Boistelle, 1988).

Under static condition, heat may cause the melting and/or dissolution of existing crystals with the resulting development of new nuclei (Metin & Hartel, 2005).

2.2.2.2 Nucleation in emulsified fat

Nucleation in emulsified fats is considerably different compared with bulk fat. In fact, unlike bulk fat where heterogeneous nucleation is predominant, in emulsified fat there should be a nucleating agent available in every droplet in order for heterogeneous nucleation to occur and this is not often the case. Homogeneous nucleation in this case will be then predominant over heterogeneous nucleation even if this will occur anyway but with a lower probability instead. In particular, there will be two populations of droplets: one which nucleates at higher temperatures (with heterogeneous nucleation) and one which nucleates at lower temperatures (with homogeneous nucleation). Moreover, in emulsions the nucleation often occurs at the fat droplet interface where surfactants are located because the structural similarity of these to the fat molecules may provide some ordering in the droplet enhancing the nucleation (Kaneko *et al.*, 1999).

2.2.2.3 Crystal growth

Once nucleation occurred, crystal nuclei grow incorporating TAGs from the liquid phase. This incorporation depends on the structural similarity of the TAGs and the crystal surface. In fact, when a growth unit (either a liquid TAG molecule or a group of TAG molecules) reach the crystal surface it will migrate across the crystal to find a correct site for its incorporation and its structural configuration will determine if it will ultimately bind the crystal lattice or return to the liquid phase. Thus, different TAG species can crystallise together and form mixed crystals but this will depend on the similarities and differences in their molecular structure (chain length, unsaturation, configuration of double bonds, and fatty acids disposition on the glycerol molecule). Generally, similar TAGs are more likely to co-crystallise, but structurally different TAGs can also co-crystallise especially in particular

conditions (e.g. very rapid growth) although they form weak crystals structures. Crystal growth in multi-compositional fats is slower than in pure TAGs because of their competition for the vacant site on the crystal lattice. Nevertheless, if stable mixed crystals are developed the crystal growth can be enhanced (Timms, 1995). For instance, Metin and Hartel (2005) observed that fat crystallisation of milkfat was notably faster than cocoa butter even if milkfat contained a wider variety of TAGs than cocoa butter and therefore they speculated this was due to the emphasised formation, in milkfat, of stable mixed crystals which enhanced the crystallisation (Timms, 1995).

As far as the crystallisation driving force exists, crystals will keep on growing. If the system reaches the phase equilibrium or it is completely crystallised, crystals will then stop growing (Hartel, 2001).

2.2.3 Effect of surfactants on fat crystallisation

Given the importance of fat crystallisation in ice cream, manufacturers should be aware of the fat crystallisation mechanism of the fat blend used and the effect of other ice cream ingredients (such as surfactants) on fat crystallisation (Goff & Hartel, 2013).

Depending on the structure similarity between the surfactant and the fat, fat crystallisation can be influenced in different ways. If the structure of the surfactant is different from the one of the fat, it can accelerate nucleation acting as a heteronucleus. Nucleation can be also promoted if the crystallisation point of the surfactant is higher than the one of the fat because the surfactant crystals (which are formed earlier) can act like seeds (Smith *et al.*, 1994; Dimick, 2000). During crystal growth, surfactants may be partially incorporated into the

growing fat crystals and act as impurities leading to imperfections in fat crystals or, if fat and surfactant are completely immiscible, this may be adsorbed on the fat crystals at crystal-liquid interface and inhibit crystal growth. On the other hand, if the fats and surfactants have a similar structure, surfactants can be adsorbed on the crystal lattice resulting in the formation of new sites for the further incorporation of TAGs so enhancing the crystallisation rate. In emulsion, the surfactant, thanks to its hydrophobic chain, absorbs at the fat interface accelerating the heterogenic nucleation (Garti &Yano, 2001).

Two kinds of surfactants are frequently used in ice cream: mono and di glycerides (MDGs) and polysorbates (Keeney, 1982) used normally in blends for their relative abilities. MDGs, being low-polar surfactants, are better foaming agents than polysorbates which in turn are more efficient in the displacement of the protein from the O/W interface (Keeney, 1982). Regarding their relative structure, MDGs have the same structure of triglycerides (glycerol backbone with attached three fatty acid molecules), but with just one or two fatty acid molecules instead. Polysorbates (such as Tween 80) have a structure similar to MAGs. Nevertheless, the fatty acid in this case is attached to a sorbitol molecule (rather than glycerol) and this is bound, in turn, to a polyoxyethylene group (Clarke, 2004).

As nowadays vegetable fats (in blends of solid and liquid fat) are being extensively used for the production of ice cream, studying the effect of surfactants on the crystallisation of different fat blends is invaluable because it can help to predict the fat network development.

2.3 Effect of formulation and microstructure on sensory perception, palatability, appetite and food intake

Both formulation and microstructure (described in details above in this review) can affect sensory perception (Bhopatkar *et al.,* 2012*;* Van Aken, 2007*).* As regards microstructure, the formation of a fat network leads to a smoother product as a direct relationship has been observed between the degree of partial coalescence and the viscosity of an emulsion (Everett 2007) and between the viscosity of an emulsion and its creaminess (Akhtar *et al*., 2005). Also, the greater the air content the higher the perceived creaminess because the softer will be consequently the product (Kilcast & Clegg, 2002). As regards formulation, solid fats are perceived as creamier than liquid fat as creaminess has also been shown to be driven by meltdown (Niranjan & Silva, 2008).

Formulation, microstructure, sensory perception and palatability have an effect on appetite (Blundell & Cooling, 2000). This is a very important aspect to consider because it is linked to overconsumption and consequently to overweight and obesity (Fig. 2.13). Regarding sensory perception, the thickness of food has an effect on food intake because we know, by experience, that viscous foods are more nutrient and energy-rich than fluids, which are typically consumed as refreshing items and may not contain nutrients (Le Magnen, 1955). In fact, by increasing the thickness and creaminess of a food product its satiety expectations will increase, influencing the actual eating behaviour (McCrickerd *et al.,* 2014). Creamier and thicker foods make us also feel physically fuller for a longer time (Bertenshaw *et al.*, 2013).

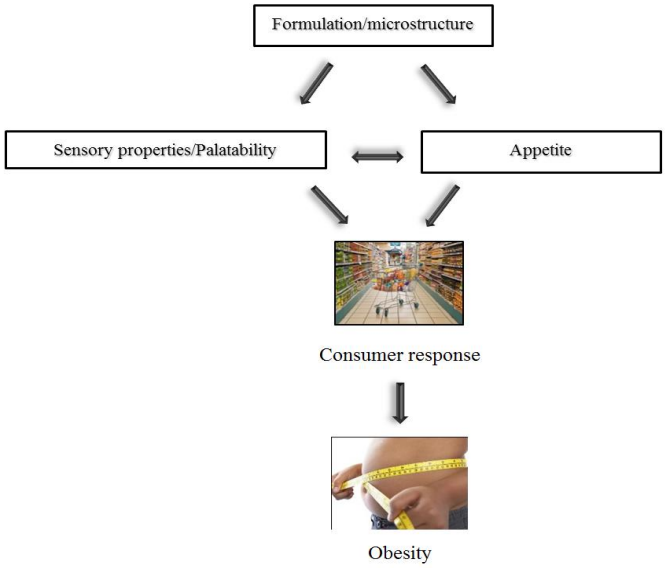

Fig. 2.13 Impact of formulation and microstructure on sensory perception and palatability of food, appetite and obesity.

In past studies, increasing the thickness and creaminess of a beverage reduced eating rate (Zhu *et al.*, 2013) and postprandial hunger (Zhu *et al.*, 2013; Mattes & Rothacker, 2001; Cassady *et al.*, 2012), increased fullness (Zhu *et al.*, 2013; McCrickerd *et al.*, 2012), expected satiety (McCrickerd *et al.*, 2012; Forde *et al.*, 2013; Hogenkamp *et al.*, 2012; Hogenkamp *et al.*, 2011), delayed gastric emptying (Zhu *et al.*, 2013; Cassady *et al.*, 2012) and reduced consequent *ad-libitum* meal intake (Chambers *et al.*, 2013; 2011; Cassady *et al.*, 2012) independently from the energy content of the beverages (McCrickerd *et al.*, 2012). Palatability has been directly correlated to an increase in food intake (Bobroff & Kissileff, 1986; Guy-Grand *et al.*, 1989; Yeomans, 1996; Yeomans *et al.*, 1997, 2004, 2005; Zandstra *et al.*, 2000; Vickers *et al.*, 2001; Robinson *et al.*, 2005). Nevertheless, this increase seems to be temporary because the overall daily intake does not vary (Yeomans *et al.*, 2001). Thus, the energy intake seems to be regulated and this regulation can last up to two days (De Casto, 2000).

Regarding microstructure, air content could affect appetite as it has been shown an inverse relationship between gastric distension and appetite (Wang *et al.*, 2008; Rolls & Roe, 2002). Last, it has been shown that formulation (for instance different fat with varying carbon chain saturation and length) can influence differently appetite and food intake (Lawton *et al*., 2000; Rolls *et al.*, 1988; Van Wymelbeke *et al*., 1998, 2001) (see later in section 2.4.4).

2.4 Appetite research methodology

An appetite study has been conducted in this research to evaluate how different formulations influence appetite feeling of appetite and food intake. Therefore it is relevant to discuss how appetite studies are normally carried out and to provide useful concepts to fully comprehend the topic.

2.4.1 Study environment

In the laboratory it is possible to assess different facets of appetite in a controlled environment. In particular, it is possible to study the effect of specific factors, incorrupt by unrelated inputs, on appetite. It is important to highlight that there are a variety of differences between a laboratory environment (with a robust control over the procedure) and a free-living situation (where no control is employed). Data collected under laboratory conditions are normally precise and accurate but the environment is not natural (people do not normally eat isolated from social stimuli); eating in an every-day condition is natural, but the data collected would not be precise because of the contamination of extraneous inputs (Blundell *et al.*, 2009).

2.4.2 Participants

Potential participants are normally screened before being able to participate in the study. In fact, it is important to make sure they like the food provided, they do not take medication known to affect appetite, they do not smoke, they do not have any food allergies or intolerances, they are not currently dieting or about to start a diet and they do not show disordered eating behaviour. Moreover, confirmed participants are normally instructed about the food and activities prior the study day (such as restraint from alcohol and vigorous

exercise and avoid any food and drink after midnight) to ensure that they are all in an equivalent state at the study day (Blundell *et al.*, 2009).

2.4.3 Understanding appetite expression – the satiety cascade

Eating behaviour is a complex interplay between psychological events (experiences of hunger, satiation and satiety) and other factors resulting from nutrient absorption, utilisation and storage (Harrold & Halford, 2013). With regard to psychological events, *Hunger* is the motivation to eat occasioned by the lack of food. It begins the eating activity which in turn leads, through feedback, the feeding episode to a cessation. *Satiation* is the process that brings an event of eating to an end whereas *satiety* is an inhibition of further eating where hunger is suppressed (Blundell *et al.*, 2001). In other words, satiation determines duration and size of the meals, whereas satiety controls the length of the inter-meal interval.

The satiety cascade (Fig. 2.14), a concept originally developed by Blundell and colleagues (1987), shows how a combination of factors influence appetite and describes the physiological, cognitive mechanisms and sensory inputs that influence satiation and satiety (Blundell *et al.*, 2001).

1) Prior consumption of food, cognitive (e.g. anticipation i.e. thinking about the consumption of food) and sensory stimuli (e.g. sight and food smell) stimulate hunger and food intake.

2) During eating, sensory and cognitive factors lead to satiation and initial satiety.

Sensory factors. Food intake increases when there is diversity of food items in a meal or a diet (Raynor & Epstein, 2001). The principal mechanism causing this phenomenon is sensory-specific satiety (Rolls, 1986; Hetherington, 1996), which is described as a reduction in liking for a food product after eating it and an increase in liking for not-yet-eaten foods (Redden, 2008). Thus, with a varied menu, satiation can take longer to occur (as it needs to manifest for all the foods) resulting in more food consumed (Raynor & Epstein, 2001).

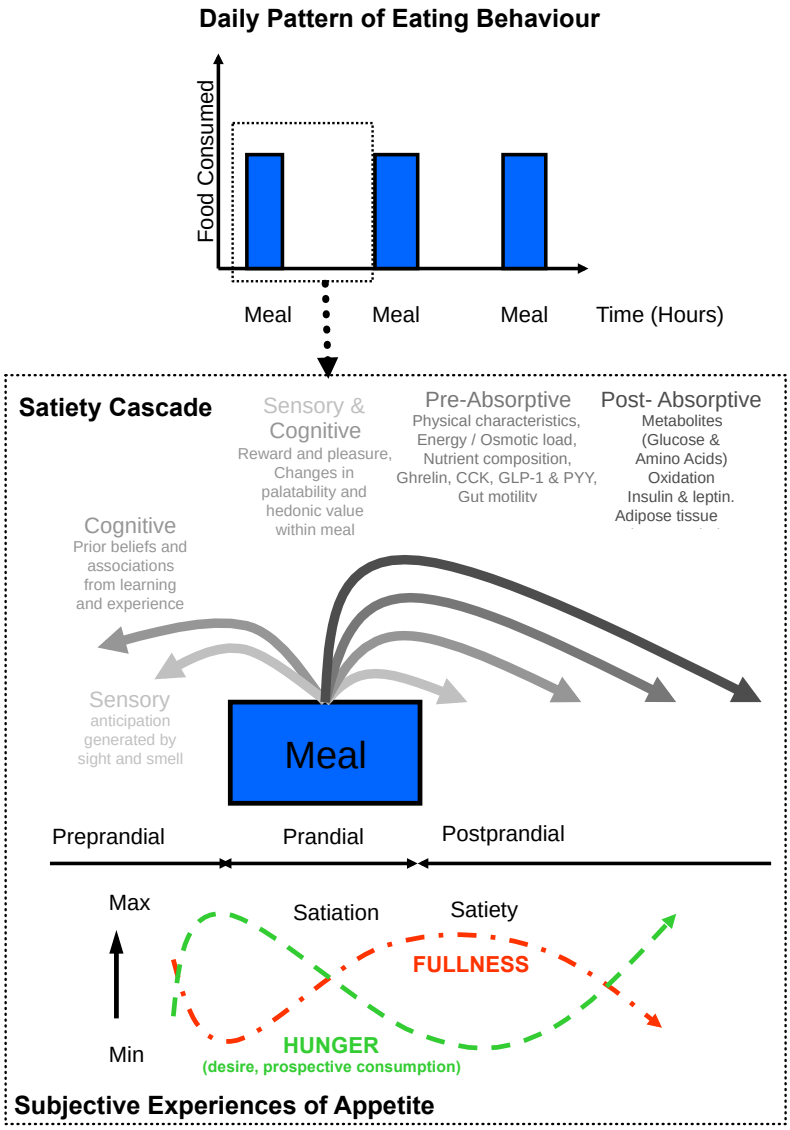

Fig. 2.14 Satiety cascade displaying the signals before (preprandial), during (prandial) and after (postprandial) food consumption involved in satiation and satiety regulation (from Harrold & Halford, 2013).

This phenomenon is sometimes defined as the "dessert effect" because appetite revives with an after meal dessert (Remick *et al.*, 2009). Note that world-wide, the increase of the variety of food items has been described as one of the causes of the obesity (Cohen, 2008).

Cognitive factors. An example of a cognitive factor influencing satiation is the "expected satiety" (Brunstrom *et al.*, 2008) of a food product. Expected satiety is normally investigated by presenting participants with a picture of a chosen standard food item in a defined amount and some pictures different in size of a comparison food item. Participants are asked to select a portion of the comparison food item which they would expect to provide the same satiety of the standard food item. In a study of Brunstrom and colleagues (2008) they showed that 200 calories of pasta and 894 calories of cashew nuts were believed to deliver the same satiety, hence the cashew nuts were expected to be 4.47 times (894/200) as filling as the pasta. The reason is probably that high fat foods are, actually and from our experience, expected to be less satiating than carbohydrate and protein-rich food (Johnstone *et al.*, 1996; Stubbs *et al.*, 1996). Nevertheless, the possibility that in the study of Brunstrom and colleagues the assessment was based on the volume of the food cannot be excluded. In fact, high fat foods are more energy dense so that a smaller quantity is needed to supply the same amount of calories of a carbohydrate or protein-rich food. Expected satiety was also found to increase with increasing familiarity of a food product (Brunstrom *et al.*, 2008) or after a piece of food has been consumed until fullness (Irvine *et al.*, 2008). Indeed, when we are about to eat a piece of food that has not be eaten before, we are normally "prudent" and we assume that it will be not filling until experience teaches us the exact satiating properties.

3) Satiety is then caused by post-ingestive events. In particular, increased gastric volume activates gut wall mechanoreceptors which detect the stomach stretch and provide the central nervous system (CNS) with an indication of the amount of food consumed (inducing satiation and satiety) (Marciani *et al.*, 2001; Hoad *et al.*, 2004). Also, gut chemoreceptors detect the macronutrient content in the food ingested and release GI hormones such as ghrelin by the stomach, cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1) by the small intestine and insulin by the pancreas (see later in section 2.4.3.2). These hormones are secreted into the blood and regulate appetite (Ritter, 2004; Woods, 2005; Huda *et al.*, 2006) generally by inducing satiety.

4) Also post-absorptive factors can cause satiety. These are, for example, nutrient metabolites secreted into the blood (such as glucose, amino acids), oxidation of nutrients and the storage of energy in stores (such as the adipose tissue).

2.4.3.1 Studying satiation and satiety

Principally, satiety is affected by the total energy and macronutrient composition of the meal ingested, whereas satiation is affected by fullness and boredom with taste (Hetherington, 1995; Tuomisto, 1998). The ways of assessment of satiation and satiety are different.

Satiation. Satiation is normally assessed with the *ad-libitum* consumption (grams and calories) of a particular experimental food. A lot of factors can influence satiation (meal termination) for instance palatability of the food, energy density and motivational state of the individual. Palatability, as previously mentioned in section 2.3, has a strong influence on food intake with more food ingested if the item is considered more palatable (De Graaf *et al.*, 1999;

De Graaf *et al.*, 2005). Thus, it is essential for the foods under assessment to be similarly liked. Energy density also influences satiation, for example in a study of Weenen and colleagues (2005) participants were asked to *ad-libitum* eat two food products: pears in syrup and cheese biscuits. Not surprisingly, participants ate 5 times more pear in syrup than cheese biscuits. The energy density of the biscuits was ~eight times greater than the one of the pears and the two items were equally liked. Therefore, if the energy density is high the food ingested will be less so that the meal terminates sooner (i.e. satiation is influenced). Nevertheless, this is not necessarily true: it has been shown that people are very slow to react to energy density changes especially for liquid foods and fast foods (Blundell *et al.*, 1996; Lissner *et al.*, 1987; Di Meglio & Mattes, 2000; Ebbeling *et al.*, 2004). In fact, these foods are easy to ingest without chewing effort, which may reduce the body's ability to associate consumption with calorie intake. Finally, motivational state (hungry vs. satiated) is also important as people eat more when they are more hungry so it is important to keep individuals with a similar satiety level when investigating satiation (Blundell *et al.*, 2010).

Satiety. The most usual method employed to assess satiety (in short-term studies) is the "preload design" which is normally carried out within a single day. The preload is a food portion (smaller than the meal) that is presented before an *ad-libitum* meal (test meal) and normally contains the ingredient whose effect on satiety is the object of investigation. Using the preload design satiety can be assessed by time (duration of the gap between the preload and the *ad-libitum* meal) or the amount (food consumed at the test meal and during the rest of the day) (Fig. 2.15). Both measures are suitable but meal size is most frequently used because it is more convenient for the experimenter (Blundell *et al.*, 2010). A within subject repeated measures design is normally used in these kind of studies (Rolls & Hammer, 1995).

Subjective appetite measures are usually required before, after and at fixed time gaps between the preload and the *ad-libitum* meal. Intake of food for the remaining part of the day is normally recorded by the participants.

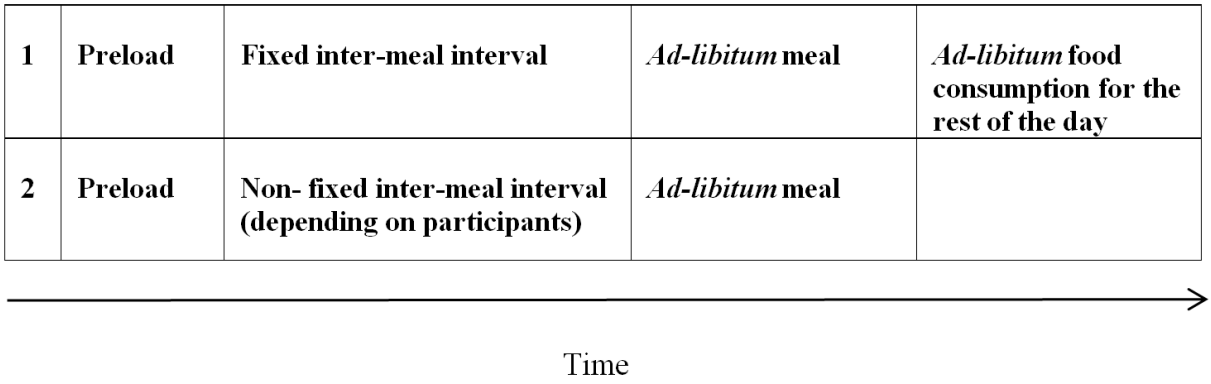

Fig. 2.15 Example of a preload study when satiety is assessed by 1) the amount of food consumed at the *ad-libitum* meal and the rest of the day, or 2) by time (duration of the interval between the preload and the *ad-libitum* meal).

Important considerations to take into account when planning a preload study are the statistical power of the study, the amount of the preload item, the gap between the preload and the *ad-libitum* meal and the *ad-libitum* meal composition. The preload should contain a sufficient amount of the ingredient to detect an effect, so that it is essential to select the right preload portion. This anyway should not be a large portion because fullness could hide any effect. The gap between the preload and the *ad-libitum* meal should be chosen based on the mechanism by which the ingredient to be tested is believed to affect satiety and food intake. In fact, a shorter time lapse allows the detection of post-ingestive or pre-absorptive mechanisms, whereas a longer time lapse assesses post-absorptive mechanisms. A pilot study can be useful to identify the right amount of preload and the right gap between the preload and the *ad-libitum* meal. The *ad-libitum* meal can be a buffet style or a single course. In the former, a large range of foods (high and low fat sweet and savoury food items) are offered to the

participants, whereas in the latter just a single plate is offered. The buffet style is useful to assess food and energy intake, food preferences and macronutrient intake. On the other hand, the single course is focused on the evaluation of just food and energy intake. Nevertheless, offering a buffet style meal is not necessarily a sensitive protocol because it, as aforementioned above, could induce overeating and delay satiation (Blundell *et al.*, 2010).

Subjective appetite states are normally assessed with a 100-mm visual analogue scale (VAS) questionnaire. This consists of a number of questions (relating the appetite state) whose answer is given by participants placing a vertical mark on a 100-mm unmarked horizontal straight line with two extreme (minimum and maximum) (Fig. 2.16).

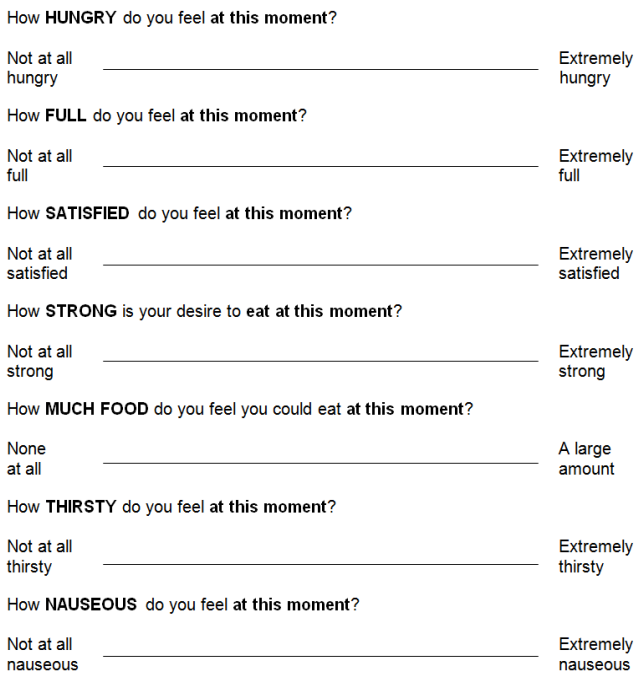

Fig. 2.16 Example of 100-mm VAS scale.

The 100-mm VAS is the most commonly used scale, even if there are some alternatives (Leathwood & Pollet, 1988; Holt *et al.*, 2001; Merill *et al.*, 2002; Drapeau *et al.*, 2007). The

most typical 100-mm VAS is the paper-and-pen VAS but this has some disadvantages. For example participants may forget to complete questions or even the entire questionnaire. A more modern 100-mm VAS is the Electronic Appetite Rating System (EARS) (Delargy *et al.,* 1996; King *et al.,* 1997). EARS is based on a mobile personal computer (Totterdell & Folkard, 1992) given to participants which automatically verifies date and time of VAS completion and identifies incomplete entries preventing the common "missed" questions. Moreover, data can be automatically downloaded to a computer (Blundell *et al.,* 2009). However, EARS are more expensive than paper-and-pen VAS and in most of the cases it is more feasible using the paper-and-pen VAS especially when the study involves a large number of participants and more than one participant is tested in one day.

The preload experimental design, despite its apparent simplicity, gathers conclusions which need a complex analysis and a deep interpretation. Generally, with an appropriate study design the methods used in appetite research have a reasonable level of reliability and repeatability (Merrill *et al.,* 2002; Stubbs *et al.,* 2001; Flint *et al.,* 2000; Raben *et al.,* 1995; Kruse, 2001). Also, plotting appetite vs. time and considering the area under the curve (AUC), produces a better repeatability and reliability than absolute appetite scores (Flint *et al.,* 2000; Kirkmeyer & Mattes, 2000). It is important to highlight that the validity of these methods is not based on their correlation with eating behaviour (Hill *et al.,* 1995). Appetite measures and eating behaviour may differ but it is expected that their correlation would be stronger under controlled conditions than free-living situation. Nevertheless, appetite measures cannot be used as an alternative to eating behaviour investigations (Blundell *et al.,* 2010).

2.4.3.2 Appetite biomarkers

As mentioned in section 2.4.3, the GI system releases hormones that control the appetite expression when food is ingested. Between them Cholecystokinin (CCK), Glucagon-Like-Peptide-1 (GLP-1), Peptide YY (PYY) and ghrelin play an important role.

CCK is released from I-cells of the small intestine upon detection of specific food components in the lumen like free fatty acids and amino acids (Barret & Raybould, 2010). CCK causes the secretion of the pancreatic juice, the contraction of the gallbladder, the relaxation of the sphincter of Oddi (see section 2.5) and the decrease of the gastric emptying enhancing, as a consequence, satiation and suppressing appetite (Barret & Raybould, 2010).

GLP-1 is secreted by the L-cells of the small intestine upon detection of carbohydrate and fat. GLP-1 principally stimulates insulin release and inhibits glucagon release, contributing to the control of blood glucose levels. In fact, blood glucose has to be kept in the range of 70-110 mg/dl and insulin/glucagon, two hormones secreted by the pancreas, maintain this homeostasis. Insulin is secreted by the pancreatic β-cells in response to high blood glucose (i.e. after a meal). In response to insulin, blood glucose is used by body cells (e.g. muscle cells, red blood cells, and adipose cells) so that its blood concentration returns to normal. On the other hand, glucagon is secreted by the pancreatic α-cells when blood glucose is low (i.e. between meals or during exercise). Glucagon exerts its effect mainly on the liver causing the release of stored glucose into the blood (Bruce, 2010). Aside from its effect on insulin and glucagon secretion, GLP-1 delays gastric emptying (Näslund *et al*., 1999) influencing, like in the case of CCK, satiation and appetite.

PYY_{3-36} is secreted also from L-cells of the small intestine in response to fatty acids, fibre and bile in the gut. Similarly to GLP-1 and CCK, PYY_{3-36} inhibits gastric empting resulting in a well-known food intake reduction (Batterham *et al*., 2002).

Ghrelin, unlike the other hormones mentioned, is produced in the stomach and it stimulates appetite, eating behaviour and gastric motility. Ghrelin is also called "the hunger hormone" and its levels increase before (Cummings *et al*., 2001) and decrease after (Callahan *et al.,* 2004) a meal.

2.4.4 Effect of different lipids on appetite and food intake (appetite studies)

It has been shown that fat carbon chain saturation has an effect on appetite and food intake. Several mechanisms could be at the core of this, as the release of CCK. In fact, polyunsaturated lipids led to the highest release of CCK followed by monounsaturated lipids, whereas saturated lipids had no effect on CCK release (Maljaars *et al.,* 2009). This is probably because CCK is secreted by the enterocytes in response to free fatty acids (Guimbaud *et al.,* 1997; Vilsbøll *et al.,* 2003): unsaturated lipids, thanks to their lower melting point, can be emulsified more efficiently, facilitating the action of the lipase and the subsequent production of free fatty acids which stimulate the I-cells to produce CCK (Small, 1991).

Nevertheless, past studies have shown contrasting results. In fact, polyunsaturated lipids incorporated into a lunch test meal, strongly reduced food intake and appetite during the rest of the day in comparison with saturated and monounsaturated lipids (Lawton *et al.,* 2000). However, a variety of studies did not show any effect of lipid unsaturation on appetite and

food intake (Strik *et al.*, 2010; Flint *et al.*, 2003; Casas-Agustench *et al.*, 2009). In this cases, the lack of effect was attributed to 1) the long interval between the fat ingestion and the subsequent meal (which could lead hunger to override any affect); or 2) the amount of lipids ingested (higher amounts are more likely to have an effect than lower quantities).

Also the carbon chain length has been shown to have an effect on appetite and food intake and this could be due to the different absorption and metabolism of these lipids. In particular, medium chain triglycerides (MCTs), due to their smaller molecular weight, are hydrolysed faster and more completely than long chain triglycerides (LCTs) and can also be absorbed intact. Moreover, MCTs reach more rapidly the liver where they are readily oxidised, causing the production of Ketone bodies (Bach & Babayan, 1982). A decrease in food intake has been associated both with hepatic fat oxidation (Langhans, 1996) and the presence of Ketone bodies (Le Foll et al., 2014), suggesting that MCTs may reduce food intake more than LCTs.

In fact, food intake significantly decreased 30 minutes after the consumption of a MCT preload in comparison with a LCT preload with no differences in appetite ratings (which suggests a post-absorptive mechanism) (Rolls *et al.*, 1988). Similarly, the incorporation of MCTs to a breakfast (Van Wymelbeke *et al*., 1998) or a lunch (Van Wymelbeke *et al.*, 2001) led to a decrease in food intake in a subsequent meal in comparison with LCTs with, again, no effect on hunger levels. Nevertheless, other studies have failed to show an effect of carbon chain length on food intake (Poppitt *et al.*, 2010; Bendixen *et al.*, 2002) highlighting again the importance in the choice of the time gap between the preload and the meal and the preload amount.

In conclusion, it is known that replacing saturated with unsaturated lipids and LCTs with MCTs may lead to a food intake reduction, even if this has not been shown consistently. Moreover, it is not clear it this effect could be seen with a normal quantity of fat, as in most of the studies a large amount of fat was employed in order to detect an effect.

2.5 Fat – digestion, absorption and metabolism

The fact that different fats influence, in a diverse way, appetite and food intake has been attributed also to their dissimilar absorption and metabolism. An overview of these will be given in this section as it is relevant to fully understand one of the results chapters.

When a food product is eaten, in the mouth (pH 5-7) its macrostructure collapses due to chewing and mixing with saliva until a bolus (mass of chewed food prior swallowing) is formed (Lucas *et al.,* 2002). In most cases, the fat in the bolus is organised into oil globules that could have already been present in the food prior to consumption or formed in the mouth with the disruption of the bulk fat (Hernell *et al.,* 1990). After being swallowed, the bolus passes through the oesophagus and enters the stomach (pH 1-3) where it is agitated causing a further disruption of dietary lipids into smaller fat droplets. This is important because it leads to a fat surface area increase facilitating the action of the gastric lipase, a digestive enzyme. This lipase is released by the gastric cells, adsorbs onto the fat droplets surface and hydrolyses TAGs to DAGs and free fatty acids (FFAs). The gastric lipase has its optimal catalytic activity at acid pH (such as in the stomach) but lipolysis is incomplete at this stage because this enzyme cannot hydrolyse the sn-2 position of the TAGs (Barret & Raybould, 2010).

The digestion of fat continues in the small intestine (pH 6-7.5). In response to food presence, bile and pancreatic juices are secreted into the duodenum thanks to the Sphincter of Oddi: the liver produces the bile which is stored in gallbladder and, when food is present in the intestine, is secreted into the cystic duct which converges to the common bile duct; the pancreatic juice, produced by the pancreas, is secreted into the pancreatic duct. The common

bile duct and the pancreatic duct converge into the Sphincter of Oddi from which bile and the pancreatic juice are released into the duodenum (Fig. 2.17).

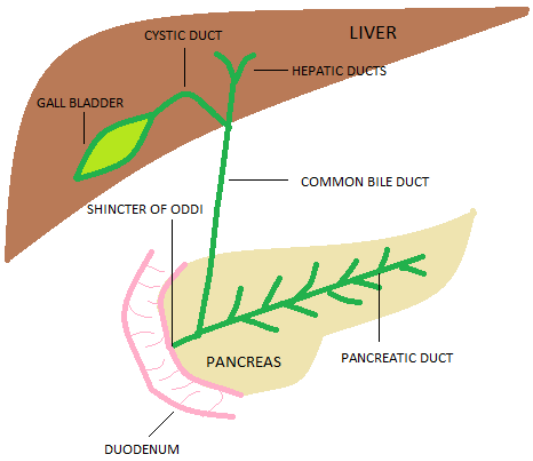

Fig. 2.17 Schematic view of ducts that transport the bile and the pancreatic juice to the duodenum.

In the duodenum the fat is emulsified thanks to the peristaltic movements and the bile. In fact, the bile contains phospholipids and bile salts (both amphipathic molecules) which, coating the fat droplets (formed thanks to the peristalsis), prevent their re-association. This results in an increased fat surface area that facilitates the action of the pancreatic lipase from the pancreatic juice. The pancreatic lipase is different from its gastric version because it is able to hydrolyse both the sn-1 and sn-2 positions of TAGs releasing FFAs and MAGs. Moreover, this enzyme is optimised for activity at neutral pH. As lipolysis proceeds, FFAs are extracted from the lipid droplets and permeate the enterocyte membranes, thanks to their lipophilicity, together with other lipolysis products.

In the smooth endoplasmic reticulum of the enterocytes FFAs are re-esterified to TAGs, phospholipids, and cholesterol esters which are all combined with apolipoproteins synthesized

in the rough endoplasmic reticulum to form chylomicrons (a structure with a lipid core of TAGs, cholesterol, phospholipid, and fat-soluble vitamin esters covered by a layer of amphipathic apolipoproteins). The chylomicrons exit the enterocyte by exocytosis and enter the lymphatic system since they are too large to permeate the intercellular spaces of blood capillaries. Chylomicrons deliver TAGs to the body becoming smaller and smaller until they enter the bloodstream and reach the liver where TAGs can be oxidised. It is important to underline that the absorption of TAGs is different with different chain lengths. For example, short- (SCTs) and medium-chain triglycerides (MCTs) thanks to their lower molecular weight are better hydrolysed by the pancreatic lipase (Fave *et al.*, 2004) and can also permeate the enterocytes without being hydrolysed. Moreover, being smaller, they can permeate the intercellular space of capillaries so they do not need to be packed into chylomicrons. They directly enter the portal system and reach the liver faster (Barret & Raybould, 2010).

2.6 Summary of literature review

This review aimed to allow a deep understanding of the results chapters of this thesis. The areas covered have been:

- Ice cream: microstructure and effect of formulation and manufactural steps on it;
- Fat crystallisation: its importance on the development of ice cream microstructure and the effect of surfactants;
- Relationship between formulation/microstructure and sensory perception/appetite;
- Overview of appetite research: how appetite studies are conducted and important concepts to fully comprehend the topic;
- Effect of different fat on appetite and food intake.

This review allowed the identification of the areas requiring further research which have been investigated in this thesis, in particular:

- The investigation of different surfactants on the thermal behaviour of a fat blend used for the production of ice cream as well as on the final product;
- The effect of different formulations (fat blends) and processing (aging step) on the microstructural and physical properties of ice cream, sensory perception and appetite.

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CHAPTER 3

Emulsifier Effects on Fat Crystallisation

Rizzo G, Norton JE, Norton IT

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Abstract

The effect of the addition of two emulsifiers differing in their molecular structure (mono and di glycerides and Tween 80) on the thermal behaviour of a bulk fat containing both solid and liquid components (75% coconut oil and 25% of sunflower oil) was investigated using differential scanning calorimetry (DSC). Different ratios of emulsifier to bulk fat were considered (emulsifier/bulk fat of 0.02, 0.05, 0.08, 0.1, 0.3, 0.6 and 1). Both the emulsifiers had an effect on the melting and crystallisation of the bulk lipid. Mono and di glycerides (MDGs), although crystallising independently of the bulk fat (i.e. the observation of the presence of independent melting peaks and enthalpies that were not dependent on the ratio of MDGs to bulk fat), were thought to act as templates for the crystallisation of the bulk fat, having an effect on the shape of the melting and crystallisation peaks. Tween 80, due to its structural properties (unsaturated carbon chain and large hydrophilic head) was thought to act as an impurity leading to the formation of less perfect crystals and a loosely packed lattice, resulting in less energy required to melt. The bulk fat and emulsifiers used this work have relevance to the ice cream industry, and could have implications for the physical properties of ice cream, particularly partial coalescence, meltdown properties, texture and sensory perception during consumption.

Keywords: DSC, Emulsifiers, Thermal behaviour, crystallisation

Industrial relevance

The fat blend studied in this work is a possible economic substitute for milk fat in the production of ice cream as it mimics milk fat in terms of partial coalescence, meltdown behaviour and flavour. As a consequence it is likely to be extensively used for this aim in the future. Studying the effect of different emulsifiers (varying in their molecular structure) on the thermal behaviour (crystallisation and melting) of this fat blend provides understanding relevant to the physical characteristics of ice cream. The emulsifiers studied lead to the formation of different fat crystal structures (i.e. differences in peak shape and enthalpy), which are likely to influence the destabilisation phenomenon (i.e. partial coalescence) and thus the properties of the final product.

3.1 Introduction

The effect of emulsifiers on fat crystallisation has been well documented (Wright *et al.*, 2000; Wright & Marangoni, 2002; Litwinenko *et al.*, 2004; Fredrick *et al.*, 2008; Basso *et al.*, 2010). Depending on the homogeneity between the emulsifier and the lipid in terms of chain length and degree of saturation, emulsifiers can retard or accelerate nucleation and crystal growth (Garti, 1988; Garti & Yano, 2001). In the ice cream industry, two emulsifiers are commonly used: Tween 80 (polysorbate 80) and mono and di glycerides (MDGs) (Goff & Hartel, 2013). These two emulsifiers are different in their structure: Tween 80 is more hydrophilic (due to the polyoxyethylated sorbitol hydrophilic head) and has a bent carbon

chain; MDGs have straight carbon chains and glycerol hydrophilic heads, making them more hydrophobic than Tween 80 (Euston, 2008).

It has been reported that polysorbates with saturated chains (such as Tween 40 or Tween 60) can act as seeds and promote fat crystallisation (reducing the free energy required for nucleation) and co-crystallise with the fat enhancing fat crystal growth. This effect is probably due to the similarity between the saturated carbon chains of the fat and the emulsifiers. In fact, Litwinenko *et al.* (2004) reported higher rate of crystal growth and shorter nucleation induction time in samples containing Tween 60 in comparison with samples without emulsifier. Sorbitan esters are similar emulsifiers to polysorbates, but with lower hydrophilicity because of the lack of the polyoxyethylene groups attached to the sorbitol molecule (Euston, 2008). The effect that these emulsifiers have on the crystallisation has been studied for a fat blend containing palm oil (Garbolino *et al.,* 2005), showing that long chain emulsifiers with at least 16 carbon atoms (sorbitan monopalmitate and sorbitan monostearate) will allow for optimal chain–chain interactions and result in co-crystallisation of the emulsifier and the fat, whose major fatty acids are palmitic and oleic acid, whereas sorbitan monolaurate has a shorter carbon chain which prevents interaction between the fat and the emulsifier.

Fredrick *et al.* (2008) showed that unsaturated monoacylglycerols (MAGs) from sunflower oil did not have an effect on the nucleation of palm oil crystals, whereas saturated MAGs (derived from palm oil) promoted nucleation. These authors suggested that the homogeneity between the fatty acids of MAGs and palm oil and their degree of saturation were the principal causes of the acceleration of palm oil crystallisation. MAGs can associate as reverse

micelles (Walstra & Van Beresteyn, 1975), which can decrease the energy barrier for the nucleation of triacylglycerols (TAGs). If the MAGs are from palm oil they can form micelles and crystallise because of their higher melting point compared to MAGs from sunflower oil. Subsequently, these MAGs micellar crystals may act as seeding material and are more effective than micellar structures alone at promoting earlier nucleation. Foubert *et al.* (2004) showed that the degree on saturation is an important factor in terms of the effect of the emulsifier on the fat. These authors investigated the influence of diacylglycerols (DAGs) and MAGs on the crystallisation of milk fat, showing that it was dependent on the acyl groups present in the additives. With stearic acyl chain the crystal growth rate was reduced, whereas an oleic acyl chain had no effect. The reason probably resides in the fact that stearic based MAGs and DAGs may be easily incorporated into the crystal lattice impeding further growth, whilst oleic based MAGs and DAGs are incorporated to a lesser extent due to their unsaturated carbon chain. The importance of the similarity between the fat and the emulsifier structure has also been highlighted by Smith *et al.* (1994) and Smith and Povey (1997) who discussed the effect of different additives on the crystallisation of a trilaurin model system. The crystal growth rate increased in the presence of monolaurin, while it was hardly affected by MAGs, whose chain length differs from lauric acid. This was probably due to the co-crystallisation of monolaurin with trilaurin, which was not possible for emulsifiers with a different carbon chain length due to structural diversity. More recently, Basso *et al.* (2010) showed that the addition of MAGs accelerated the crystallisation of palm oil by increasing the number of crystallisation seeds (heteronuclei).

To conclude, there is a well-documented effect of the emulsifiers on fat crystallisation. In particular, depending upon the affinity between the emulsifier and the fat (saturation and

carbon chain length) emulsifiers can interact with the fat favouring or interfering with the fat crystallisation. The aim of this study was to investigate the thermal behaviour of a fat blend of 75% coconut oil and 25% sunflower oil in presence of Tween 80 and MDGs. The intention for the work was to investigate both a bulk fat blend and emulsifiers that have relevance for the production of ice cream; as such this was considered a starting point to understand the effect in an emulsified ice cream.

This is a novel area of investigation for two reasons: 1) the thermal behaviour of this blend has not been investigated previously and an understanding of the effect of the presence of liquid oil on the crystallisation of coconut oil is required as it can decrease its crystallisation and increase its melting temperature (Norton *et al.,* 2009), and 2) the effect of these two emulsifiers on the thermal behaviour of this fat blend has also not been investigated previously, and the effect of chain length and hydrophobicity of the head is interesting and useful for the scientific community as it is likely to have an impact upon the microstructure of ice cream.

Our hypothesis is that Tween 80 interferes with the fat crystallisation (due to the unsaturated carbon chain and large hydrophilic head), whereas MDGs co-crystallise with the fat (due to the structural homogeneity with the bulk fat), favouring its crystallisation. The results are likely to have many applications in the ice cream industry. This fat blend is an economic substitute to milk fat as it mimics milk fat it in terms of partial coalescence, meltdown behaviour and flavour.

3.2 Materials and methods

3.2.1 Materials

Sunflower oil was purchased from a retailer (Sainsbury's, UK); coconut oil was purchased at Akoma International LTD (UK). MDGs (product number: 149563) were purchased at Danisco (UK) Ltd and Tween 80 (product number: 9005656) was purchased at Croda LTD (EU). MDGs were palm based (saturated 16-carbon chain) and mono glycerides represent more than 60%. The most abundant fatty acid in coconut oil and sunflower oil are lauric acid (saturated 12-carbon chain) and linoleic acid (unsaturated 18-carbon chain) respectively.

3.2.2 Preparation of the fat-emulsifier blends

The fat blend used was a blend of coconut oil 75% and sunflower oil 25% (bulk fat). Emulsifiers, Tween 80 and MDGs were dispersed at different ratios (emulsifier/bulk fat of 0.02, 0.05, 0.08, 0.1, 0.3, 0.6 and 1) in the melted bulk fat and stirred with a magnetic stirrer at 80 °C for approximately 20 min, until a homogeneous sample was obtained.

3.2.3. Differential scanning calorimetry

The effect of the emulsifiers on the thermal behaviour of the bulk fat used was determined using differential scanning calorimetry (DSC), a Perkin Elmer DSC Series 7 (UK), with thermal analysis software (Pyris). Nitrogen was used as a purge gas, at a flow rate of 30 ml/minute. The thermal behaviour of MDGs was also measured. The samples (8-10 mg) were loaded into Perkin Elmer 40 µl capacity aluminium pans, and sealed with aluminium covers; an empty pan was used as a reference. The following thermal program was used: holding isothermally at 70 °C for 10 minutes, cooling from 70 °C to -30 °C at 10 °C/minute and then

heating from -30 °C to 70 °C at 10 °C/minute. The ΔH (J/g) was calculated using the thermal analysis software (Pyris). The DSC scans shown in all figures have been normalised according to total mass and mass of crystalline material (i.e. amount of coconut oil and MDGs). In order to determine if the ΔH (J/g) of crystallisation and melting of MDGs were independent from those of the bulk fat, predicted (theoretical ΔH according to the mass of crystallising material) and experimental enthalpies for samples containing different MDGs to bulk fat ratios were determined.

3.3 Results and discussion

3.3.1 Thermal behaviour of the bulk fat in presence of MDGs

In this section the effect of the addition of MDGs on thermal behaviour of the bulk fat (specifically the shape of the melting and crystallisation peaks, peak temperature and enthalpy) will be discussed.

As can be observed, on cooling the bulk fat crystallised in two peaks, the first at ~-5.5 °C and the second at ~-16.5 °C (see **1** and **2** in Fig. 3.1) and on heating there was a main peak at ~19 °C with shoulder at ~6 °C (see Fig. 3.2). Very similar results were obtained by Tan and Che Man (2002) who studied the thermal behaviour of coconut oil with DSC using the same scan rate. Our melting and crystallisation peaks are slightly lower due to the effect that the liquid oil has on the crystallisation of solid fat, a well-known phenomenon (Norton *et al.*, 2009). We can assume that the two crystallisation peaks and the presence of a shoulder in the melting curve is due to the presence of two different TAGs, a higher and a lower melting fraction, where the higher fraction exhibits slower melting and more rapid crystallisation (Tan & Che

Man, 2002). The MDGs melted between ~50 °C and ~60 °C and crystallised between ~40 °C and ~55 °C (see Fig. 3.1 and 3.2), thus at higher temperatures than the bulk fat.

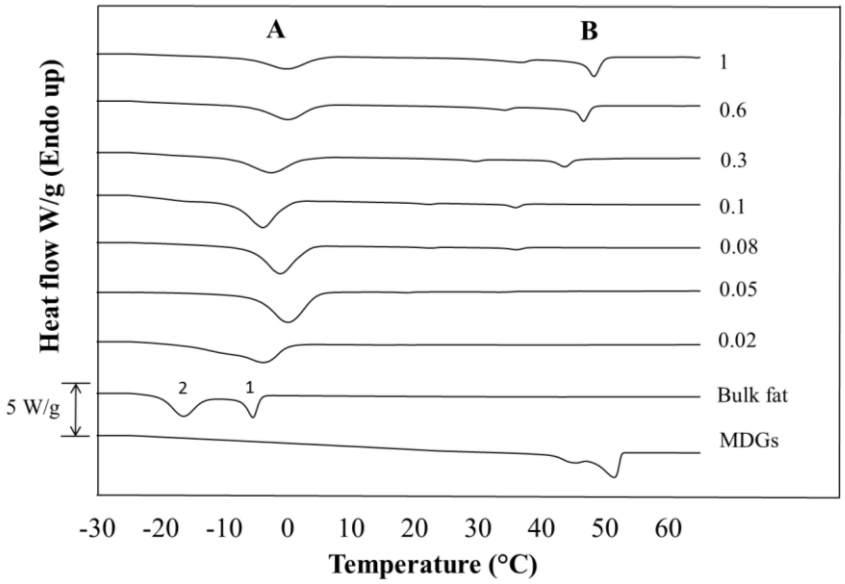

Fig. 3.1 Thermal behaviour of MDGs, bulk fat, and different ratios MDGs to bulk fat during cooling from 70 °C to -30 °C at a scan rate of 10 °C/minute.

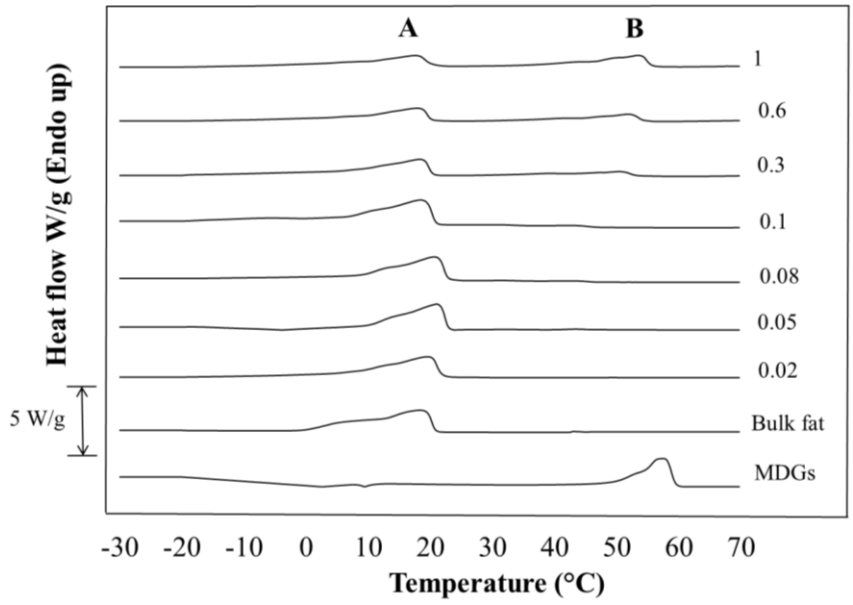

Fig. 3.2 Thermal behaviour of MDGs, bulk fat, and different ratios MDGs to bulk fat during heating from -30 °C to 70 °C at a scan rate of 10 °C/minute.

The thermal behaviour of the bulk fat changed with the addition of different quantities of MDGs. In the crystallisation curves (see Fig. 3.1) the two exothermal peaks of the bulk fat were replaced by a single peak at around ~0-5°C. Moreover, with the addition of greater quantities of MDGs a second peak appears on both melting and crystallisation, one in the position of the bulk fat peak (see '**A**' in Fig. 3.1 and 3.2), and the other at a higher temperature representative of the MDGs (~35-50 °C for the crystallisation and ~40-57 °C for the melting thermograms; see '**B**' in Fig. 3.1 and 3.2). The melting and crystallisation peaks in position '**B**' (see Fig. 3.3B) shifted to higher temperature with the addition of MDGs (logarithmic R^2= 0.97 and 0.93 for melting and crystallisation, respectively). However, the peak temperatures were always lower than the MDGs alone, probably due to the effect that the sunflower oil exerted on it.

Given the presence of the peaks in position '**B**' it was assumed that there was independent melting and crystallisation of the bulk fat and the MDGs. In order to have a clearer understanding of the behaviour observed, experimental enthalpies of peaks in position '**B**' were calculated and compared to predicted enthalpies calculated assuming that this peak was as a result of melting or crystallisation of the MDGs alone. As the experimentally measured enthalpies were very similar to the predicted ΔH's (see Fig. 3.4) it was concluded that MDGs melt and crystallise independently from the bulk fat, with the peak in position '**B**' being a result of the melting or crystallisation of the MDGs only.

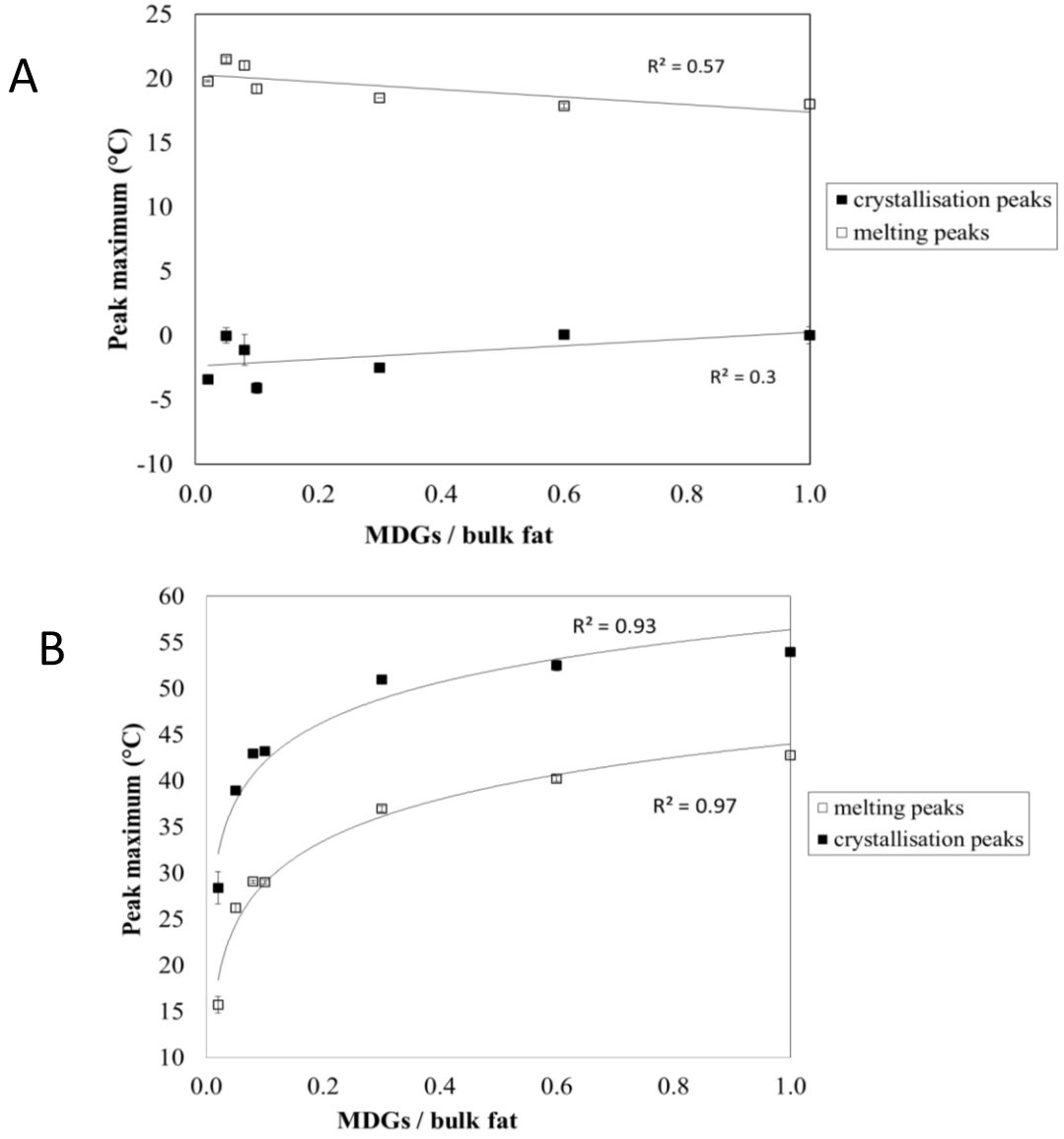

Fig. 3.3 Crystallisation and melting peak maximums in position A (A) and B (B) (see Fig. 3.1 and 3.2) as a function of MDGs to bulk fat ratio during cooling from 70 °C to -30 °C and heating from -30 °C to 70 °C at a scan rate of 10 °C/minute. The graph shows means (n=6) ± one standard deviation.

Nevertheless, on the addition of MDGs there was still an effect on the melting and crystallisation of the bulk fat as the shape of the curves in position '**A**' changed (see Fig. 3.1 and 3.2), even if the melting peak maximum (see Fig. 3.3A) and the enthalpies (see Fig. 3.5) did not change significantly (linear R^2 = 0.57 and 0.3 for melting and crystallisation peak maximums and linear R^2 = 0.08 and 0.01 for melting and crystallisation ΔH, respectively,

indicating poor correlations between the ratio of MDGs to bulk fat for both peak maximum and ΔH).

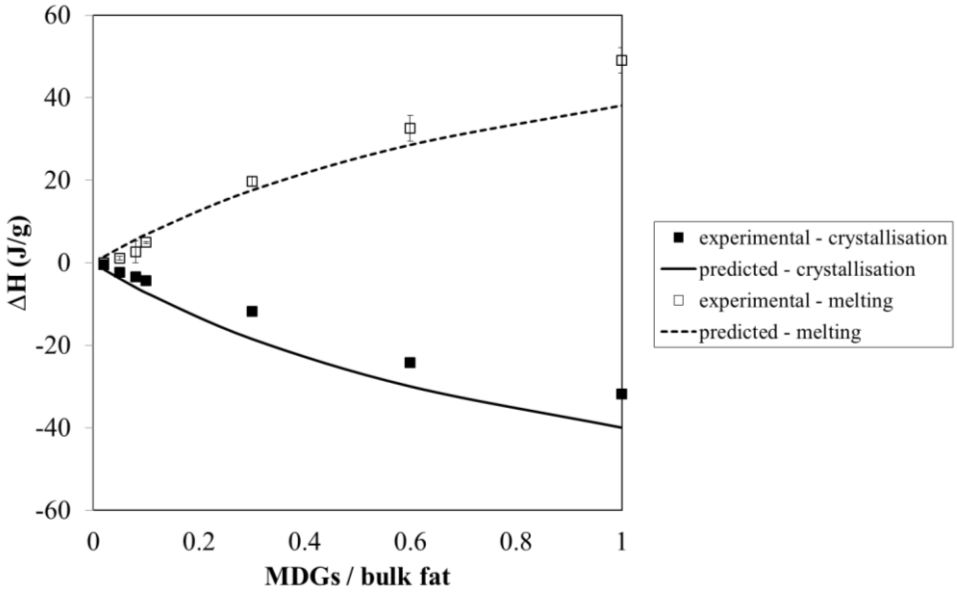

Fig. 3.4 Predicted and experimental ΔH's of the melting and crystallisation peaks in position B (see Fig. 3.1 and 3.2) as a function of MDGs to bulk fat ratio during cooling from 70 °C to -30 °C and heating from -30 °C to 70 °C at a scan rate of 10 °C/minute. The graph shows means (n=6) ± one standard deviation.

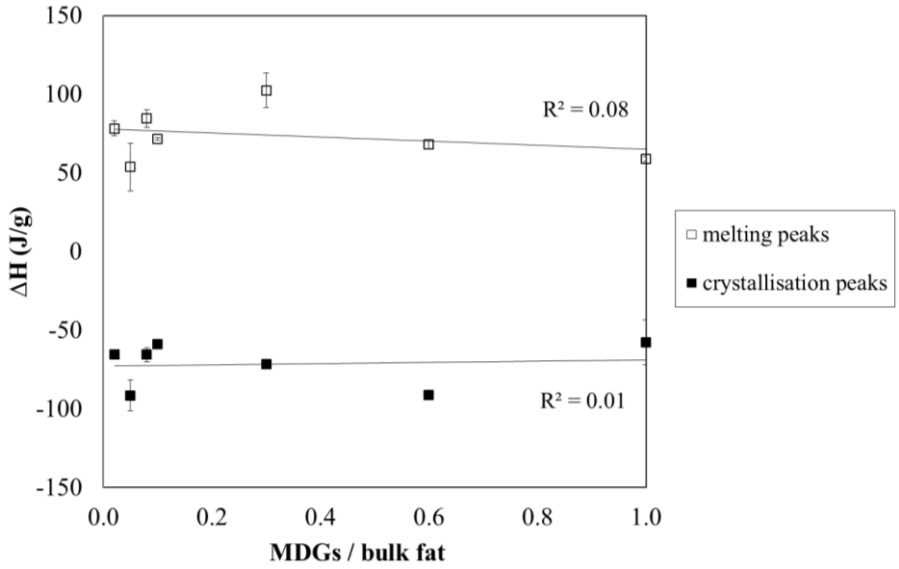

Fig. 3.5 ΔH (J/g of total crystalline material) of the crystallisation peaks in position A (Fig. 3.1) and melting peaks in position A (Fig. 3.2) as a function of MDGs to bulk fat ratio during cooling from 70 °C to -30 °C and heating from -30 °C to 70 °C at a scan rate of 10 °C/minute. The graph shows means (n=6) ± one standard deviation.

These results suggest the presence of two phenomena: an independent melting and crystallisation of MDGs and the bulk fat and a templating effect exerted by MDGs. MDGs crystallise first in the form of reverse micelles (Fredrick *et al.*, 2008) which act as templates for the crystallisation of the bulk fat, resulting in more rapid growth of the bulk fat crystals. A schematic representing this effect is shown in Fig. 3.6A. This hypothesis is supported by the earlier crystallisation of the bulk fat in the presence of MDGs than without this emulsifier (see Fig. 3.1). As already mentioned, without MDGs two crystallisation peaks are observed for the bulk fat due to the presence of two TAGs fractions. In presence of MDGs we observe one peak because when the bulk fat crystallises the MDGs micellar crystals are included in the lattice. This is in accordance with findings reported in the literature (Basso *et al.*, 2010, Foubert *et al.*, 2004). Nevertheless, our results also show an independent melting and crystallisation of bulk fat and MDGs that has not been showed previously, and represent a novel area for future investigations.

MDGs are currently used in the ice cream production. In addition to providing understanding of the effect of this emulsifier on the thermal behaviour of the bulk fat, this study is useful in the context of ice cream production: with the addition of MDGs the fat crystallisation occurs earlier, so the fat will be completely crystallised by the end of the commercial freezing process (~5 °C to -8 °C). Earlier crystallisation enhances the destabilisation phenomenon. It is likely that the fat droplets would have then large protruding crystals, which would easily interact with other fat droplets giving rise to partial coalescence. Partial coalescence is in turn important because it contributes to some of the characteristics of the final product, such as the speed of melting, the degree of shape retention during melting and smoothness during

consumption (Goff & Hartel, 2013). The effect of MDGs on the thermal behaviour and structure of ice cream would be an interesting area for future research.

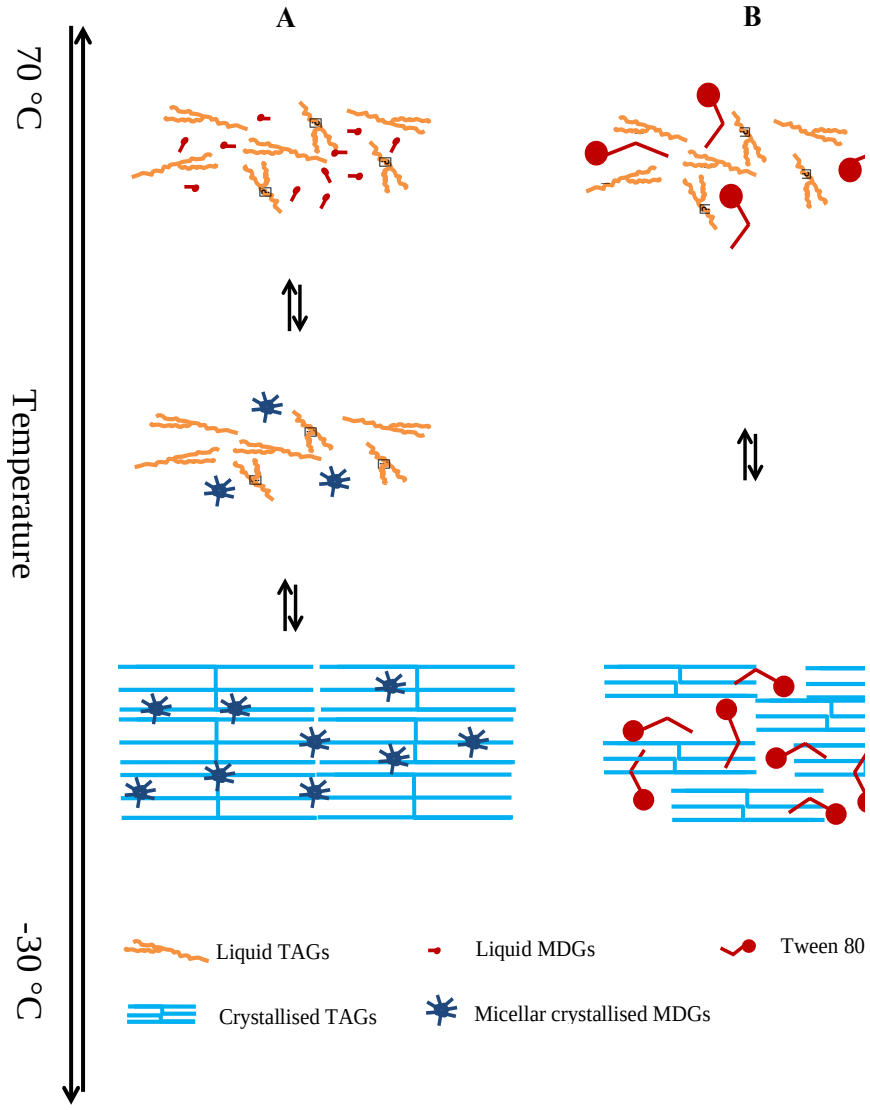

Fig. 3.6 Schematic representation depicting the interaction between the emulsifiers and the lipid during crystallisation on cooling from 70 °C to -30 °C (A: MDGs, B: Tween 80).

3.3.2 Thermal behaviour of the bulk fat in presence of Tween 80

In this section the effect of the addition of Tween 80 on thermal behaviour of the bulk fat (specifically the shape of the melting and crystallisation peaks, peak temperature and enthalpy) will be discussed.

Fig. 3.7 and 3.8 show the thermal behaviour of the bulk fat with the addition of increasing amounts of Tween 80. As can be observed, the general shape of the curves (i.e. two peaks) did not modify with the addition of Tween 80. However, increasing the ratio of Tween 80 to bulk fat, the exothermic and endothermic energy of melting and crystallisation did differ. As can be seen in Fig. 3.9 increasing the amount of Tween 80 decreased the amount of energy required to melt the fat (linear R^2= 0.83 indicating a strong relationship between Tween 80 to bulk fat ratio and ΔH) and decreased the amount of energy released during crystallisation (linear R^2= 0.74 indicating a strong relationship between Tween 80 to bulk fat ratio and ΔH).

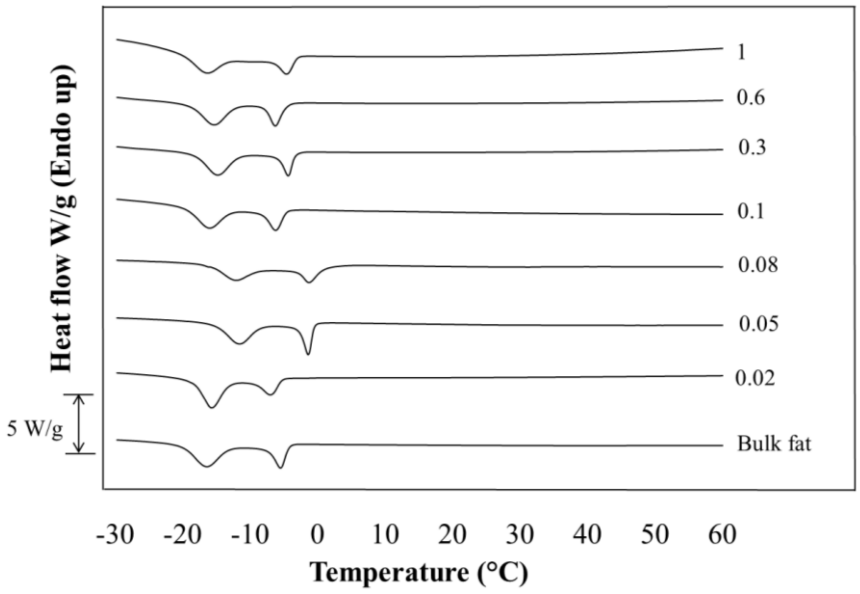

Fig. 3.7 Thermal behaviour of bulk fat and different ratios Tween 80 to bulk fat during cooling from 70 °C to -30 °C at a scan rate of 10 °C/minute.

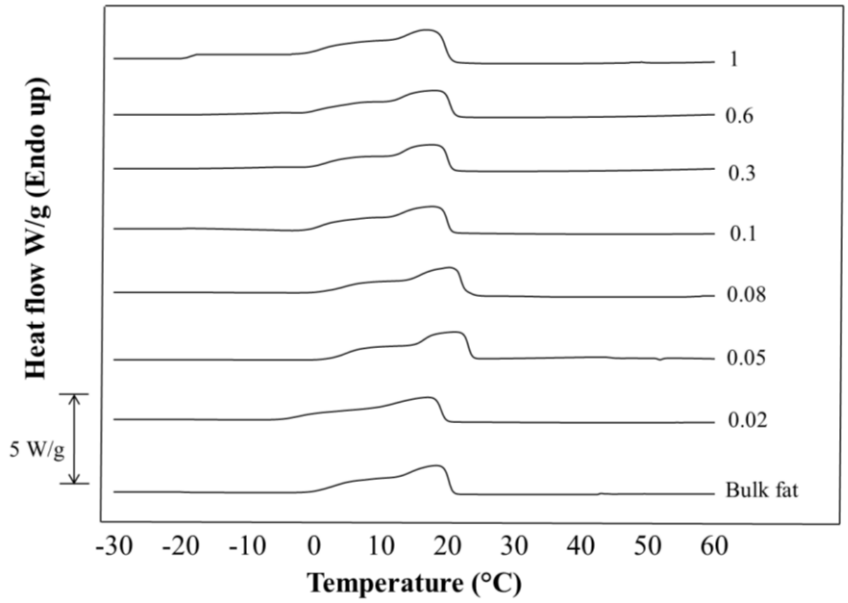

Fig. 3.8 Thermal behaviour of bulk fat and different ratios Tween 80 to bulk fat during heating from -30 °C to 70 °C at a scan rate of 10 °C/minute.

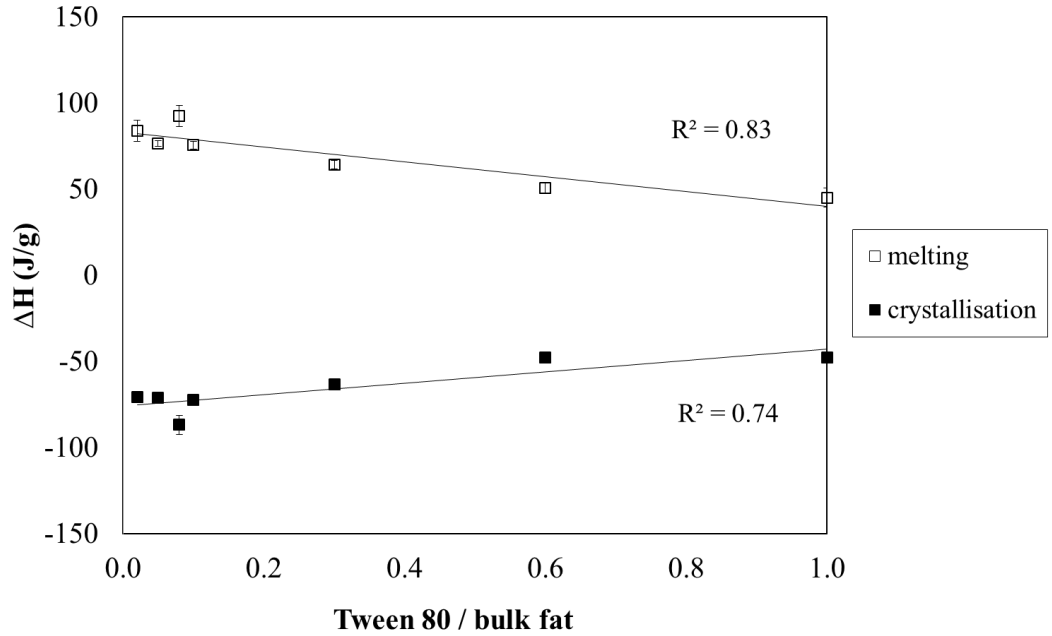

Fig. 3.9 ΔH (J/g of crystalline material) of melting and crystallisation peaks (see Fig. 3.7 and 3.8) as a function of increasing ratio of Tween 80 to bulk fat during cooling from 70 °C to -30 °C and heating from -30 °C to 70 °C at a scan rate of 10 °C/minute. The graph shows means (n=6) ± one standard deviation.

It is hypothesised that Tween 80 acts as a liquid impurity that is incorporated into the lattice during crystallisation. As a consequence it leads to the formation of less perfect crystals. The imperfection of these crystals would explain the decrease in the ΔH of the exothermic and endothermic peaks: the energy required and released is lower in lattices with more loosely packed crystals. Tween 80 has a large hydrophilic head (due to the polyoxyethyleted sorbitol) and a kinked carbon chain (because it is unsaturated), and both these features interfere with the crystallisation of bulk fat.

It should be highlighted that the effect of MDGs and Tween 80 on the thermal behaviour of the bulk fat was different. MDGs crystallised independently from the bulk fat (i.e. the energy required to melt and crystallise the bulk fat did not change). On the other hand, Tween 80 acted as a liquid impurity decreasing the energy required to melt and crystallise the bulk fat.

In the literature, unlike MDGs, there are few studies considering the effect of Tween 80 on fat crystallisation, but it is known that Tween 80 can delay fat crystallisation (Dickinson & McClements, 1996). Whilst this work does not indicate a delay in the crystallisation, it does suggest interference of this emulsifier with crystal packing. A schematic representation of the effect of Tween 80 on lipid crystallisation is shown in Fig. 3.6B.

These results, in addition to those obtained for MDGs are useful not only to understand the effect of the emulsifier on the thermal behaviour of the bulk fat, but also provide information relevant to ice cream production. As Tween 80 results in the formation of less perfect crystals it is likely to decrease fat destabilisation: the crystals protruding from the fat globules will be less structured and this could lower their interaction with the surrounding fat droplets

decreasing the partial coalescence phenomenon and having a negative influence on the final product.

3.4 Conclusions

Both the emulsifiers investigated have an effect on the melting and crystallisation of the bulk fat. MDGs melt and crystallise independently from the bulk fat, but have an effect on its thermal behaviour, acting as templates for the crystallisation of the bulk fat. Tween 80 acts as an impurity, leading to the formation of less perfect crystals in the bulk fat. When used for the production of ice cream MDGs could enhance fat destabilisation more than Tween 80 (because of the more structured fat crystals developed) and so lead to a better final product in terms of fat network, meltdown behaviour and stand-up properties. For this reason, MDGs have been chosen to carry on this research on an ice cream system: in the next chapter this surfactant was compared with some of its esters to investigate the effect of the HLB number on microstructural and physical properties of ice cream.

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CHAPTER 4

Effect of Formulation on Ice Cream Microstructure and Physical Properties

Rizzo G, Gonzalez Espinosa Y, Norton JE, Norton IT

Abstract

The effect of different surfactants varying in their hydrophilic–lipophilic balance (HLB) number on the microstructural and physical properties of ice cream (hardness, overrun, meltdown behaviour, shape retention and fat droplet size distribution) was investigated.

Hardness measurements showed that both low and high HLB number surfactants led to the formation of a more structured fat network. The hydrophilic nature of high HLB number surfactants limits their adsorption to the fat globule and so these dissociate during shearing causing poor coating of the fat droplets and promoting fat destabilisation. Very low HLB number surfactants have a less steric-bulky hydrophilic head so that thinner layers between fat globules can be formed bringing the fat globules in close proximity and making them prone to fat destabilisation. The presence of a more structured fat network in these samples was confirmed by a slower meltdown rate and a higher fat agglomeration measured by light scattering. There were no differences in overrun and shape retention between samples, suggesting that the differences observed in the fat network had a slight impact on ice cream properties.

Results from this study can be useful for ice cream manufactures in the choice of ice cream formulation.

Keywords: Ice cream, HLB number, surfactants, fat network

Industrial relevance

This study can be a useful reference for ice cream manufacturers when selecting the surfactant to employ in their ice cream formulation. Both surfactants with low (i.e. 2-3) and high (i.e. 10-12) HLB numbers lead to a product with a more structured fat network in comparison with surfactants with intermediate HLB numbers (4-5 and 6-8). As the fat network has been associated in past studies with creaminess, these surfactants should be preferentially chosen because they could lead to an increase of the palatability of the final product and also the sales.

4.1 Introduction

Ice cream is a complex food product in which the oil-in-water emulsion is frozen and foamed giving rise to a group of dispersed phases (fat droplets, ice crystals and air bubbles) all contained within the same continuous phase (Goff, 1997).

In ice cream, emulsifying agents are required because, being surface-active substances, they absorb onto the fat droplets reducing the free energy at the surface between the immiscible phases (by reducing the interfacial tension) and avoiding droplets aggregation (McClements, 2009). Ice cream formulations contain mainly two emulsifying agents, proteins (complex macromolecules) and surfactants (lipid-like molecules). Surfactants are better emulsifying agents than proteins for a variety of reasons. It is known that effective emulsifying agents must adsorb onto emulsion droplets quite rapidly (the faster the adsorption rate, the smaller

the size of the droplets). The absorption is primarily dictated by the molecular weight of the emulsifying agents. Surfactants, possessing a lower molecular weight, have greater mobility through the bulk in comparison with proteins allowing for a more rapid adsorption. Once absorbed, it is the interfacial layer formed by the emulsifying agents to stabilise the emulsion lowering the interfacial tension and avoiding coalescence. Proteins, upon absorption, are slower than surfactants to form an interfacial layer because of their re-alignment (they rearrange themselves to the most entropically stable state with the hydrophobic residues in contact with the fat and the hydrophilic residues in contact with the continuous phase). On the other hand, surfactants, having a simpler structure, allow for a more rapid interfacial layer formation. Moreover surfactants, thanks to their structure, can form a better packed interfacial layer than proteins, limiting the contact between the immiscible phases, i.e. lowering more the interfacial tension. Once the interfacial layer has been formed, the Gibbs-Marangoni effect can cause emulsion destabilisation. In fact, when two droplets approach each other, the continuous phase is forced out of the gap separating them. When this happens, the liquid drags some of the emulsifiers along the droplet surface, leading to the formation of a region (on the droplet surface) where the emulsifier concentration is low. This causes an increase of the interfacial tension which is thermodynamically unfavourable. For this reason, emulsifier molecules flow toward that region dragging some of the continuous phase along with them. This motion increases the fat droplets stability against coalescence. Surfactants, being more mobile than proteins at the interface can better contrast the Gibbs-Marangoni effect and so better stabilise the emulsion (McClements, 2009).

However, in ice cream formulation there is roughly a protein/surfactant mass ratio of ~12 so that during homogenisation the principal emulsifying agents are proteins, as it has been shown

by measuring the absorbed proteins at the fat surface in a variety of studies (Courthaudon *et al.*, 1991; Euston *et al.*, 1995; Euston *et al.*, 1996). This probably happens because proteins interact more strongly with the fat in comparison with surfactants (Wilde, 2009). In fact, homogenisation is normally carried out at temperatures above the surfactant melting point (~ 60 ºC in ice cream for example), so that they are fluid-like as it has been shown by florescence (Clark *et al.*, 1989). After homogenisation (during cooling and aging), as the surfactants begin to crystallise, they can adsorb more strongly onto the fat droplets and so displace the proteins (Clarke, 2004). For instance, Goff *et al.* (1987) showed a higher quantity of absorbed proteins at the fat surface in unaged than in aged ice creams. Barfod *et al.* (1991) also showed that the amount of proteins at the fat surface decreased during aging and that, in presence of surfactants, the interfacial tension decreased proportionally with a decrease of temperature.

The displacement of the proteins by the surfactants, explained in detail by Morris and Gunning (2008), is an important phenomenon because it promotes fat destabilisation and the formation of a fat network (Goff & Hartel, 2013a). The proteins at the fat interface interact with each other forming a network whose defects can lead to the absorption of the surfactants. The surfactant domains will grow gradually causing first the compression of the protein network that initially remains connected at the interface causing a temporary increase of the interfacial thickness. Nevertheless, the protein network will progressively extend into the aqueous phase until it will be completely expelled into it leaving an interfacial layer occupied solely by surfactants (Mackie *et al.*, 2000) and so thinner than the one developed in presence of proteins only (Fredrick *et al.*, 2010). With this thin interfacial layer between fat droplets it

will be easier for the fat crystals, during freezing, to pierce the surrounding fat droplets leading to the development of a fat network.

During the freezing process an important phenomenon called fat destabilisation takes place and in particular partial coalescence (Goff & Hartel, 2013a). Partial coalescence of the fat globules only occurs when the fat droplets are partially crystalline and when the shear applied leads them to collide. When this happens, the protruding crystals of the fat droplets can penetrate into the surrounding droplets resulting in a junction (Goff, 1997; Walstra, 2003; Fredrick *et al.,* 2010). Differently from full coalescence, the spherical shape of fat droplets is maintained because of their partial crystalline nature and subsequently a fat network is formed (Goff 1997, 2002; Pawar *et al.,* 2012).

The hydrophilic–lipophilic balance (HLB) concept, introduced in 1949 by Griffin, is an important parameter to consider because it is an expression of the attraction of surfactants for the two immiscible phases (Griffin, 1949). The HLB scale normally ranges from 0 to 20; low numbers (2-6) correspond to more hydrophobic surfactants, whereas high numbers (11-15) are characteristic of more hydrophilic surfactants (Bergenståhl B, 2008; Kruglyakov PM, 2000). Most of the studies investigating the effect of surfactants with different HLB number on ice cream have been addressed to understand their effect on fat aggregation and melting rates. Results from these investigations have shown that surfactants with high HLB numbers, compared to those having low HLB numbers, led to higher degree of fat aggregation (Govin & Leeder, 1971; Lin & Leeder, 1974; Goff & Jordan, 1989) and, as a consequence, produce ice creams with lower meltdown rates (Govin & Leeder, 1971). Explanation to this tendency has been that low HLB number surfactants strongly associate to the fat globule, whereas high

HLB number surfactants are only weakly attached to them so that they can dissociate during the freezing step, removing the protection of the droplets and promoting fat destabilisation. This topic has not recently been investigated even when the effect of surfactants with different HLB number on other parameters such as hardness, shape retention, and overrun (i.e. air incorporation) is unknown. The focus, in most of the studies has been instead to understand the effect of the surfactants according their degree of carbon chain unsaturation (Granger *et al.* 2004, 2005; Méndez-Velasco & Goff, 2012).

The aim of this work was therefore to 1) further investigate the effect of surfactants varying in their HLB number but having the same carbon chain on fat destabilisation and meltdown rate and 2) to explore their effect on hardness, overrun and shape retention, as they have not been previously considered. Our hypothesis it that surfactants which form a thin interfacial layer around the droplets (i.e. emulsifiers with high HLB number, as it has been shown by others authors, but also low HLB numbers due to a lower steric repulsion) would lead to the formation of a stronger fat network (higher hardness) which subsequently would lead to a slower meltdown rate and a better shape retention.

4.2 Material and methods

4.2.1 Ingredients

Sunflower oil (SO), sucrose and skim milk powder were purchased from a retailer (Sainsbury's, UK); coconut oil (CO) was from Akoma International LTD (UK). Acetic acid ester of mono glycerides (ACETEM, HLB = 2 – 3, product number: 052799), distilled mono glycerides (DMG, HLB = 4 – 5, product number: 026754), lactic acid ester of mono

glycerides (LACTEM, HLB = 6 – 8, product number: 052566), citric acid ester of mono glycerides (CITREM, HLB = 10 – 12, product number: 093224) and guar gum (product number: 810034) were purchased at Danisco LTD (UK). All the surfactants were palm based, i.e. with a saturated sixteen carbon chain.

4.2.2 Ice cream mix formulation and processing

The ice cream mix composition used in this study is reported in Table 4.1. For ice cream preparation all ingredients were mixed together in the required amounts and were pasteurised at 70°C for 20 minutes.

Table 4.1 Ice cream mix composition.

Ingredient	Amount
Fat (75% CO, 25% SO)	10 wt%
Skim milk powder	11 wt%
Sucrose	12 wt%
Stabiliser (Guar Gum)	0.3 wt%
Surfactant	0.2 wt%
Water	60.5 wt%

Pasteurised mix was emulsified for 20 minutes using a high shear mixer (Silverson L5M, Silverson Machines, LTD, UK) operated at maximum speed (~8500 rpm) with a 0.8 mm pore emulsor screen. Using this method four different mixes were prepared by varying the surfactants (ACETEM, DMG, LACTEM and CITREM).

After the emulsification process the mix was cooled down to 5 °C in an ice water bath and aged at 5 °C for 24 hours. Once aging period was completed, the ice cream was subjected to the freezing process using an ice cream machine (soft served ice cream machine, model 191/P

of Carpigiani, UK). Finally the ice cream produced was stored in the freezer at -25 °C for 24 hours.

4.2.3 Hardness

A Texture Analyser (TA-XT *Plus*, Exponent, Stable Micro Systems LTD, UK) with a 30 kg load cell and a 45° acrylic conical probe was employed to determine the hardness of the samples at -16 °C with a penetration test. Fresh ice cream samples were located into 100 ml (max diameter: 58mm; height: 71mm) beakers filled to the top; therefore sample height was in all cases 71 mm. The surface was made even with the aid of a spatula and the samples were stored at -25 °C. After 24 hours the samples were moved to -16 °C. After another 24 hours at -16 °C the samples were quickly transported one-by-one to the equipment and measured on a work surface at -5 °C to minimize variability due to sample warming. The trigger force used was 3g and the hardness was calculated as the maximum force (Fig. 4.1) required to penetrate a distance of 25 mm at 2 mm/second. Ten measurements were taken for each sample.

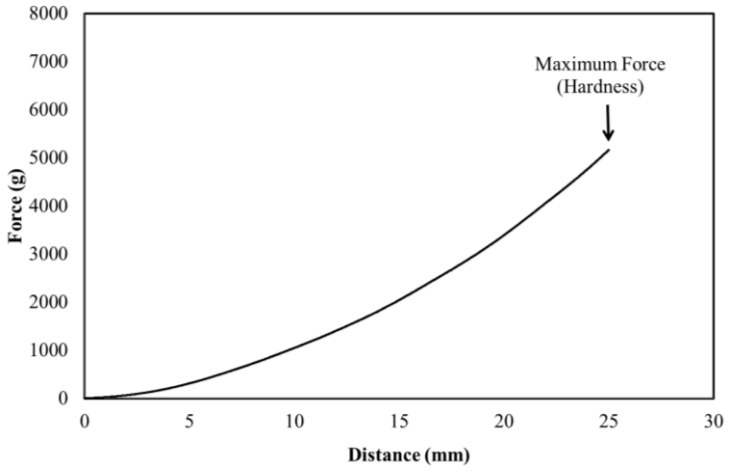

Fig. 4.1 Graph showing hardness (maximum force required to penetrate a distance of 25 mm).

4.2.4 Overrun

The amount of air incorporated into the ice cream matrix (overrun, i.e. increase in volume of mix used to produce the ice cream) was estimated by weighing the ice cream mix and the frozen ice cream in a fixed volume container (250 ml) and then using Equation 4.1 (Goff & Hartel, 2013b)

$$\boldsymbol{Overrun} = \frac{\boldsymbol{weightof\ ice\ cream\ mix - weigth\ of\ ice\ cream}}{\boldsymbol{weight\ of\ ice\ cream}}\ \boldsymbol{x}\ \mathbf{100}\% \qquad \textit{Equation (4.1)}$$

4.2.5 Meltdown test

The meltdown test was adapted from the method of Koxholt *et al.* (2001). All experiments were performed in an acclimatised room at 20°C. The apparatus used consisted of a balance, a beaker, a tripod stand, a plastic funnel and a stainless steel grid (dimensions 160 × 160 × 1 mm; aperture 1.5 mm) mounted as displayed in Fig. 4.2.

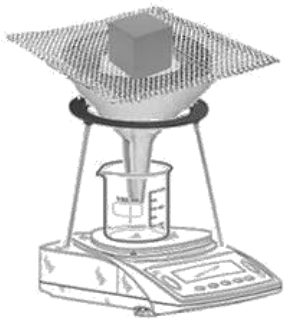

Fig. 42 Diagram showing full set up of apparatus to perform meltdown tests of ice cream.

For preparation of the samples, the ice cream mix obtained from the ice cream machine was put, into containers of cubical shape (5 cm × 5 cm) easy to disassemble and were stored at -25 °C for 24 hours. For each test, the frozen sample was removed from the container, weighted

and immediately placed on the grid. As the ice cream melted, the sample was collected on the beaker and the weight recorded automatically. Recordings were taken every 10 minutes over a 3 hours period. Tests were replicated three times per sample. The percentage of ice cream melted was estimated as the ratio between the mass of the drip loss (in grams) and the mass of ice cream prior to the melting test (in grams) for every time point.

4.2.6 Fat droplet size distribution

Droplet size distributions obtained by dynamic light scattering, using a MasterSizer HYDRO 2000 (Malvern Instruments LTD, UK) for ice cream mix (after the aging step) and for ice cream melted (ice cream, after 24 hours at -25 °C, were placed at 5 °C and allowed to melt for 60 minutes prior measurement. The refractive indexes (measured with an Electronic Refractometer – Rodolph Research Analytical J357) were 1.33 and 1.46 for water and fat, respectively. Each sample was analysed in triplicate at room temperature and the average determined.

4.2.7 Statistical analysis

Analyses were performed using the statistical program "SPSS" for Windows Version 22. One-way between subject Analysis Of Variance (ANOVA) was conducted for hardness, overrun and volume weighted mean with condition (ACETEM, DMG, LACTEM, and CITREM) as within-subject factor. In cases of violated homogeneity of variances (significant Levene test) Welch correction was reported. Contrast effects were assessed using Tukey's test where significant main effect was evident.

4.3 Results and discussion

4.3.1 Hardness

As already mentioned, surfactants with different HLB number are attracted in different ways by oil and water so that they can influence differently the destabilisation of the fat droplets and the fat network formation. The fat network developed provides load bearing structures so that it can be evaluated by measuring the hardness (Tharp *et al.*, 1998; Muse & Hartel, 2004).

As it can been seen from Fig. 4.3, ice creams made with ACETEM and CITREM as surfactants were significantly harder compared to samples produced using LACTEM and DMG (see supplementary material for detailed statistical results). These results suggest the formation of a stronger fat network in samples containing high (i.e. CITREM) and low (i.e. ACETEM) HLB number surfactants in comparison with the other samples.

It can be presumed that this is due to the behaviour of these surfactants at the fat interface during the freezing process as follows. High HLB number surfactants, as suggested in previous studies (Govin & Leeder, 1971; Lin & Leeder, 1974; Goff & Jordan, 1989), being more hydrophilic would attach weakly to the fat droplet and would be more inclined to dissociate during freezing process by the shear applied. Consequently, the fat droplets would be more exposed to destabilisation.

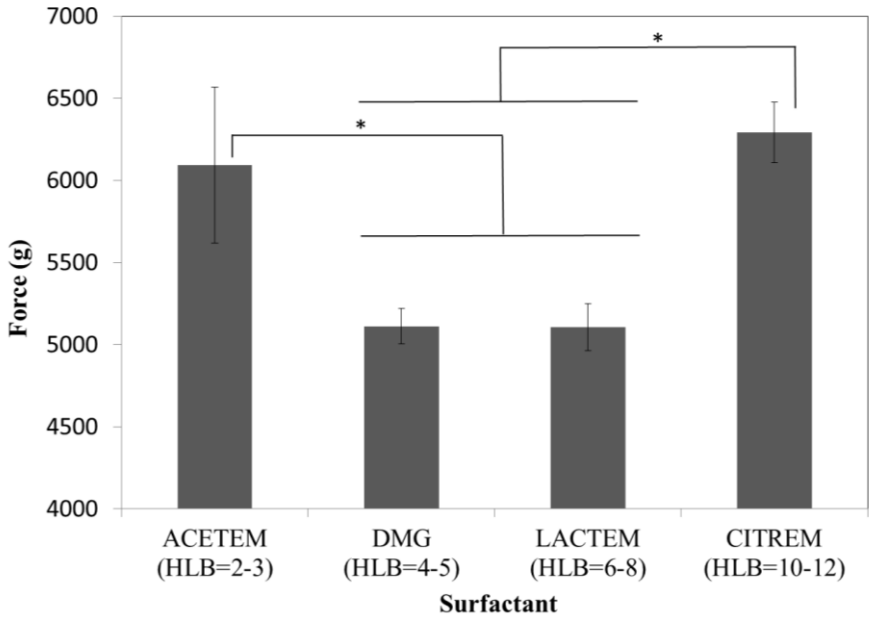

Fig. 4.3 Hardness of ice creams made with different surfactants. The graph shows means (n=10) ± one standard deviation. Significant differences (p≤ 0.05) are marked with an asterisk.

In the present work also the surfactant with very low HLB number (i.e. ACETEM) led to the formation of a fat network comparable to the one developed with the surfactant with high HLB number (i.e. CITREM). We suggest that, given the case of a single layer interface, if the HLB number is very low and the carbon chain is saturated (as in the case of ACETEM), the surfactant would be strongly attached to the fat globules, the interface layer would be very thin and the fat destabilisation would be therefore promoted. In fact, as the carbon chain is saturated, the molecule is more flexible and this flexibility will entail a close packing structure. Moreover, the very low HLB number denotes that 1) the surfactant hydrophilicity is quite low and 2) the steric hindrance of the surfactant hydrophilic head is not pronounced.

A schematic representation of the role of the surfactants' hydrophilic heads in partial coalescence can be observed in Fig. 4.4. In this figure it can be noticed that in the presence of surfactants with high and low HLB number (i.e. ACETEM and CITREM) the interfacial layer

between fat globules will be thinner (in the case of CITREM because the surfactant probably dissociate from the fat droplet during freezing) than the one developed in the presence of DMG and LACTEM. With a thin interfacial layer the fat crystals of a fat globule can easily pierce other fat globules leading to partial coalescence (i.e. the formation of a fat network). Surfactants with intermediate HLB number give rise to a thicker interfacial layer whose thickness increases as the HLB number increases. The thicker the interfacial layer the poorer will be the fat destabilisation, as the fat crystals cannot freely reach the other droplets for piercing.

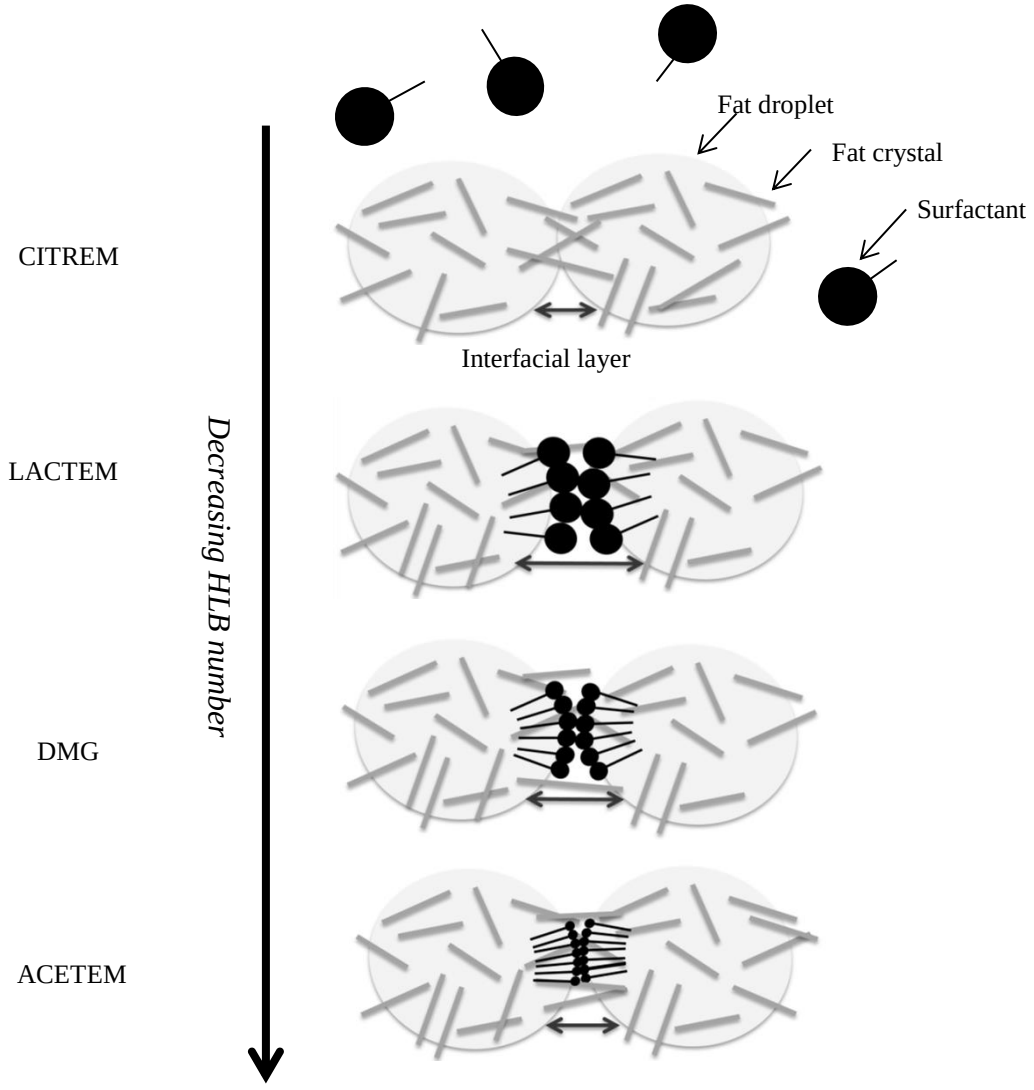

Fig. 4.4 Schematic representation of the role of the surfactant's hydrophilic heads in the partial coalescence phenomenon (not drawn to scale).

It is important to highlight that in the past studies just fat aggregation was measured through for example, spectrophotometry whereas in this study with hardness data it was possible to measure the fat network as a specific form of fat destabilisation.

4.3.2 Overrun

The incorporation of air happens during the freezing step thanks to the shear applied. Once incorporated the air cells must be entrapped into the matrix thanks to molecules that stabilise them against merging. If this does not happen, the air bubbles will merge, increase in dimension and exit the matrix. In ice cream these molecules are principally proteins and fat both in the form of aggregates or single droplets, as was shown in cryo-scanning electron microscopy and transmission electron microscopy by Goff *et al.* (1999).

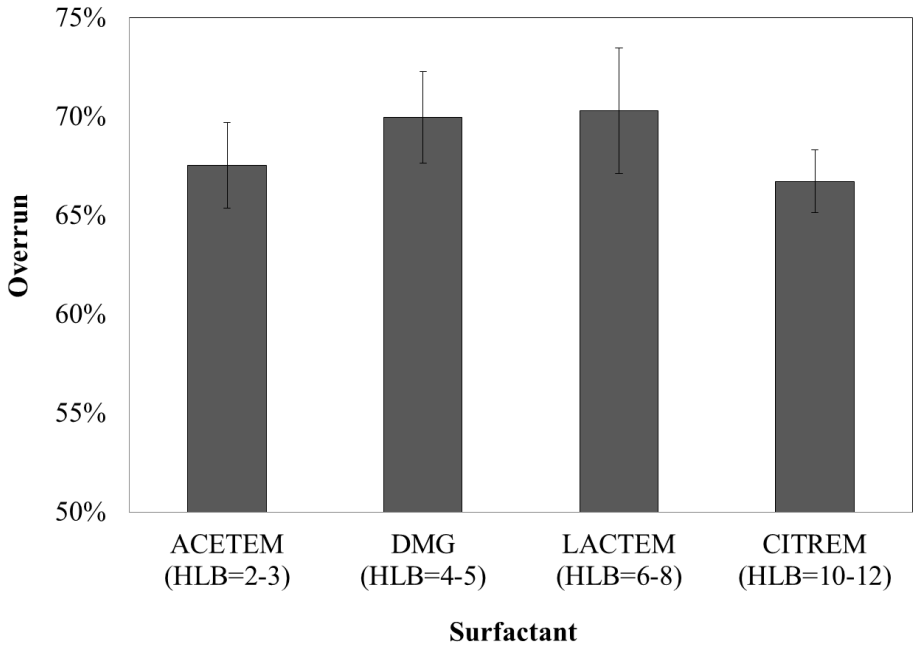

Fig. 4.5 Overrun of ice creams made with different surfactants. The graph shows means (n=3) ± one standard deviation.

The results obtained in this study showed no significant differences (see supplementary material for detailed statistical results) between samples as all reached similar increase in volume (~ 70% overrun) (Fig. 4.5). This suggests that the extent of fat destabilisation does not influence the incorporation of air.

In fact, it is true that a network of partially coalesced fat globules form a layer around the air bubbles' interface stabilising them (Chang & Hartel, 2002) but a direct relationship between fat destabilisation and overrun was not shown in other studies (Goff *et al.* 1999; Sung & Goff, 2010). We think that this happens because the interfacial layer separating the air bubbles (i.e. the steric repulsion) does not change radically with different degree of fat destabilisation.

4.3.3 Meltdown behaviour and shape retention

The fat network influences the meltdown rate and the shape retention of ice cream (Muse & Hartel, 2004; Bollinger *el al.,* 2000) so these have been investigated and results are displayed in Fig. 4.6.

It is shown by Fig. 4.6A that ice creams made with low (i.e. ACETEM) and high (i.e. CITREM) HLB number surfactants melted more slowly in comparison with the other samples. This was due to the more structured fat network indicated in Fig. 4.3. In fact, with a more structured fat network, it is expected that the path undertaken by the water phase to flow and leave the matrix would be more tortuous resulting in slower rates of meltdown. Moreover, the fat network supports the air bubbles during melting resulting in greater retention of shape.

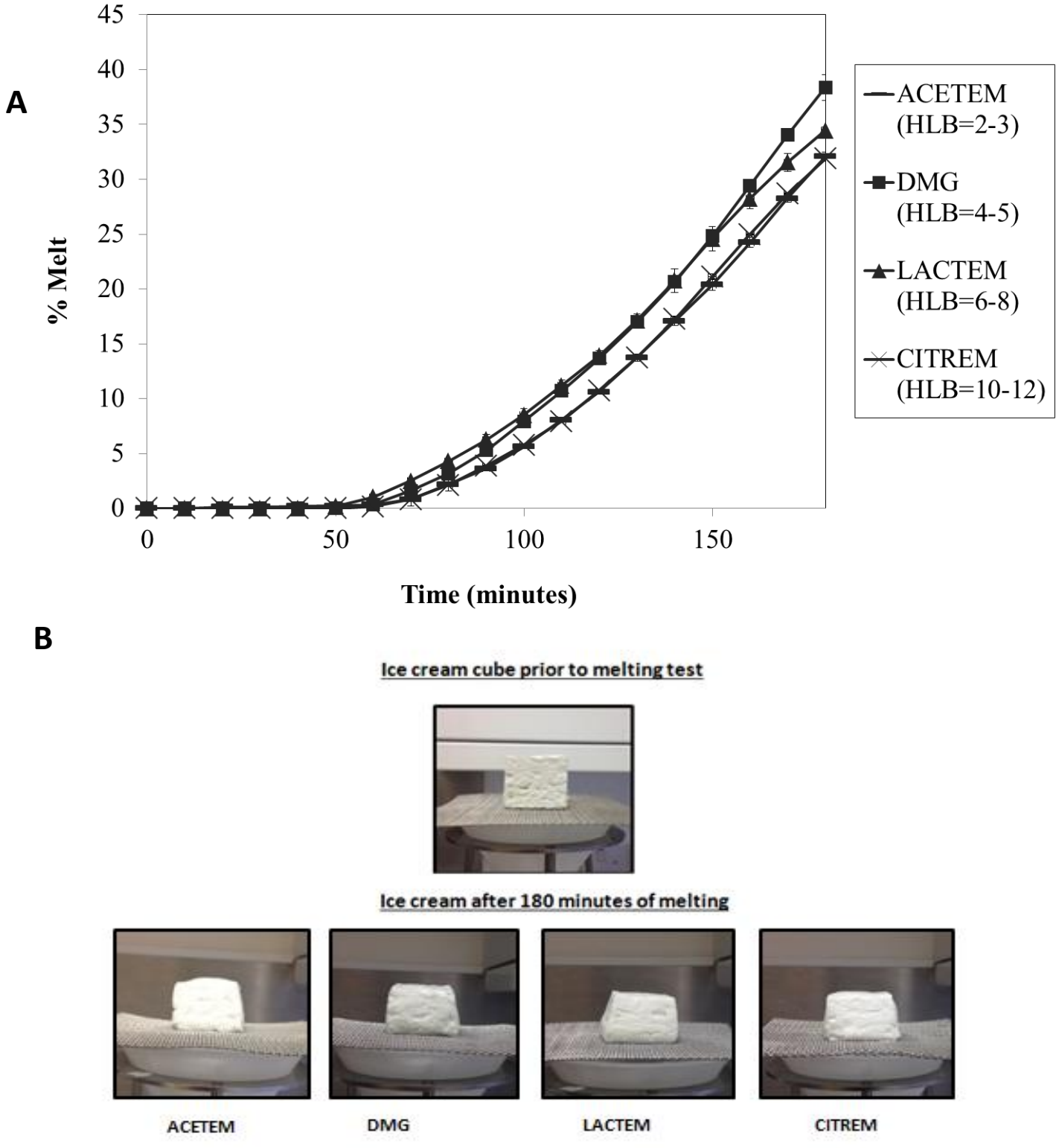

Fig. 4.6 Meltdown rate (A) and shape retention (B) of ice creams made with different surfactants. The graph shows means (n=3) ± one standard deviation.

Govin and Leeder (1971) showed that high HLB number (~12) surfactants led to a product with a lower meltdown rate in comparison with low HLB number (~2) surfactants and attributed this to a higher fat destabilisation taking place. Among the surfactants used in this study, we have seen that not only the high HLB number surfactant (i.e. CITREM) led to a low meltdown rate but also the one at the lowest HLB number extreme (i.e. ACETEM). The discrepancy with the study of Govin and Leeder could be due to the differences in the surfactants carbon chain used in their study. In fact, as aforementioned, saturated carbon chain surfactants, given the case of a single layer interface, could pack more closely (due to their structural flexibility) giving rise to a very thin interfacial layer and enhancing fat destabilisation.

Surprisingly, no difference was observed in the shape retention (Fig.4.6B) of ice creams made with different surfactants suggesting that the fat network variations were too small to be appreciated visually.

4.3.4 Fat destabilisation

In this section the fat droplet size distribution of ice cream mix and ice cream melted (samples after the freezing step, stored at -25 °C for 24 hours and melted at 5 °C for 60 minutes prior measurement) will be shown. The fat droplet size distribution is a measure of fat destabilisation so that it was relevant to investigate this. Nevertheless, it should be highlighted that laser diffraction technique cannot distinguish between scattering by single particles and by clusters of particles. For this reason, this technique is not very useful if used alone but if combined with other measurements (such as hardness, meltdown behaviour, and shape retention) becomes a useful tool for getting a more complete picture of the developed

microstructure of ice cream. The mean of cumulative weighted distribution ($d_{4,3}$) was used with the aim of more clearly compare the destabilisation levels of the ice cream melted samples. As we can see in Fig. 4.7A, the type of surfactant used had not an effect of the fat droplet size distribution of the ice cream mixes which was between ~0.5 and ~5 μm (mono-modal distribution).

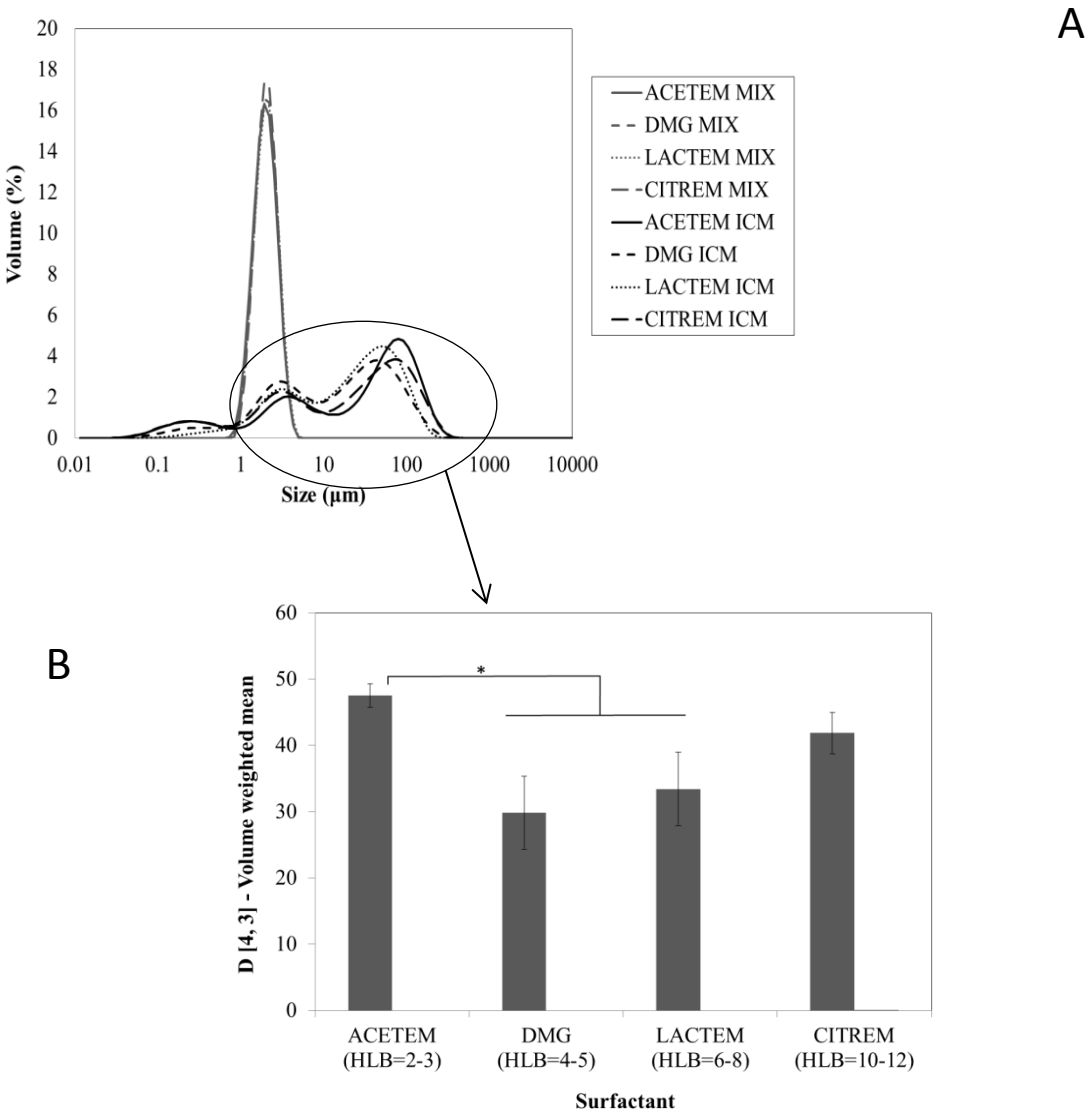

Fig. 4.7 A- fat droplet size distribution of ice cream mix and ice cream melted in the presence of different surfactants; B- weighted mean diameters ($D_{4,3}$) of ice cream melted made with different surfactants. The graph shows means (n=3) ± one standard deviation. Significant differences ($p \leq 0.05$) are marked with an asterisk.

In fact, as explained in the introduction of this chapter, the surfactants in ice cream are not used to emulsify the mix but to de-emulsify it instead.

As it can be observed from Fig. 4.7A&B different emulsifiers had an effect of the fat destabilisation taking place during freezing. The ice creams made with ACETEM and CITREM had a significantly more pronounced fat destabilisation compared to the ice creams made with DMG and LACTEM even if this was just a trend for CITREM in comparison with DMG and LACTEM (see supplementary material for detailed statistical results).

This is expected to be due to the more structured fat network, as shown by a higher hardness of these samples (Fig. 4.3). These results are partially supported by previous works (Govin & Leeder, 1971; Lin & Leeder, 1974; Goff & Jordan, 1989) where it was reported that surfactants with high HLB number led to higher fat aggregation than those with low HLB number. The different behaviour of low and high HLB number surfactants observed in those studies was probably a result of the use of a mix of saturated and unsaturated carbon chain surfactants (Govin & Leeder, 1971; Lin & Leeder, 1974). Different carbon chain saturation could lead, in fact, to different interfacial layers as proposed schematically in Fig. 4.8 and thus influence differently fat destabilisation. On the other hand, the comparison between saturated surfactants was made just for higher HLB numbers (i.e. higher steric hindrance) (Goff & Jordan, 1989).

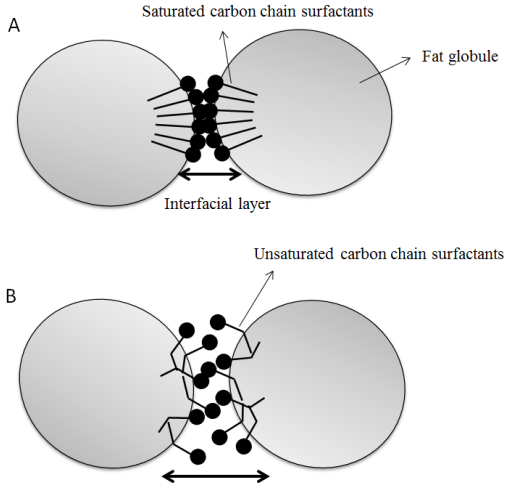

Fig. 4.8 Schematic representation of the interfacial layer between fat droplets developed with saturated and unsaturated carbon chain surfactants (not drawn to scale).

4.3.5 Overall findings

The effect of different surfactants on ice cream properties is summarised in Table 4.2. In particular, it has been shown that surfactants with high and low HLB number and a saturated carbon chain led to the formation of a more structured fat network that exhibited lower meltdown rates in comparison with samples made with intermediate HLB number surfactants.

Table 4.2. Effect of surfactants on ice cream properties as influenced by HLB number. Surfactants sharing the same and letter and framed with dashed line are not different in term of the parameter considered. HLB numbers: ACETEM (2-3), DMG (4-5), LACTEM (6-8), CITREM (10-12).

PARAMETER	Low → High			
Hardness	LACTEM (a)	DMG (a)	ACETEM (b)	CITREM (b)
Overrun	LACTEM (a)	DMG (a)	ACETEM (a)	CITREM (a)
Meltdown	ACETEM (a)	CITREM (a)	LACTEM (b)	DMG (b)
Fat aggregation	LACTEM (a)	DMG (a)	ACETEM (b)	CITREM (b)

4.4 Conclusions

In this study it has been showed that employing different formulations has an effect on the microstructural and physical properties of ice cream because it influences the formation of the fat network. Surfactants with very low and high HLB number are suitable to produce an ice cream with a more structured fat network that will give good stand up properties when exposed to ambient temperature. Differences observed could have an impact on sensory perception because a direct relationship has been observed between partial coalescence and creaminess (Akhtar *et al.,* 2005). These results could be a practical reference to ice cream manufactures to select the right formulation and process to employ.

This chapter evaluated the role of different surfactants on ice cream when keeping constant the fat blend used for its production (75% coconut oil and 25% sunflower oil). Conversely, in the next chapter the surfactant will be kept constant (DMG) while changing the fat blend used.

4.5 Supplementary material

Hardness

Condition * Hardness, main effect: F [3, 13]= 33.139, p< 0.0001;

Interaction: ACETEM * DMG p< 0.0001;

Interaction: ACETEM * LACTEM p< 0.0001;

Interaction: ACETEM * CITREM p= 0.520;

Interaction: DMG * LACTEM p= 1;

Interaction: DMG * CITREM p< 0.0001;

Interaction: LACTEM * CITREM p< 0.0001.

Overrun

Condition * Overrun, main effect: F [3, 8]= 1.673, p= 0.249.

$D_{4,3}$

Condition * $D_{4,3}$, main effect: F [3, 8]= 7.774, p= 0.012;

Interaction: ACETEM * DMG p= 0.015;

Interaction: ACETEM * LACTEM p= 0.045;

Interaction: ACETEM * CITREM p= 0.558;

Interaction: DMG * LACTEM p= 0.772;

Interaction: DMG * CITREM p= 0.055;

Interaction: LACTEM * CITREM p= 0.195.

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CHAPTER 5

Effect of Solid Fat Content and Aging on Microstructural, Physical Properties of Ice Cream and Sensory Perception

Rizzo G, Norton JE, Gonzalez Espinosa Y, Masic U, Halford JCG, Norton IT

Abstract

The effect of different fat blends varying in the ratio of coconut oil to sunflower oil (CO:SO) as well as the effect of aging on the microstructural and physical properties of ice cream have been investigated along with their effect on sensory perception and palatability of the final product. Measured hardness showed that, as the solid fat content (SFC) increases, the ice cream fat network became more structured leading to a slower meltdown rate and good shape retention. Nevertheless, with a high SFC (87%) results suggested that the fat was organised into discrete agglomerates rather than taking part into a network. This was probably caused by the low concentration of liquid fat indispensable for the occurrence of partial coalescence. Aging of the ice cream mix had a minimal influence on the microstructural and physical properties of ice cream so that this step could be avoided decreasing ice cream production time. The sensory study has shown that untrained panellists, probably due to their low discrimination ability and the lack of familiarity with the terms used, could perceive differences between samples just with some sensory attributes and when there were large differences among samples microstructures. Solid fat content had a more dominant effect on the palatability of ice cream than the microstructure developed.

Keywords: ice cream, vegetable fat blends, aging, coconut oil, sensory perception

Industrial relevance

This study can be a useful reference for ice cream manufacturers when selecting the fat blend and the process to use. It was shown that ice cream with a solid fat content (SFC) of 60.5% had the most structured fat network and that the aging of the ice cream mix, having a minimal effect on the final product, could be avoided thus reducing ice cream production time. A reduction of about 40% in SFC, in comparison with a standard ice cream made with milk fat, might be possible without affecting consumers' sensory response and resulting in a healthier product. Palatability of the ice cream was principally influenced by the formulation rather than the microstructure developed.

5.1 Introduction

Ice cream consists of different phases: fat, air, ice, and a concentrated unfrozen serum (Goff, 1997). The three-dimensional structure that provides structural integrity to ice cream evolves during the freezing process. During this step fat destabilisation takes place and in particular partial coalescence, a type of coalescence happening when fat droplets are partially crystalline. In this condition, when the shear forces involved in the freezing step lead individual droplets to collide, their protruding crystals can penetrate into each other resulting in their junction while maintaining their structural identity, with the formation of a fat network (Goff, 1997; Walstra, 2003; Fredrick *et al.*, 2010). The presence of a fat network in ice cream can affect its textural properties and sensory perception. In fact, direct relationship

has been observed between the degree of partial coalescence and the viscosity of an emulsion (Everett 2007) and between the viscosity of an emulsion and its creaminess (Akhtar *et at.*, 2005).

Modifications in the formulation and/or the process have been the scope of many investigations looking to optimize the final product. For example, it has been shown that using different types of fat affects the microstructural and physical properties of the ice cream (Goff *et al.*, 2007; Sung & Goff, 2010; Méndez-Velasco & Goff, 2012) influencing partial coalescence and thus the formation of a fat network. In fact, for partial coalescence to occur both solid and liquid fat are required; as it is not favourable for the fat crystals protruding from a fat globules to be in contact with the water continuous phase, they penetrate the surrounding fat droplets in their liquid portion with the formation of a semi-solid junction. It is the presence of the fat crystals that avoid the fusion of the droplets (Fredrick *et al.*, 2010; McClements, 2016) and the presence of liquid fat to allow the junction between the droplets. If no liquid is present, no junction will be formed (Walstra, 2003; Boode *et al.*, 1993; Boode & Walstra, 1993).

The type of fat also tends to affect 1) the stabilization of air bubbles, where solid fat droplets stabilise the air bubbles at the interface thus affecting the incorporation of air into the microstructure (overrun) (Clarke 2004; Sung & Goff, 2010; Méndez-Velasco & Goff, 2012; Adleman & Hartel, 2001), 2) the meltdown properties (Tharp *et al.*, 1998; Bolliger *et al.*, 2000; Muse & Hartel, 2004), where the presence of a fat network reduce the meltdown rate enhancing the tortuosity of the path undertaken by the serum phase to leave the ice cream

matrix (Goff & Hartel, 2013a), and 3) the hardness of ice cream (Tharp *et al.*, 1998; Muse & Hartel, 2004).

All the steps of the manufacture of ice cream have a relevant influence in the final structure development. One of these steps is "aging" in which the ice cream mix is kept at 5 °C for 16 to 24 hours before undergoing the freezing step. During aging three phenomena occur; 1) the hydration of the stabilizer, 2) the initial crystallization of fat (Goff & Hartel, 2013b; Adleman & Hartel 2001), depending on the fat blend used and 3) the displacement of the proteins by the surfactants at the fat globule interface (Goff, 1997; Barfod *et al.*, 1991; Gelin *et al.*, 1996; Chen *et al.*, 1993). Both the displacement of the proteins by the surfactants and the fat crystallisation are important for the formation of a fat network. In fact, the surfactants provide a thinner interfacial layer between droplets (reducing steric repulsion) (Fredrick *et al.*, 2010) and consequently the fat crystals can more easily pierce the surrounding droplets (Boode & Walstra 1993; Boode *et al.*, 1993).

Since a relationship between microstructure of food and sensory perception is well known (Bhopatkar *et al.*, 2012*;* Van Aken, 2007*)*, it is expected that microstructural changes in ice cream, such as modifications in the fat network, caused by different fat types, have an influence on sensory attributes such as creaminess, thickness, fattiness and hardness of ice cream as well as in the general palatability of the product (Fredrick *et al.*, 2010).

The aims of the present work were 1) to further study the effect of fat blends varying in their SFC on the microstructural and physical properties of ice cream, 2) to investigate the effect of

aging, and 3) to evaluate the effect of different fat blends on sensory perception and palatability of the final product.

This is a novel area of investigation because of the nature of the vegetable fat used and the effect of aging. Coconut oil (instead of palm oil used previously in the literature) has been chosen due to its benefits; it could suppress fat deposition (Gunstone, 2013; Nagao & Yanagita, 2010) and it can prevent the tropical deforestation. Also, when different fat blends are used, aging is an important aspect to consider because the amount of crystalline fat has a significant impact on final structure of the ice cream (Goff, 1988, 1997). Moreover, the effect of different fat blends of solid and liquid fat is not fully understood in terms of, for example, of its actual solid fat content, shape retention of ice cream, fat network formation, fat organisation at air bubble surface and especially sensory perception.

5.2 Material and methods

5.2.1 Ingredients

Sunflower oil, sugar and skim milk powder were purchased from a local retailer (Sainsbury's, UK); coconut oil was purchased from Akoma International LTD (UK). Distilled mono glycerides (DMG, product number: 026754) and guar gum (product number: 810034) were purchased at Danisco LTD (UK).

5.2.2 Ice Cream formulation and processing

The formulation employed to prepare all ice cream mixes is reported in Table 5.1. The 10% of fat contained in the formulation was added as a blend of coconut oil (CO) and sunflower

oil (SO). Five different mixes were prepared varying the ratios of CO to SO (CO:SO) as follows 0CO:100SO, 25CO:75SO, 50CO:50SO, 75CO:25SO, and 100CO:0SO.

Table 5.1 Ice cream mix composition. All the percentages are in weight.

Ingredient	Percentage (%)
Fat	10
Skim milk powder	11
Sucrose	12
Stabiliser (Guar gum)	0.3
Surfactant (DMG)	0.2
Water	60.5

For the preparation of the mixes, all ingredients (together with the corresponding fat blend used in each case) were carefully weighed, mixed and pasteurised at 70 °C for 20 minutes. After pasteurisation, the mix was emulsified for 20 minutes in a high shear mixer (Silverson L5M, Silverson Machines LTD, UK) operated at the maximum speed (~8500 rpm) with a 0.8 mm pore emulsor screen. The emulsified mix was then allowed to cool down to 5 °C in an ice water bath. The cooling rate was determined to be 0.3 ± 0.5 °C/minute. After cooling the sample, two different routes were followed in the process in order to evaluate the effect of aging. In the first case cooled mix was immediately subjected to freezing using an ice cream machine (soft served ice cream machine, model 191/P of Carpigiani, UK). For the second approach cooled mixed was left to age for a 2 hours period at 5 °C prior the freezing step. The time of aging was selected based on previous reports which showed that 1) the major solid fat content (SFC) increase occurs during the first 2 hours of aging with no significant changes observed after this period (Barford *et al.,* 1991), and 2) the displacement of the proteins by the surfactants is expected to be completed after this time (Mackie *et al.,* 2000). Unaged and aged (A) ice cream samples were stored in the freezer at -25 °C.

5.2.3 Differential scanning calorimetry (DSC)

The thermal behaviour of the different fat blends in presence of the surfactant (DMG) was initially determined using Differential Scanning Calorimetry (DSC) at a rate of 2 °C/minute. Samples that crystallized at 5 °C (aging temperature) or below were then analysed with a Differential Scanning Micro Calorimetry (μDSC) at a rate of 0.3 °C/minute to increase precision.

The DSC was a Perkin Elmer Series 8000 (UK), with thermal analysis software (Pyris). Nitrogen was used as a purge gas, at a flow rate of 30 ml/minute. The samples (5-8 mg) were loaded into Perkin Elmer 40 μl capacity aluminium pans, and sealed with aluminium covers; an empty pan was used as a reference. The following thermal program was used: holding isothermally at 70 °C for 20 minutes and cooling from 70 °C to -30 °C at 2 °C/minute. The DSC scans shown in all figures have been normalized according to total mass of crystalline material (i.e. amount of CO). The μDSC was a high-sensitivity SETARAM μDSC7 Evo Microcalorimeter (SETARAM Instrumentation, France). Samples (10-15 mg) were loaded into stainless steel cells and empty cells were used as reference. The program used was the same of the DSC, but at a rate of 0.3 °C/minute.

5.2.4 Nuclear magnetic resonance (NMR)

Solid fat content (SFC) of the fat blends was measured by pulsed Nuclear Magnetic Resonance (pNMR) (Minispec PC/120 series NMR, Bruker, Germany) using an indirect method which uses the signal from the liquid protons only. The 2-cm-tubes were filled with the fat blends at 70 °C, weighed, and inserted into NMR where the SFC was measured when the temperature was equilibrated at 5 °C (after ~30 minutes) and subsequently when the temperature was equilibrated at 50 °C. Samples were measured in duplicate and the average

calculated. The SFC was calculated using equation 5.1 (AOCS, 2009) by 1) measuring the signal of the melted sample (50 °C) with the signal of the same sample after cooling (5 °C) and 2) comparing it with a standard oil (ST) known to be liquid at both temperatures (the standard oil used in this study was sunflower oil, both liquid at 50 °C and at 5°C). :

$$\mathbf{SFC}\ (\%) = \mathbf{100} - \left(\frac{\mathbf{Sample_{5\,°C}} \times \mathbf{ST_{50\,°C}}}{\mathbf{Sample_{50\,°C}} \times \mathbf{ST_{5\,°C}}}\right)\mathbf{x100} \qquad \textbf{Equation (5.1)}$$

Note that the SFC was measured in presence of the surfactant (DMG) as it is well known that this molecule can influence it (Dimmick, 1999; Miskandar *et al.*, 2006; Rizzo *et al.*, 2015).

5.2.5 Hardness

A Texture Analyzer (TA-XT Plus, Exponent, Stable Micro Systems LTD, UK) fitted with a 30 kg load cell and a 45° acrylic conical probe was employed to conduct a penetration test, to measure the hardness of the ice cream at -16 °C. Fresh ice cream samples were located into 100 ml (max diameter: 58mm; height: 71mm) beakers filled to the top; therefore the sample height was in all cases 71 mm. The surface was made even with the aid of a spatula and the samples were stored at -25 °C. After 24 hours the samples were moved to -16 °C for another 24 hours. After this period, the samples were tested one-by-one using the textural analyser. Measurements were carried out at ambient temperature and the samples were placed on a work surface set at -5 °C to minimize variability due to sample warming. Ten measurements were taken per sample. The test was set to start automatically by using a trigger force equals to 3g as this ensured the test started immediately after the probe had made contact with the sample. The hardness was determined as the maximum force required to penetrate a distance of 25 mm at 2 mm/second.

5.2.6 Overrun

The amount of air incorporated into the ice cream matrix (overrun) was estimated using equation 5.2 (Goff & Hartel, 2013c) where weights of ice cream mix and ice cream where determined by weighting an equal volume (250 ml) of each one.

$$\boldsymbol{Overrun} = \frac{\textbf{weightof ice cream mix} - \textbf{weigth of ice cream}}{\textbf{weight of ice cream}} \textbf{ x } \mathbf{100}\% \qquad \textbf{Equation (5.2)}$$

5.2.7 Microscopy

Cryogenic Scanning Electron Microscopy (cryo-SEM) was conducted with a XL-30 FEG ESEM, Philips - UK fitted with a Polaron PolarPrep 2000 cryo preparation chamber. The sample was frozen to < -80 °C with the aid of liquid nitrogen, covered with gold to allow electrical conductivity, and an image was produced by scanning with a focused electron beam.

5.2.8 Melting test

Evaluation of meltdown was conducted adapting the method of Koxholt *et al*. (2001). All experiments were conducted at ambient temperature (20 °C). The apparatus used was a balance, a beaker, a tripod stand, a plastic funnel and a grid (material: stainless steel; size: 16 cm × 16 cm; thickness 1 mm; square side: 1.5 mm). Ice cream samples were taken from the ice cream machine, placed in disassemble cubical containers (5 cm × 5 cm) and left at -25 °C for 24 hours before being placed at ambient temperature on the grid which was placed on the plastic funnel. The funnel was held by the tripod stand on the balance. As the ice cream melted, it passed through the grid, the funnel and finally was collected in the beaker (placed on the balance). The weight displaced on the balance was automatically recorded every 10

minutes for 3 hours. Three repeats were taken per sample and the percentage of ice cream melted was estimated as the ratio between the mass of the drip loss (in grams) and the mass of ice cream prior to the melting test (in grams) for every time point.

5.2.9 Fat droplet size distribution

Droplet size distributions of both ice cream and ice cream mix were measured by dynamic light scattering, using a MasterSizer HYDRO 2000 (Malvern Instruments LTD, UK). The droplet size distribution of the mix was measured at 5 °C after the cooling step and after the aging step. The ice cream (both from aged and unaged mixes), after 24 hours at -25 °C, was placed at 5 °C, allowed to melt for 60 minutes and then measured. The refractive index (measured with an Electronic Refractometer – Rodolph Research Analytical J357) was 1.33 for water and 1.474, 1.47, 1.465, 1.460 and 1.455 for the different CO:SO ratios 0CO:100SO, 25CO:75SO, 50CO:50SO, 75CO:25SO, and 100CO:0SO, respectively. Measurements were performed at ambient temperature and repeated in triplicate.

5.2.10 Sensory and palatability study

Thirty healthy volunteers (twenty males and ten females) were recruited to the study through advertisements at the University of Birmingham. Selection criteria for recruitment of volunteers were based on people being aged between 18 and 55 years with a BMI between 18.5 and 25, non-smokers and without any diet restriction or diet disorder (scoring less than 4 on the Dutch Eating behaviour Questionnaire Restraint - Van Strien *et al.*, 1986) and free from food allergies. Ethical approval was obtained by the University of Birmingham Research Ethics Committee before performing the study and it was conducted in accordance with the standards expressed in the Helsinki Declaration. Written informed consent was obtained from

all subjects. Sample number was determined with power calculations using G*Power for a repeated measures design using a medium (0.25) effect size and powering to 90% power. Calculations indicated that 21 participants were required. 30 participants were recruited to account for any possible withdrawal or exclusion.

A single blind within-subjects design was employed to assess the sensory attributes of all unaged ice creams (creaminess, thickness, fattiness, meltdown rate and hardness) and their palatability (liking). A validated visual analogue scales (VAS) (Flint *et al.,* 2000) of 100 mm line with two extreme anchors: "not at all" and "extremely" was provided to participants who were instructed to draw a vertical line on the scale to indicate their ratings after sample evaluation. Prior to the sensory test, a brief warm-up session was conducted to familiarise assessors with testing procedures. In this session participants were provided with a sheet defining the sensory attributes (Table 5.2) to ensure evaluation procedure for each attribute was fully understood. In addition, a pre-test using a warm-up sample (one of the ice cream samples randomly chosen and different for each participants and each session) was performed prior to the actual assessment.

Samples of ice creams were provided in a transparent plastic shot cup, which was stored at -25 °C and kept at -16 °C for 24 hours before sensory assessment. The samples were served one-by-one and in a randomised order. Participants were instructed to consume only one spoon of ice cream (of an agreed size) and, right after swallowing it, to proceed to rate the sample using the VAS questionnaires provided.

Table 5.2 Sensory attributes and definitions employed to perform sensory assessments of ice cream samples.

Sensory attribute	Definition
Thickness	In a scale from water to honey a thick product is similar to honey in terms of its consistency perceived in the mouth
Creaminess	A creamy product is perceived in the mouth with a homogenous soft and smooth texture
Hardness	A hard product is perceived with a very stiff structure which requires a high force to be compressed in the mouth
Fattiness	A fatty product where a high content of fat can be perceived in the mouth
Meltdown speed	The speed required for the product to melt when manipulated in the mouth

In between samples participants were instructed to use still water as a mouthwash and to spit the water into a provided container.

Statistical analyses were performed using SPSS statistical software for Windows Version 22. As the data were found to be not normally distributed, a non-parametric Friedman (Field, 2013) test was employed to determine if an overall statistically significant difference existed between samples analysed and a post-hoc Wilcoxon Signed-Rank test was performed as a paired comparison test between samples.

5.3 Results and discussion

5.3.1 Characterisation of the fat blends used: thermal profiles and SFC

The thermal behaviour of the fat blends in the presence of 2% of DMG was investigated to determine the crystallisation state of the fat blends (if liquid or crystalline) prior being subjected to the aging step (at 5 °C) in order to determine if the effect of aging (if observed) was due to the fat crystallisation. In fact, as already mentioned in the introduction, during aging a variety of phenomena occur including fat crystallisation and the displacement of the proteins by the surfactants both of which enhance the partial coalescence phenomenon. Nevertheless, the fat crystallisation will depend on the fat blend used: for fat blends that crystallise at a temperature higher or lower than 5 °C, if an aging effect was observed this would not be due to the fat crystallisation because the fat would be completely crystallised or liquid at aging temperature. On the other hand, for fat blends that crystallise around 5 °C, if an aging effect was observed this could be due to the fat crystallisation.

All the fat blends were first scanned using DSC with a cooling rate of 2 °C/minute. Although this cooling rate was higher than that measured in the real cooling step of ice cream production (0.3 ± 0.5 °C/minute), it offered a quick glance into the crystallisation behaviour of the fat blends. Nevertheless, it is well known that fats crystallise at higher temperatures using slower cooling rate (Tan & Che Man, 2002). For this reason, fat blends with a crystallisation temperature around 5 °C (aging temperature) were further analysed using µDSC with a cooling rate of 0.3 °C/minute. As observed in Fig. 5.1A, fat blends with ratios of 100CO:0SO and 75CO:25SO are already crystallised by the time of the aging step (5 °C).

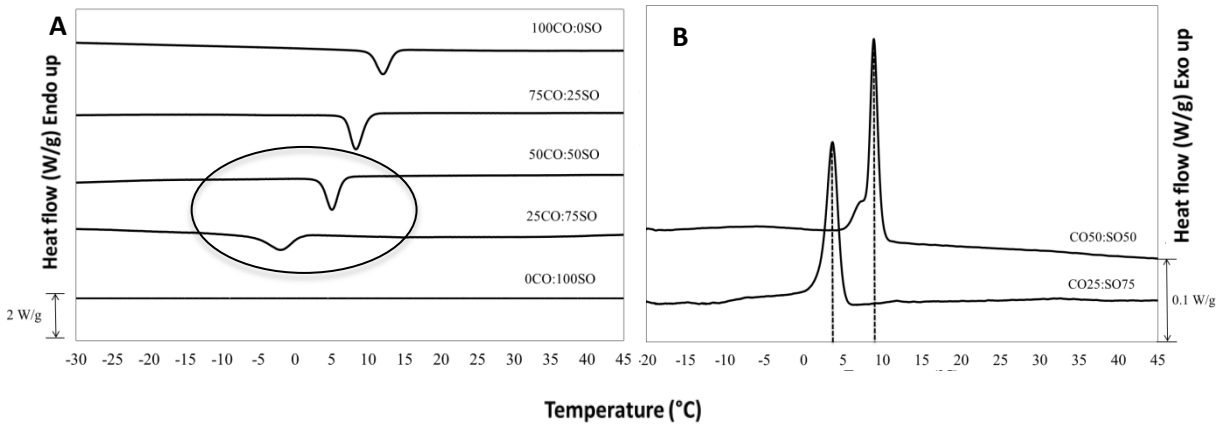


Fig. 5.1 Thermal behaviour of different CO:SO ratios fat blends in presence of 2% of surfactant (DMG) during cooling at 2 °C/minute (A) and 0.3 °C/minute (B).

As such, if the aging step had an effect it will not be due to fat crystallisation in these cases. Blends with ratios of 50CO:50SO and 25CO:75SO (which crystallised around 5 °C – see circle in Fig. 5.1A) were further investigated using µDSC using a cooling rate similar to the one of the production of ice cream (see Fig. 5.1B). The fat blend 50CO:50SO was already crystallised at 5 °C, whereas 25CO:75SO just starts crystallising. Therefore, if the aging step had an effect, in ice creams with a ratio of 50CO:50SO it would not be due to fat crystallisation, whereas in samples with a ratio of 25CO:75SO fat crystallisation might contribute to this.

The thermal behaviour of the fat blends, although being important as fully explained above, does not give any information about the actual SFC. This is an important parameter to consider because, as previously mentioned in the introduction, it influences partial coalescence and so the formation of the fat network (Fredrick *et al.*, 2010). In this study the SFC was measured for each fat blend by NMR, as explained in the material and methods section 5.2.4. As expected, the SFC increased proportionally with an increase of CO (R^2 = 0.99) (Fig. 5.2).

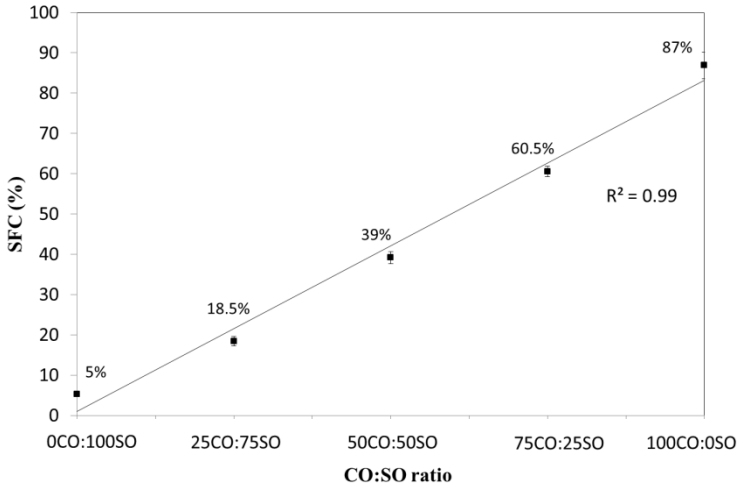


Fig. 5.2 SFC (%) of CO:SO blends in presence of surfactant (2% DMG), as measured by NMR. The graph shows means (n=2) ± one standard deviation.

Notice that the SFC was not 100% for a ratio of 100CO:0SO and was not zero for 0CO:100SO ratio. This was probably due to the presence of some saturated fatty acids in SO and some unsaturated fatty acids in CO.

Systematic errors of NMR, even if minimal, cannot be disregarded. In particular, errors relative to the use of not suitable reference oil are lower than 2% (Haighton *et al.*, 1972). Moreover, a 0.5% lower SFC could be measured because, on crystallising, the iodine value of the remaining liquid increases (Haighton *et al.*, 1971).

The SFC influences the physical properties of ice cream such as hardness and overrun, therefore these have been investigated and discussed in the following sections.

5.3.2 Hardness

SFC directly influences occurrence of partial coalescence and the subsequent formation of a fat network. The fat network can be evaluated by measuring hardness, so consequentially this was investigated.

As appreciated in Fig. 5.3, the ice cream hardness increased proportionally with the SFC (R^2 is 0.96 for unaged samples and 0.81 for aged samples).

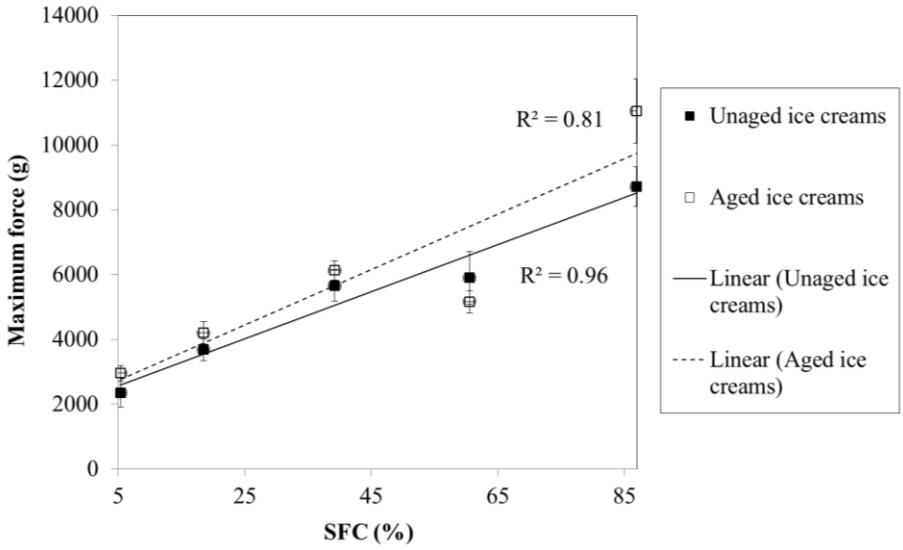


Fig. 5.3 Hardness of aged and unaged ice creams with different SFC fat blends. The graph shows means (n=10) ± one standard deviation.

This increase is due to a stronger fat network developed which provides more load bearing structures per unit volume. Our results are in line with both Tharp *et al.* (1998) and Muse and Hartel (2004) who showed that the hardness of ice cream increased as the level of destabilised fat increased. Nevertheless, different outcomes in the study of Abd-El-rahman *et al.* (1997) were shown. In fact, these authors investigated the hardness of ice creams made with different milk fat fractions and different extent of fat agglomerations. No differences in hardness

among samples were shown and this was attributed to the fact that the majority of the fat in these samples was in a crystalline state at the testing temperature (~-16 °C).

As already mentioned in the introduction section, the aging step would enhance the fat partial coalescence so a hardness increase in aged samples was expected. Nevertheless, as it can be seen in Fig. 5.3, there were no changes in hardness for any of the aged samples except for the sample with a SFC of 87% where the hardness increased. These results suggest that aging did not enhance the fat network formation in any of the samples (except the one with a SFC of 87%) or if it did, this enhancement was too slight to produce an effect in hardness.

5.3.3 Overrun

The SFC is important also for the incorporation of air during freezing: solid fat droplets are more effective at stabilizing air bubbles (Eisner *et al*., 2007; Hotrum *et al*., 2004).

As it can be seen in Fig. 5.4A, the overrun was directly correlated to the SFC (R^2 is 0.89 for unaged samples and 0.94 for aged samples). This suggests that the solid fat directly influences air incorporation. Air is introduced during the freezing process thanks to the shear applied. During this process, solid fat would provide 1) a more solid-like interfacial layer between air bubbles, which sterically stabilise air bubbles against coalescence and 2) an enhancement of the system viscosity (Munk & Andersen, 2015), which stabilises the microstructure kinetically (air bubbles become less prone to migrate and exit the matrix, limiting overrun). The interpretation of these results is supported by the SEM images of the air bubble surfaces of unaged samples (Fig. 5.4B). These images show that the surface of the air bubbles of samples with low SFC (5%) appeared smooth, whereas as the SFC increased, more structured fat droplets were observed. In fact, with low SFC the fat droplets, being more liquid-like,

could spread on the air bubble surface (forming a thin membrane between air bubbles). Increasing the SFC, the fat droplets become more solid-like, forming a more structured membrane which stabilises better the air bubbles against coalescence (i.e. increasing overrun).

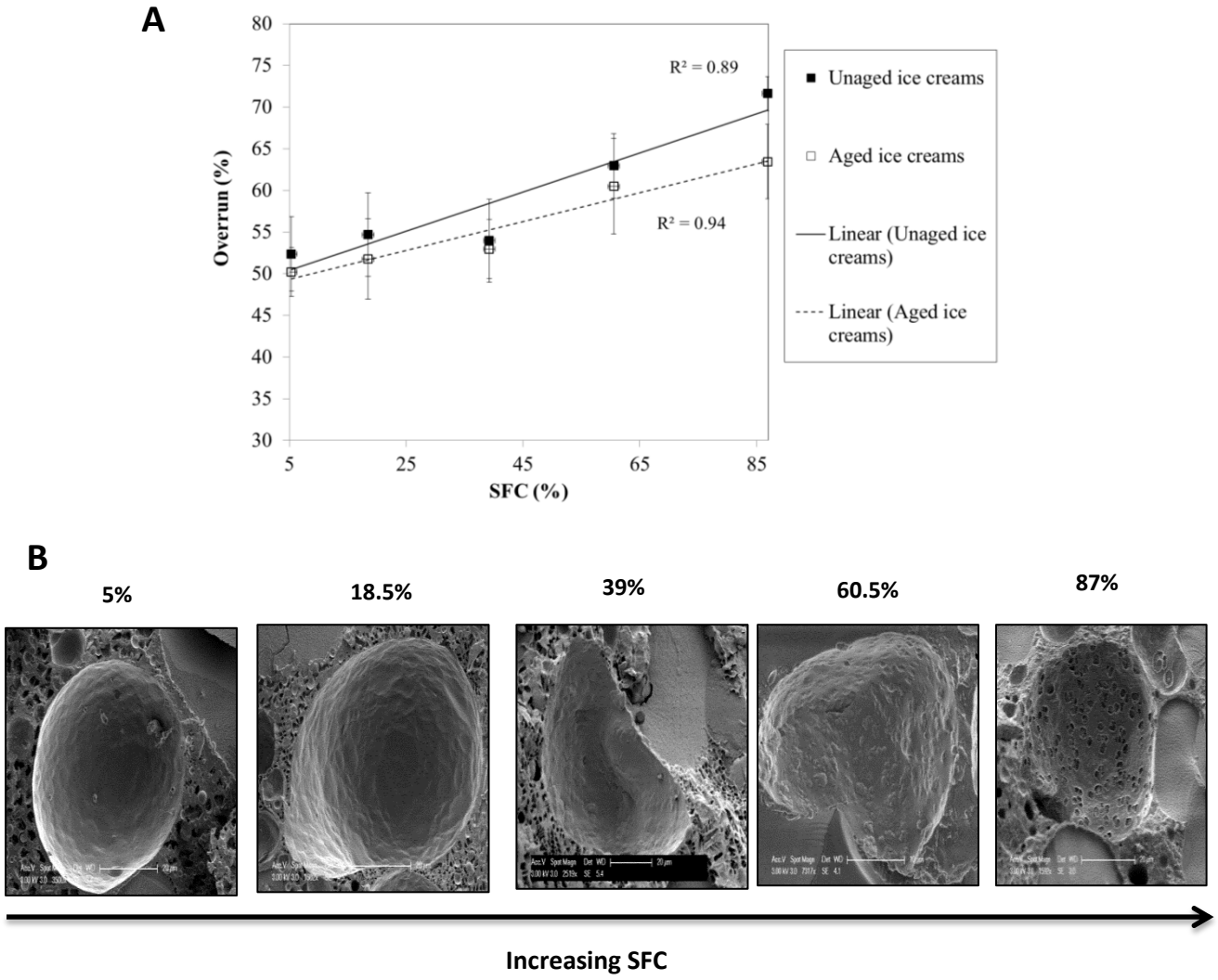


Fig. 5.4 A-Overrun of aged and unaged ice creams made with different SFC fat blends. The graph shows means (n=3) ± one standard deviation. B-SEM images showing the air bubble surface of unaged ice creams with varying SFC.

Similar results have been shown by Sung and Goff (2010) and Méndez-Velasco and Goff (2012) whereas Adleman and Hartel (2001) reported that the solid fat negatively affects the overrun. This was attributed to the protrusion of the solid fat crystals located in the surface of the air bubble that can pierce other air bubbles and cause their merging. This difference could

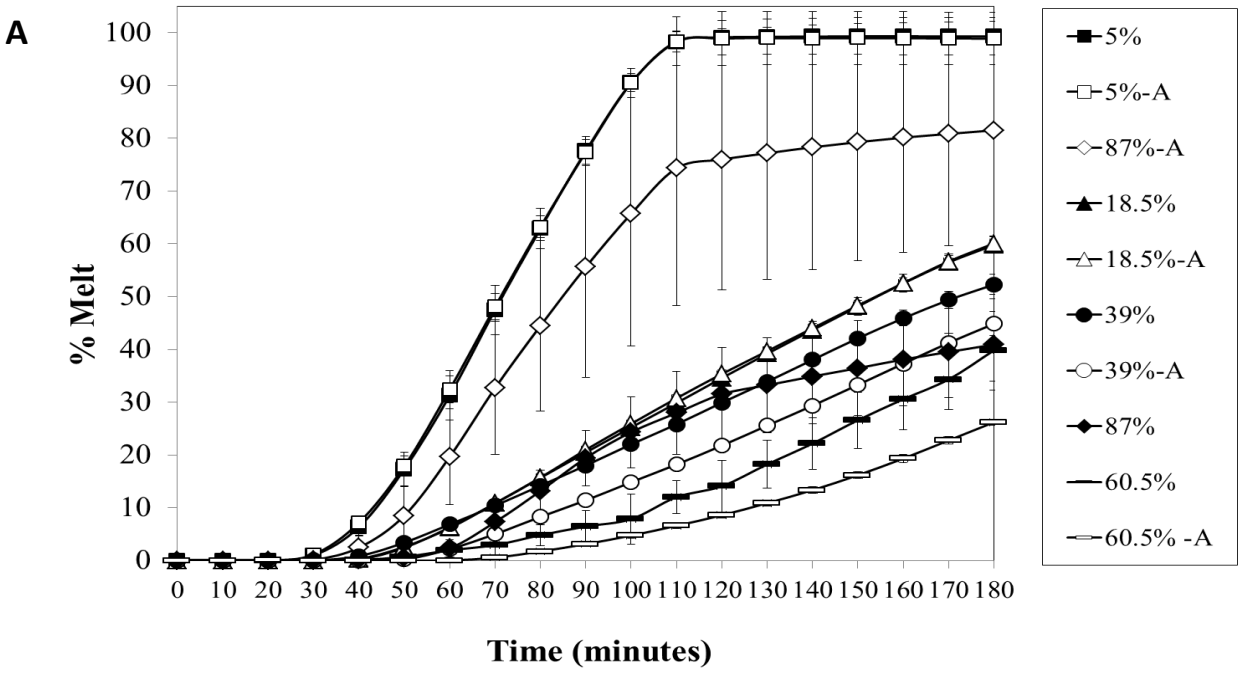
be a product of the more saturated fat fraction used by these authors which could have led to the production of harder protruding fat crystals (De Greyt & Kellens, 2001).

As depicted in Fig. 5.4A, aging did not influence significantly the overrun of any of the samples except the sample with a SFC of 87% where the overrun decreased. Aging would enhance the fat network formation which would stabilise the air bubbles increasing overrun (Chang & Hartel, 2002). Nevertheless, in this study no differences in fat network among aged and unaged samples were noticed and this could be the cause for the absence of an effect on overrun. Also, it was shown to be more the amount of solid fat, rather than its extent of destabilisation to influence the incorporation of air (Goff *et al.,* 1999; Sung & Goff, 2010).

5.3.4 Meltdown behaviour and shape retention

In ice cream the developed fat network influences the meltdown properties because it provides 1) a more tortuous path to be undertaken by the serum phase when exiting the matrix, slowing down ice cream melting rate (Bolliger *et al.,* 2000; Muse & Hartel, 2004), and 2) a structural support, enhancing ice cream shape retention when exposed to melting conditions (Tharp *et al.,* 1998; Bolliger *et al.,* 2000).

If we consider the unaged ice creams (Fig. 5.5A), the sample with a SFC of 5% had the fastest melting rate. This is due to the lack of a fat network developed (due to the low SFC, Fig. 5.2).



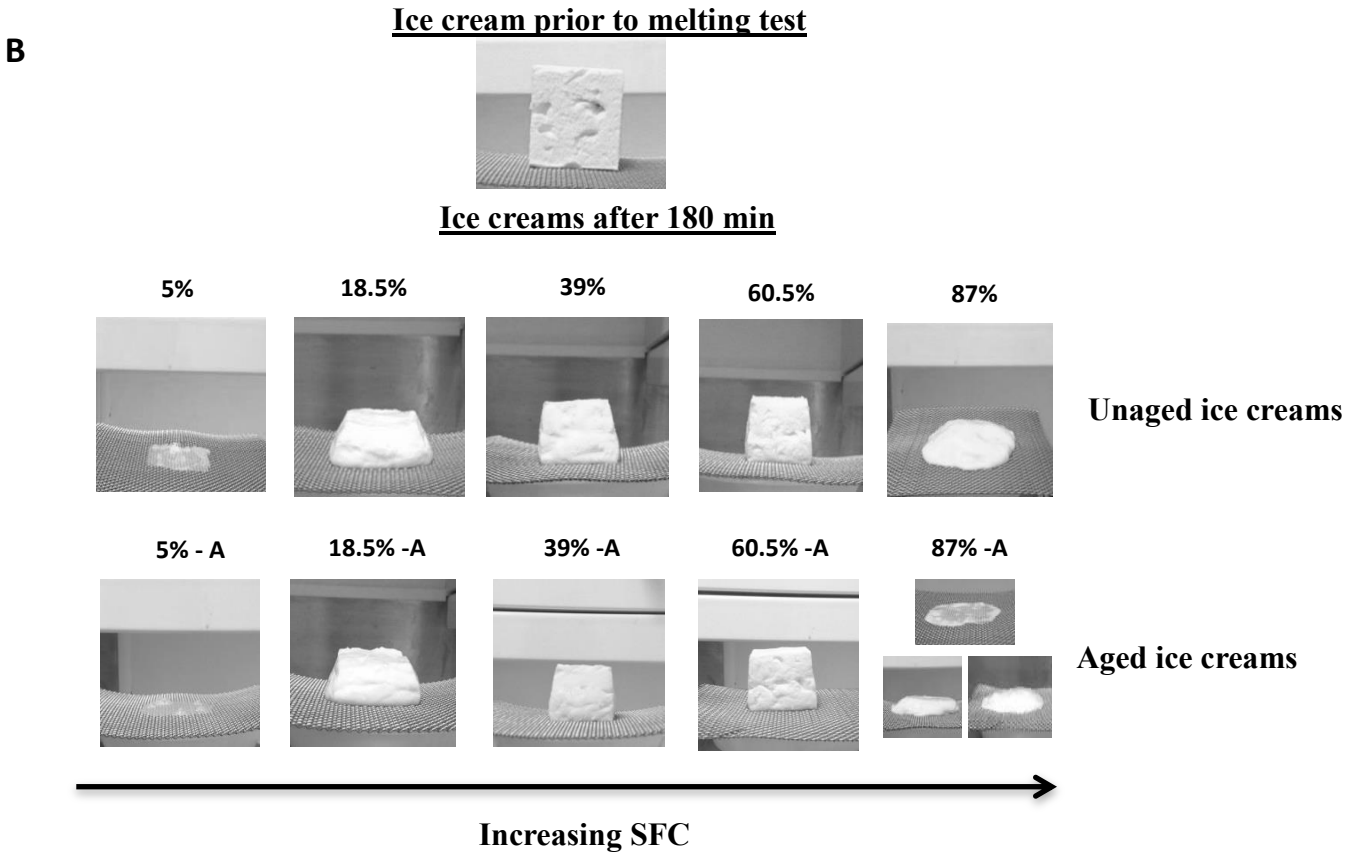


Fig. 5.5 A- Meltdown rate of aged (-A) and unaged ice creams with different SFC fat blends. The graph shows means (n=3) ± one standard deviation. B- Images showing the shape retention of unaged and aged (-A) ice cream made with different SFC.

Increasing the amount of solid fat (i.e. samples with a SFC of 18.5%, 39% and 60.5%) there was a decrease in the melting rate. This is due to the more structured fat network developed as suggested by hardness (Fig. 5.3). In the sample with a SFC of 87% the meltdown rate was comparable to the ice cream with a SFC of 39%.

To better understand this behaviour the shape retention of the ice creams at the beginning and at the end of the melting test has been observed (Fig. 5.5B). As expected, due to the lack of a fat network, the sample with a SFC of 5% had completely disappeared at the end of the melting test. In the samples containing a SFC of 18.5%, 39% and 60.5% an enhancement in the shape retention was observed as the SFC increase, because of an increased fat network developed (Fig. 5.3). The sample with a SFC of 87% did not retain its shape, spreading on the grid, but not passing through it. This was unexpected due to the hardness (Fig. 5.3) suggesting the development of the most structured fat network and the low meltdown rate observed (Fig. 5.5A). In this case, it could be possible that, due to the high SFC, the fat present was organized into discrete agglomerates rather than taking part into a fat network. In fact, it is known that partial coalescence only occurs if the fat is just partially crystalline because the solid fat crystals need to penetrate into the liquid fat and form a semi-solid junction (Fredrick *et al.*, 2010). If none or just too little liquid is present, there will be no wetting of the crystals and the junction will not be formed (Walstra, 2003). With very high SFC the fat droplets would undergo flocculation because rigid droplets cannot merge (McClements, 2016). The presence of these discrete agglomerations could result in the incorporation of smaller-size air bubbles with the formation of a more compact internal structure as proposed schematically in Fig. 5.6.

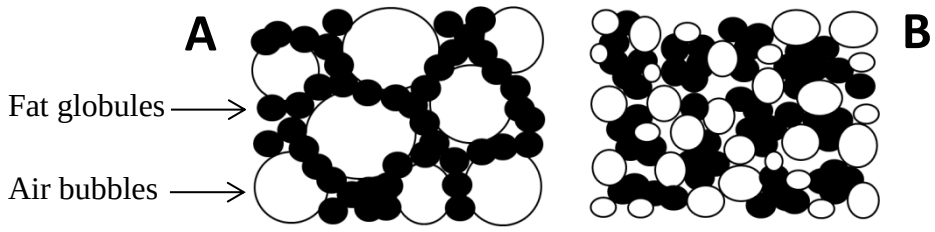


Fig. 5.6 Schematic representation of proposed microstructure of samples containing A- fat blends of SO and CO (i.e. a SFC of 18.5, 39 and 60.5%) B- just CO (i.e. a SFC of 87%) (not drawn to scale).

This interpretation is supported by previous studies showing 1) a high fat flocculation (by observing the melted samples with the confocal microscope) when high solid fat was used for the production of ice cream (Goh *et al.*, 2006); and 2) smaller air bubbles sizes when the concentration of solid fat in ice cream increased (Sung & Goff, 2010). Moreover, the presence of this compact structure would explain 1) the high hardness observed for this sample because when performing the penetration test the hard agglomerated fat would oppose a great resistance to the probe and 2) the low meltdown rate because there will be a more tortuous path through which the serum must undergo while exiting the matrix.

Aging led to a lower meltdown rate of samples with a SFC of 39% and 60.5%. This suggests the formation of a more structured fat network in the aged samples with this SFC. Nevertheless, hardness of aged and unaged samples (Fig. 5.3) and their shape retention did not change (Fig. 5.5B). Therefore, it is not clear if in these samples there was a development of a more structured fat network. It is possible that this was the case but the differences were too small to be observed visually or by hardness measurements. In the sample with a SFC of 87%, aging caused the increase of the meltdown rate. In this case, a great standard deviation can be observed and the macrostructure of the ice cream after the melting test (Fig. 5.5B) was very different among samples. This suggests the formation of an inhomogeneous product.

Investigating the droplet size distribution of ice cream samples in the next section will clarify this point.

5.3.5 Investigation of destabilised fat

Partial coalescence, full coalescence and flocculation are all forms of fat destabilisation. Fat destabilisation can be evaluated by investigating the droplet size distribution of the ice cream mix and the ice cream melted. Nonetheless, it is important to highlight that results from the droplet size distribution only are not very conclusive since the laser diffraction technique cannot differentiate between scattering by single particles and cluster of particles.

Ice cream mixes. All unaged ice cream mixes, and all but one of aged ice cream mixes (i.e. 87%-A), had the same mono-modal distribution with a peak between ~0.5 and ~5 μm (Fig. 5.7), indicating the fat type did not affect the formation of the emulsion and the aging step did not influence the emulsion stability.

For the 87%-A sample there was an additional peak appearing between ~15 and ~480 μm (Fig. 5.7E) which suggests the presence of fat aggregates developed during the aging step.

This is probably because, as the amount of solid fat is high (Fig. 5.2) and CO is already in crystallised form at 5 °C (Fig. 5.1), the probability for the droplets to interact with each other increases so that the fat droplets may form fat aggregates during aging.

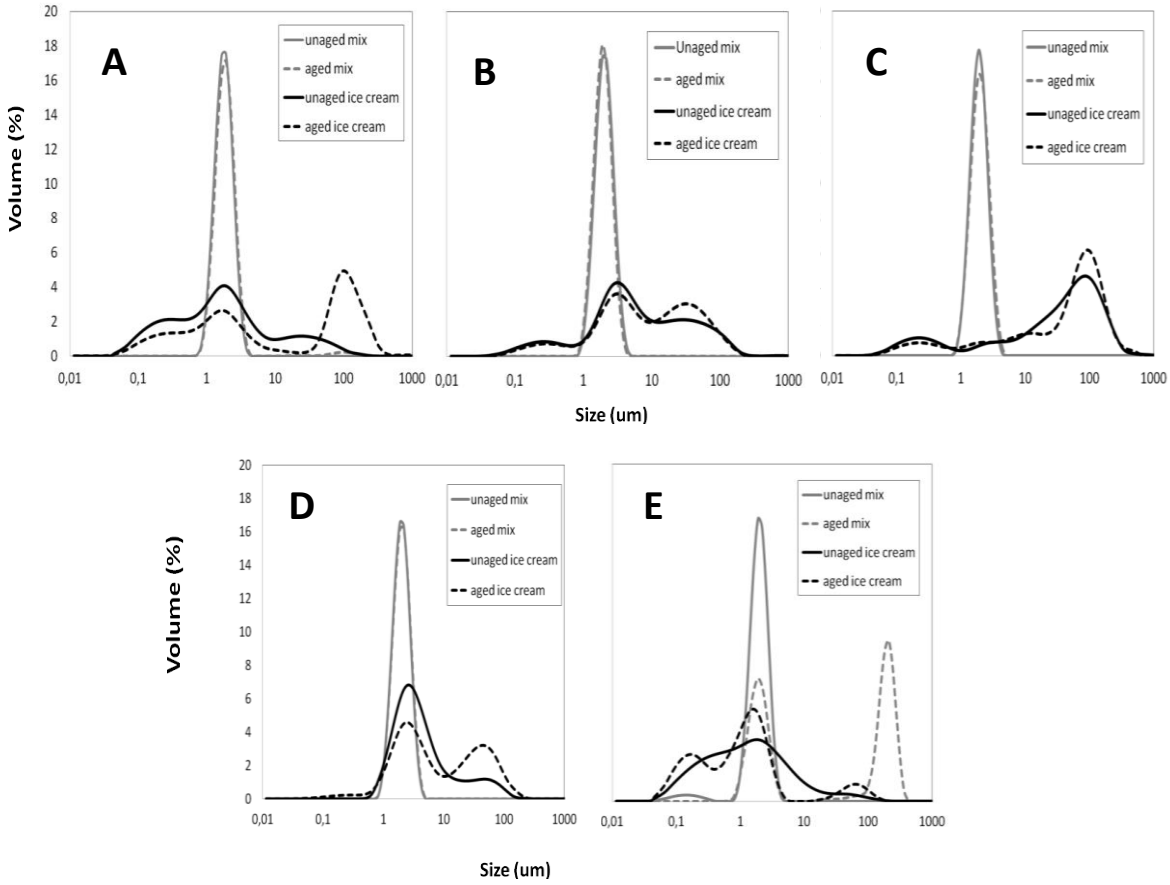


Fig. 5.7 Droplet size distribution of ice creams and ice cream mixes with different SFC, with or without an aging step. Individual graphs represent different SFC: A) 5%; B) 18.5%; C) 39%; D) 60.5%; E) 87%.

Ice creams. For the ice creams, differences in droplet size distribution profiles were observed depending on both the fat blend used and whether the ice cream mix was aged or not.

Unaged and aged ice cream samples prepared using a fat blend of 18.5, 39.0 and 60.5 % SFC (Fig. 5.7B, 5.7C and 5.7D) showed very similar size distribution profiles skewed towards higher values of the distribution profiles of the original mix. The bimodal peaks are a clear sign that fat destabilisation has taken place where this could be the product of partial coalescence or full coalescence depending on the fat blend. In particular, it is expected that when the concentration of liquid fat is high more probably full coalescence will take place.

Aging led to an increase of fat destabilisation. This could be associated with the formation of a more structured fat network in samples with a SFC of 39% and 60.5% as a slower meltdown rate was observed (Fig. 5.5A).

With no addition of CO (i.e. a SFC of 5%, Fig. 5.7A) and when the ice cream mix was unaged the fat destabilisation was not developed. This can be due both to 1) the lack of aging: the fat droplets would be coated by a thick proteinaceous layer providing high stearic repulsion, and 2) the absence of solid fat which is important for partial coalescence occurrence. The aging step led to an increase in the fat destabilisation attributed to full coalescence since barely any solid fat is present.

When no liquid fat was added (i.e. a SFC of 87%, Fig. 5.7F) and the ice cream mix was unaged the fat destabilisation was not developed. This, again, can be due both to 1) the lack of aging and 2) the absence of liquid fat which is important for partial coalescence occurrence. This result is in line with the poor shape retention observed for this sample (Fig. 5.5B) and justifies the assumption that in this sample the fat is organised into discrete aggregates more than forming a network, as proposed in the previous section. With aging, the droplet size distribution suggested that the aggregates developed during the aging step partially broke. This could be due to the shear applied during the freezing process and it is likely to lead to an inhomogeneous sample, as proposed in the previous section. Probably in this sample, as the fat droplets in the mix are taking part into aggregates (developed during the aging step), it is unlikely for them to form a fat network during the freezing process. Moreover, fat aggregates already present in the mix before entering the freezing process could partially impede the aeration of the sample during freezing, so that explaining the decrease in overrun observed in

this sample (Fig. 5.4). The higher melting rate of these samples in comparison with the unaged ones (Fig. 5.5A) could then have been caused by 1) the reduced overrun reported for this sample which causes a high rate of heat transfer and 2) the consequent less tortuous path through which the serum must undergo.

5.3.6 Sensory perception and palatability of unaged ice cream samples

Since a relationship between microstructure of food and sensory perception/palatability is well known (Bhopatkar *et al.*, 2012*;* Van Aken, 2007*),* a sensory and palatability study has been performed in this research employing untrained panellists. A summary of the results obtained from sensory and palatability test is presented in Table 5.3.

Table 5.3 Mean scores (±SEM) for sensory and palatability assessments of unaged ice creams provided. Means in a row without a common letter differ ($p \leq 0.05$).

Parameter to be evaluated	SFC					p-value
	5%	**18.5%**	**39%**	**60.5%**	**87%**	
Creaminess	6.68 (0.45)	5.78 (0.38)	5.75 (0.38)	6.42 (0.32)	6.79 (0.31)	0.54
Thickness	5.14 (0.42)	5.50 (0.36)	4.87 (0.37)	5.68 (0.40)	5.97 (0.41)	0.172
Fattiness	5.64 (0.44)	5.43 (0.33)	4.53 (0.35)	5.48 (0.34)	5.60 (0.30)	0.134
Hardness	2.86 (0.31)[a]	5.00 (0.38)[b]	5.28 (0.41)[b]	5.59 (0.45)[b]	5.79 (0.42)[b]	<0.0005*
Meltdown speed	6.72 (0.34)[a]	4.97 (0.32)[b]	5.43 (0.37)[b]	4.69 (0.44)[b]	4.68 (0.40)[b]	<0.0005*
Liking	5.05 (0.51)[a]	5.32 (0.37)[a]	5.07 (0.39)[a]	6.21 (0.38)[b]	6.24 (0.37)[b]	0.005*

In previous studies it has been shown that untrained panellists led to results with higher standard deviation, lower repeatability and less significant parameters. On the other hand, trained panellists have a better discrimination ability due to the fact that they are familiar with

the sensory attributes they are going to evaluate (Losó *et al.*, 2012). Nevertheless, untrained panellists are used in a variety of studies because they reflect the consumers' response (Wheeler *et al.*, 2004) so that will produce more appealing results from an industrial and economical point of view.

Creaminess, fattiness, thickness. There were no significant differences among conditions in the perception of creaminess, thickness and fattiness (χ 2(4)= 9.309, p= 0.054; (χ 2(4)= 6.389, p= 0.172 and χ 2(4)= 7.045, p= 0.134 respectively). This was unexpected because the ice creams evaluated by the panellists had a different fat network structure (as shown by physical measurement of this study) and previous studies have shown that in oil-in-water food emulsions partial coalescence has been correlated to an increase in creaminess, thickness and fattiness (Fredrick *et al.*, 2010).

Meltdown speed and hardness. Significant differences were observed for the perceived meltdown speed (χ 2(4)= 20.482, p< 0.0005) and hardness (χ 2(4)= 46.067, p< 0.0005) among conditions.

In this study meltdown behaviour and hardness have been measured physically (see section 5.3.2 and 5.3.4). Nevertheless, it should be highlighted that comparison between physical and sensory measurements should be approximate in this case due to differences in the testing conditions. For sensory measurements ice cream previously kept at -16 °C was placed in the mouth where was subjected to body temperature (~37 °C) and to the shearing action of the tongue (both of which could be contributor factors for melting). On the contrary, for physical measurements ice cream originally at -25 °C was allowed to meltdown at room temperature

(20 °C) to evaluate the meltdown properties or acclimatise at -16 °C and then subjected to hardness measurements.

Samples with a SFC of 5% were perceived to melt faster in the mouth than the rest of the samples (see supplementary material for statistic results). This could be due to the fact that SO was in liquid state at -16 °C (before ice cream was placed in the mouth). Indeed, in this case just the melting of ice crystals would occur in the mouth whereas in the other samples containing CO also this would melt. Similarly, regarding hardness, samples with a SFC of 5% were perceived less hard than all the other samples (see supplementary material for statistic results). This agrees with the physical measurement of the hardness (Fig. 5.3) where sample with a SFC of 5% was the softest one. No differences were perceived among the other ice creams (with SFC of 18.5, 39 and 60.5%) both in terms of meltdown speed and hardness probably because 1) the physical measurement did not show radical differences, 2) the samples could have been swallowed before they were completely melted and therefore differences might be not completely appreciated, and 3) the panellist were untrained.

In a standard ice cream (containing milk fat) the SFC is around 60% (Goff *et al.,* 2007). With this sensory study we have shown that it could be possible to reduce the SFC of about 40% without affecting the consumers' sensory perception. This would involve a remarkable impact on global health, as saturated fat has always been associated with serious diseases such as obesity (Casas-Agustench *et al*., 2014) or heart conditions (Ruiz-Núñez *et al.,* 2016).

Palatability (liking). Liking of samples differed significantly among conditions (χ 2(4)=14.908, p= 0.005), with post hoc analysis showed that samples with a SFC of 60.5% and

87% were more liked than samples with a SFC of 5%, 18.5% and 39% (see supplementary material for statistic results). These results suggest that it is more the quantity of solid fat rather than the fat network developed to influence product palatability. In fact, previously in this chapter it was shown that samples with a SFC of 87% did not develop a structured fat network. Nevertheless, participants liked the most this sample

5.3.7 Overall findings

This study highlighted that the use of fat blends with different SFC had an effect on microstructural, physical properties, some sensory attributes and the palatability of ice cream.

As the SFC increased from 5% to 60.5%, the ice cream fat network became more structured. With an excessively high SFC (87%) the results suggested the organisation of the fat into discrete agglomerates rather than into a network.

The aging step was shown to have a minimal effect on the final product. In this study the aging time was selected accurately as explained in section 5.2.2 so that the lack of aging effect is unlikely to be due to the aging time chosen, even if this could be further investigated. Aging was shown just to slow down the meltdown of samples with a SFC of 39% and 60.5% but it is was not clear if this was due to the development of a more structured fat network as no differences in hardness were shown. In the ice cream with a high SFC (87%) the aging step had a detrimental effect because it caused the formation of fat aggregates in the mix that were partially broken down during the freezing by shearing action and resulted in an inhomogeneous product.

Key findings from sensory study have shown that untrained panellists, due to their low discrimination ability and the lack of familiarity with the definitions used, could perceive differences between samples just for some sensory attributes and when there were large differences among samples microstructures. Solid fat present has a more dominant effect on liking of ice cream than the microstructure developed.

5.4 Conclusions

Results from this study have led to the conclusion that 1) the aging step, having a minimal effect, could be avoided decreasing ice cream production time, 2) a reduction of about 40% in saturated fat in comparison with a standard ice cream (made with milk fat) might be possible without affecting consumers' sensory perception response, and 3) the palatability of the ice cream was principally influenced by its formulation.

The results obtained using CO as solid fat could be comparable but may not extrapolate directly to other fat sources such as palm oil due to the different composition. The use of CO over palm oil has the advantage of being a healthier fat due to its high concentration of medium chain triglycerides (Gunstone, 2008) and the additional benefit of providing a more sustainable ingredient for the production of ice cream.

As a next step the effect of the ice creams produced on appetite and food intake will be investigated. In fact, the fat source used contained CO and SO (composed mainly by medium chain triglycerides and long chain triglycerides respectively) and these fats were shown to

have a different effect on appetite and food intake as it will be fully explained in the next chapter.

5.5 Supplementary Materials

Liking

Interaction: 60.5%*5% Z= -2.201, p= 0.028

Interaction: 87%*5% Z= -2.433, p= 0.015

Interaction: 60.5%*18.5% Z= -1.965, p= 0.049

Interaction: 87%*18.5% Z= -2.387, p= 0.017

Interaction: 60.5%*39% Z= -2.952, p= 0.003

Interaction: 87%*39% Z= -2.530, p= 0.011

Meltdown

Interaction: 5%*18.5% Z= -3.428, p= 0.001

Interaction: 5%*39% Z= -3.024, p= 0.002

Interaction: 5%*60.5% Z= -3.456, p= 0.001

Interaction: 5%*87% Z= -3.476, p= 0.001

Hardness

Interaction: 5%*18.5% Z= -4.134, $p < 0.0005$

Interaction: 5%*39% Z= -4.207, $p < 0.0005$

Interaction: 5%*60.5% Z= -4.556, $p < 0.0005$

Interaction: 5%*87% Z= -4.330, $p < 0.0005$

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CHAPTER 6

Coconut and Sunflower Oil Ratios in Ice Cream Influence Subsequent Food Selection and Intake

Rizzo G, Masic U, Harrold JA, Norton JE, Halford JCG

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Abstract

The effect of coconut oil (CO, containing mainly medium chain triglycerides - MCTs) and sunflower oil (SO, containing mainly long chain triglycerides - LCTs) used as fat source (10% fat ice cream) in different ratios (25% CO and 75% SO - 25CO:75SO, 50% CO and 50% SO - 50CO:50SO, 75% CO and 25% SO - 75CO:25SO) was investigated to assess differences in appetite and *ad-libitum* (evening and snack) food intake using a single blind design. 36 healthy female participants consumed a fixed portion (150g) of ice cream 45 minutes before an *ad-libitum* dinner and snacks. Appetite sensations were tracked across the day. Participants ate significantly less fat after 75CO:25SO than 25CO:75SO (p= 0.007) and there was also a trend for lower fat intake in this condition as compared to 50CO:50SO (p= 0.068). High fat savoury intake significantly decreased after 75CO:25SO in comparison with both 25CO:75SO (p= 0.038) and 50CO:50SO (p= 0.008). Calorie intake from snacks was also found to be significantly lower after 25CO:75SO and 50CO:50SO than 75CO:25SO (p= 0.021 and 0.030 respectively). There was no effect of condition on appetite or desire ratings over the day. Eating a standard portion of ice cream containing different ratios of MCTs and LCTs can modestly influence acute food selection and intake, with MCTs manifesting their effect earlier and LCTs later due to differences in the absorption and metabolism of these lipids. However, the differences evident in the present study were small, and require further research before firm conclusions can be drawn.

Keywords: Ice cream, medium chain triglycerides, long chain triglycerides, food intake, appetite.

Industrial relevance

Given the obesity epidemic, the results of this study are invaluable because they give an overview of which could be the contribution of the food industries in the fight against this problem. It has been shown that changing the type of fat in ice cream rather than its amount (which would not affect the acceptability of the product) could be a promising way to decrease food intake. Different absorption and metabolism mechanisms of fats influence the time gap between the ice cream ingestion and the subsequent food intake reduction. Medium chain triglycerides manifest their effect earlier, whereas long chain triglycerides later. Depending on the country of residence of the food industries and the habits (in terms of the ice cream consumption), the formulation of ice cream should be manipulated in order to influence food intake.

6.1 Introduction

Fats are an important source of energy and should account for 30% of daily calorie intake (Zúñiga & Troncoso, 2012) due to their essential role in the absorption of fat-soluble vitamins. Most fatty foods are energy dense and palatable, but they exert a weak effect on satiety and satiation compared with protein- and carbohydrate-rich foods (Gerstein *et al.,* 2004; Johnstone *et al.,* 1996; Karhunen *et al.,* 2008; Chambers *et al.,* 2015). The consumption of a high fat diet may therefore contribute to weight gain and obesity, which is linked to a variety of co-morbidities (Lee, 2013). One means of preventing the potential for weight gain

from fat sources is by replacing or reducing the amount of fat in food. This usually leads to a considerable reduction in palatability which is likely to reduce consumption (German & Watzke, 2004). Another possible approach may be to maintain the fat content and vary instead the type of fat consumed to one that may enhance satiation and satiety. For instance, using fats with different carbon chain lengths or saturation levels may influence pre- and post-absorptive mechanisms (Beardshall *et al.,* 1989; Lawton *et al.,* 2000; Feltrin *et al.,* 2008; Rolls *et al.,* 1988; Van Wymelbeke *et al.,* 1998, 2001). This would maintain palatability and intake while altering satiety and satiation properties to potentially reduce subsequent intake.

Low fat diets are a generally accepted means of weight loss, but recent meta-analyses suggest they are a poor means of weight loss maintenance (Tobias *et al.,* 2015) due to their low palatability which may contribute to low levels of satisfaction and therefore adherence (Hetherington *et al.,* 2013; Halford & Harrold, 2012). Instead, it may be more useful to maintain healthy levels of functional fats within the diet which are palatable and act to increase satiation and satiety whilst also decreasing food intake. For instance, it has been shown that unsaturated fats, in comparison to saturated fats, lead to a greater release of satiety-related gastrointestinal hormones such as GLP-1 and CCK (Beardshall *et al.,* 1989; Hirasawa *et al.,* 2005) and are absorbed and oxidised faster than saturated fats (Small, 1991). However, fat saturation has rarely been shown to have an effect on food intake (Lawton *et al.,* 2000), with many more experiments finding no such effect (Flint *et al.,* 2003; Casas-Agustench *et al.,* 2009; Strik *et al.,* 2010). Fats with different chain lengths are also absorbed and metabolised differently. In particular, medium chain triglycerides (MCTs) are hydrolysed faster and more completely than long chain triglycerides (LCTs) due to their smaller molecular weight, thus increasing lipase efficiency and allowing them to be absorbed intact.

Unlike LCTs, which are packed into chylomicrons and enter the lymphatic system, MCTs enter the portal system and reach the liver more rapidly where they are readily oxidised, causing the production of Ketone bodies (Bach & Babayan, 1982). A decrease in food intake has been associated both with hepatic fat oxidation (Langhans, 1996) and the presence of Ketone bodies (Le Foll *et al.,* 2014), suggesting that MCTs may reduce food intake more than LCTs. Indeed, a variety of studies have shown that an intestinal infusion (Feltrin *et al.,* 2008), a preload (Rolls *et al.,* 1988) or a meal (Van Wymelbeke *et al.,* 1998, 2001) containing MCTs led to a reduction in food intake in a subsequent meal as compared to LCTs. Nevertheless, other authors have failed to show an effect of carbon chain length on food intake and appetite after a substantial delay (210-300 minutes) between the manipulation and subsequent meal; this is likely due to hunger overriding any observable effect (Poppitt *et al.,* 2010; Bendixen *et al.,* 2002).

Ice cream is a highly palatable, high-fat dessert comprised of a solid foam made up of air bubbles, ice crystals, and a network of fat globules surrounded by an unfrozen serum of sugars, proteins, polysaccharides and water (Goff, 1997). The fats employed to make up ice cream can be unsaturated or saturated, allowing for a stable food matrix to compare MCTs (such as coconut oil - CO) to LCTs (such as sunflower oil - SO).

In the previous literature, standard quantities of fat were in the range of 30-40g (Lawton *et al.,* 2000; Van Wymelbeke *et al.,* 1998, 2001; Rolls *et al.,* 1988), which exceeds the amounts normally found in foods. This may be problematic as, firstly, such quantities are not realistic to incorporate into everyday use; and secondly, these amounts of fat may be more harmful than helpful in the long term (Lee, 2013). The present research assesses the effects of different

fats (CO, containing mainly MCTs and SO, containing mainly unsaturated LCTs) in different ratios (25% CO and 75% SO - 25CO:75SO, 50% CO and 50% SO - 50CO:50SO, 75% CO and 25% SO - 75CO:25SO) as part of a fixed portion ice cream; a palatable, well accepted, complex food product with 10% (15g) fat (a standard ice cream fat content) to determine how differing fat ratios influence appetite, *ad-libitum* dinner and snack intake. Such research in this area is novel because it assesses the effect of these fats when ingested in more typical quantities. It is important to highlight that in this study, as well as in other studies (Rolls *et al.,* 1988; Van Wymelbeke *et al.,* 2001; Barbera *et al.,* 2000), fats with both different chain length (MCTs and LCTs) and saturation (in particular saturated MCTs and unsaturated LCTs) were compared because 1) much research comparing fatty acid saturation levels (when keeping the chain length constant) on appetite and food intake has not shown any difference in effect; 2) MCTs have been shown to reduce food intake in comparison with both unsaturated and saturated LCTs (Van Wymelbeke *et al.,* 1998) and 3) a variety of food products (including ice cream) use a combination of vegetable-based saturated fat (like CO and palm oil, rich in MCTs) and vegetable-based unsaturated fat (like SO, rich in unsaturated LCTs). Thus understanding the effects of such fats in differing ratios on appetite and energy intake are invaluable. We predicted that due to the faster absorption of MCTs, the high ratio MCT condition would elicit a reduction in appetite and food intake more strongly than the high ratio LCT condition.

6.2 Material and methods

6.2.1 Participants

Thirty six healthy female volunteers were recruited to the study through advertisements at the University of Liverpool. Volunteers were asked to provide informed consent and were then screened. Exclusion at the screening session included: volunteers aged <18 years or >55 years; with a BMI <18.5 kgm^{-2} or >25 kgm^{-2}; who were taking medication known to affect appetite; who disliked more than 25% of the study foods; who were smokers or had recently stopped smoking; who reported food allergies or intolerances; who were currently dieting or about to embark a diet; who had significantly changed their physical activity in the past 4 weeks or intended to change it during the course of the study; who did not eat breakfast regularly; who dislike coconut flavoured ice cream; and who showed disordered eating behaviours (score > 4 on the Dutch Eating Behaviour Questionnaire Restraint, DEBQ-R (Van Strien *et al.*, 1986) or >27 on the Binge Eating Scale, BES (Gormally *et al.*, 1982)). The study was conducted in accordance to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the University of Liverpool Committee on Research Ethics. Written informed consent was obtained from all subjects. Participants were compensated for their time and travel to the laboratory.

6.2.2 Study foods

6.2.2.1 Study products

The study products were three fixed quantity ice cream portions (150 g) different in ratios of CO to SO; 25% CO and 75% SO (25CO:75SO), 50% CO and 50% SO (50CO:50SO), 75% CO and 25% SO (75CO:25SO). Ice cream ingredients are shown in Table 6.1 and the

nutritional profile is shown in Table 6.2. Each ice cream portion provided 270 calories, 6 grams of proteins, 15 grams of fats and 27 grams of carbohydrates. The typical composition of the fats used was as follows; SO is composed of palmitic acid (16:0; 5%), stearic acid (18:0; 6%), oleic acid (18:1; 30%), linoleic acid (18:2; 59%), whereas CO of caproic acid (6:0; 0.4-0.6%), caprylic acid (8:0; 7-9%), capric acid (10:0; 6-8%), lauric acid (12:0; 46-50 %), myristic acid (14:0; 17-19%), palmitic acid (16:0; 8-10 %), stearic acid (18:0; 2-3 %), oleic acid (18:1; 5-7 %), linoleic acid (18:2; 1-2%). A separate pilot sensory test with thirty participants showed that the ice creams used were sensory matched for creaminess, thickness, hardness, meltdown speed (time taken to melt in the mouth) and fattiness using VAS scale measures.

Table 6.1 Ice cream composition.

Ingredient	Percentage (wt%)
Fat	10
Skim milk powder	11
Sucrose	12
Guar gum	0.3
Distilled mono glycerides	0.2
Water	60.5

Table 6.2 Nutritional profile of ice cream provided (g – grams; Kcal – calories; CHO - carbohydrate).

Typical values	100 g contains
Energy	180 Kcal
Protein	4 g
Fat	10 g
CHO	18 g

6.2.2.2 Test meals and snack box

All participants were provided with a fixed-load breakfast, fixed-load lunch, fixed-load ice cream and *ad-libitum* dinner and snacks. A preliminary pilot study was conducted to adjust the fixed load and *ad-libitum* meal quantities to ensure the participants could comfortably consume the fixed load meals and that the *ad-libitum* items were more than they could possibly eat in one sitting. The nutritional profile of the fixed-load meals is shown in Table 6.3. 250g of water was provided for breakfast (as either tea, coffee or pure water) and lunch and 500g water was provided for dinner. If participants requested tea or coffee at breakfast they received the same beverage on each study day (with sugar or sweetener if requested). The *ad-libitum* dinner provided a range of high and low fat savoury and sweet options which consisted of pasta with bolognese sauce, medium grated cheese, garlic bread, strawberry jelly and chocolate mousse. After the dinner, participants were given a snack box containing a range of pre-weighed high and low fat sweet and savoury options (see Table 6.4 for nutritional information of the snacks provided). Participants were instructed to consume as much or little of these food products as they wished for the rest of the evening, to save the packages and/or the peel of the products eaten in the snack box and to return the pack on their next visit. Snack intake was used as a measure of 'snacking' behaviour and to cover all eating occasions (breakfast, lunch, dinner and snacks).

Table 6.3 Nutritional profile of the fixed-load meals (g – grams; Kcal – calories; CHO - carbohydrate).

Meal	Energy (Kcal)	Protein (g)	CHO (g)	Fat (g)
Breakfast	415	12	5	11
Lunch	337	14	45	10

Table 6.4 Nutritional profile of the snack box food products provided (g – grams; Kcal – calories; CHO - carbohydrate). Weight of the fruit could vary.

Item	Energy (Kcal)	Protein (g)	CHO (g)	Fat (g)
Cheese crackers (HFSV) – 25g	131	2.7	12.9	7.5
Salt and vinegar rice crackers (LFSV) – 22g	89	1.5	17	1.6
Caramel biscuit chocolate bar (HFSW) – 23g	114	1	14.9	5.5
Marshmallow – 250g	825	11.5	195	trace
Fruit (Apple/Banana) ~100g	~55/84	~0.3/1.2	~13.8/20.3	~0.2/0.3

6.2.3 Study design

A single blind within-subjects design was used to assess the effect of ice creams containing different CO to SO ratios (25CO:75SO, 50CO:50SO, 75CO:25SO) on subsequent *ad-libitum* dinner and snack intake and the experience of appetite. Each study visit was separated by one week and participants were provided with the three conditions in a randomised order. Power calculations were performed using G*Power for a repeated measures design using a medium (0.25) effect size and powering to 90% power which indicated that 30 participants were required. 40 participants were recruited to prevent any possible withdrawal or exclusions.

6.2.4 Appetite, palatability and sensory measures

Participants' appetite ratings (hunger, fullness, prospective consumption, desire to eat, satisfaction), palatability of the meals (pleasantness, fillingness, saltiness, familiarity, palatability, sweetness and tastiness of the food) and sensory attributes of the different ice creams (creaminess, thickness, meltdown speed, sweetness, fattiness) were evaluated using validated visual analogue scales (VAS) (Flint *et al.*, 2000) made up of 100 mm line with two extreme anchors: "not at all" and "extremely". Participants were asked to draw a vertical line

to indicate their ratings. Appetite VAS were completed before and after each meal and at hourly intervals throughout the test day. Palatability and sensory ratings were included to ensure acceptance of the product and to determine whether any sensory differences between the ice creams were perceived which may influence appetite such as creaminess ("How creamy was the ice cream?"), fattiness ("How fatty was the ice cream?"), thickness ("How thick was the ice cream?"), and meltdown speed ("How long did the ice cream take to melt in your mouth?").

6.2.5 Universal eating monitor (UEM)

The Sussex Ingestion Pattern Monitor (SIPM) is a Universal Eating Monitor (UEM) which uses an automated method to measure food intake and subjective ratings of appetite and palatability. The SIPM is made up of a hidden scale connected to a computer, which measures the weight of the plate at 2-second intervals as the participant consumes their meal. Participants' appetite ratings before and after ice cream consumption as well as palatability and the sensory attributes of the different ice creams were evaluated using on-screen visual analogue scales (VAS). The use of mixed paper and pen and computerised VAS has been validated elsewhere (Thomas *et al.,* 2013).

6.2.6 Procedure

A schematic representation of the study is shown in Fig. 6.1 and uses a standardised approach used widely in the literature (Lawton *et al.*, 2000; Harrold *et al.,* 2014). Participants were asked to keep each pre-study evening similar in terms of exercise and food intake and to avoid both alcohol consumption and vigorous exercise. They were also asked to record their food intake and activities in a provided standardised diary from 5 pm the day preceding the study

visit to ensure compliance. Participants were instructed not to eat or drink anything except water from midnight the day preceding the study visit. Preceding each meal at the study centre, participants were seated in individual cubicles. They were given appetite VAS questionnaires before being served a meal (fixed-load breakfast, lunch, preload or *ad-libitum* dinner). For the breakfast and lunch, participants were asked to consume the entire meal within 20 minutes. After each meal participants completed further appetite and sensory VAS questionnaires. After breakfast and lunch, participants were free to leave the study centre and were instructed not to eat or drink anything except the water provided by the researcher until they returned for their next meal. They were provided with VAS questionnaires to complete hourly until their next meal. Lunch was provided 4 hours after the breakfast and the preload was given 3 hours and 15 minutes after lunch. After ice cream consumption and VAS questionnaire completion participants were asked to remain in a waiting room before being served the *ad-libitum* dinner 45 minutes after they received the preload. Participants were asked to eat and drink from the choice of foods and water offered until they felt comfortably full, taking as long as they wished. Following dinner, participants were given a snack box with instructions to eat as much or as little of the food items provided as they wished for the rest of the evening. Participants were also given a retrospective appetite questionnaire and a gastrointestinal questionnaire to complete before retiring to bed. Participants were asked not to consume any alcohol for the rest of the evening.

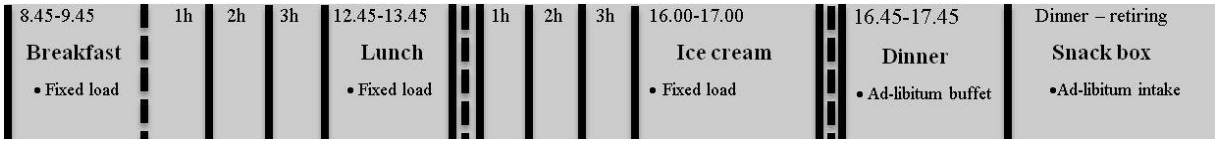

Fig. 6.1 Schematic representation of the study design. Solid lines represent appetite VAS scale completion; dash lines represent palatability/sensory VAS scale completion.

6.2.7 Statistical analysis

Analyses were performed using SPSS for Windows Version 22. One-way within subject repeated measures Analysis Of Variance (ANOVA) were conducted for appetite ratings with condition (25CO:75SO, 50CO:50SO and 75CO:25SO) and time (pre-ice cream, post-ice cream, and pre-dinner) as within-subject factors. Area under the curve (AUC) hunger, sensory meal ratings and retrospective appetite and the GI questionnaire were also assessed in this way. Intake at the *ad-libitum* meal and of the snack box provided was analysed in terms of grams, calories and macronutrients consumed. Total intake of *ad-libitum* dinner and snack box was also analysed (calories and grams consumed). Exact amounts consumed were calculated weighting the food (comprised of crockery/packets) before and after the eating episodes. Condition order was also analysed as a between-subjects factor. In cases of violated sphericty, Greenhouse Geisser values were reported. Contrast effects were assessed using paired samples t-tests where significant interactions were evident. Bonferroni corrected values are provided where sphericity assumptions were violated. All data are presented as means ± standard error of the mean (SEM).

6.3 Results

6.3.1 Participants

In total, 72 participants were screened and 40 were recruited. Three participants withdrew for personal reasons with a total of 37 participants who completed the study. One participant was excluded during the analysis as an outlier (due to intake exceeding 2 standard deviations above the mean), resulting in 36 available cases. The demographic and anthropometric characteristics of the completing participants are shown in Table 6.5.

Table 6.5 Means (±SEM) of gender, age, anthropometrics, and psychometric trait characteristics of participants.

	Participant characteristics
Gender	Female
Age	29.7 (4)
Height (cm)	149.8 (6.2)
Weight (kg)	66.4 (4.9)
BMI	21.7 (0.3)
DEBQ- Restraint	2.4 (0.1)
Binge Eating Score	7.7 (1)

6.3.2 Sensory perception and palatability of ice cream

The sensory and palatability ratings of the ice cream are shown in Table 6.6. There was no effect of condition on tastiness, pleasantness, sweetness, meltdown speed and fattiness. A significant effect of condition was found for creaminess (ANOVA main effect: $F [2, 68]= 3.302$, $p= 0.043$) and thickness (ANOVA main effect: $F [2, 68]= 3.333$, $p= 0.042$). In particular, ratio 75CO:25SO was perceived as significantly creamier than 50CO:50SO ($t [34]= -2.485$, $p= 0.018$) and there was a trend for a creamier perception of this ratio as compared to 25CO:75SO ($t [35]= -1.810$, $p= 0.079$). Ratio 75CO:25SO was also rated as significantly thicker than 25CO:75SO ($t [35]= -2.150$, $p= 0.039$) and 50CO:50SO ($t [34]= -2.461$, $p= 0.019$). These results differed from our pilot sensory test. This may be due to participants receiving a larger quantity of ice cream in the present experiment which meant that the ice cream may have partially melted, making certain sensory attributes (such as creaminess or thickness) more prominent.

Table 6.6 Means (±SEM) of sensory and palatability assessments of ice creams provided. Means in a row without a common letter differ ($p \leq 0.05$).

	Ratio		
	25CO:75SO	**50CO:50SO**	**75CO:25SO**
Tastiness	76.3 (4.2)	76.3 (4.2)	76.2 (4.4)
Pleasantness	79.5 (3.8)	77.2 (4.3)	79 (4.2)
Creaminess	71.3 (3.5) [ab]	69.2 (4.4)[a]	78.8 (3.4) [b]
Sweetness	63.5 (3.8)	62.2 (4.1)	65.9 (3.9)
Meltdown speed	34.1 (4.1)	38.6 (4.2)	36.3 (4.1)
Fattiness	47.7 (5.1)	47.6 (5)	50.9 (4.5)
Thickness	63.5 (4) [a]	64.8 (3.9) [a]	72 (3) [b]

6.3.3 *Ad-libitum* meal intake

Dinner intake is shown in Table 6.7. There was a significant difference between conditions in both the total consumption of fat (from main meal and dessert) and high fat savoury (HFSV) food selection (ANOVA main effect for fat: F [2, 70]= 3.774, p= 0.028 and HFSV: F [2, 70]= 0.4333, p= 0.017) with participants consuming significantly less fat after 75CO:25SO than 25CO:75SO (t [35]= 2.879, p= 0.007) and a trend for lower fat intake after this ratio in comparison with 50CO:50SO (t [35]= 1.883, p= 0.068). The consumption of HFSV options significantly decreased after 75CO:25SO as compared to both 25CO:75SO (t [35]= 2.153, p=0.038) and 50CO:50SO (t [35]= 2.800, p= 0.008). Dinner calorie intake also decreased as CO concentration increased but this was only found to be a trend in the data (F [2, 70]= 0.822, p= 0.444).

Table 6.7 Means (±SEM) of energy (g - grams; and Kcal - calories) and macronutrient (PRO – protein; CHO – carbohydrate; and fat) intake, food selection (HFSV – high fat savoury; LFSV – low fat savoury; HFSW – high fat sweet; LFSW – low fat sweet) of dinner (main meal and dessert) items provided. Means in a row without a common letter differ (p ≤ 0.05).

	Ratio		
	25CO:75SO	**50CO:50SO**	**75CO:25SO**
Dinner (g)	591.7 (31.4)	587(30.993)	562.7 (31.3)
Dinner (Kcal)	1980.6 (123.8)	1957.4 (120.3)	1883.6 (120.5)
PRO (g)	74.7 (4.4)	74 (4.3)	70.5 (4.2)
PRO (%)	15.2 (0.2)	15.3 (0.1)	15.2 (0.2)
CHO (g)	385.8 (24)	384.7 (23.1)	368.3 (23.1)
CHO (%)	78.1 (0.6)	79 (0.7)	78.7 (0.8)
Fat (g)	31.419 (1.5)[a]	30.9 (1.5) [ab]	28.8 (1.4) [b]
Fat (%)	15.2 (0.7)	15(0.6)	14.8 (0.7)
HFSV (g)	89.3 (4.9) [a]	90.7 (4) [a]	81.6 (4.3) [b]
LFSV (g)	431.3 (29.6)	429.3 (28.4)	412.8 (28.5)
HFSW (g)	33.7 (4.8)	27.8 (4.7)	24.8 (4.6)
LFSW (g)	37.4 (8.8)	39 (8.9)	43.5 (9.3)

Snack energy intake (Table 6.8) significantly differed by condition (ANOVA main effect: F [2, 70]= 4.137, p= 0.020) with fewer calories consumed after 25CO:75SO and 50CO:50SO as compared to 75CO:25SO. Indeed, participants ate significantly less protein, carbohydrate and reduced their low fat sweet (LFSW) food selection after these conditions as compared to 75CO:25SO. Fruit consumption was also significantly higher after 50CO:50SO and a trend was also apparent after 25CO:75SO as compared to 75CO:25SO (see supplementary materials for detailed results). This suggests that the higher calorie intake at the dinner was compensated for in subsequent snack intake after 25CO:75SO and 50CO:50SO, with lower energy intake and healthier snack choices.

Table 6.8 Means (±SEM) of energy (g - grams; and Kcal - calories) and macronutrient (PRO – protein; CHO – carbohydrate; and fat) intake, food selection (HFSV – high fat savoury; LFSV – low fat savoury; HFSW – high fat sweet; LFSW – low fat sweet) of snack box items provided. Means in a row without a common letter differ ($p \leq 0.05$).

	Ratio		
	25CO:75SO	**50CO:50SO**	**75CO:25SO**
Snack Box (g)	141.5 (16.2)	144.7 (16.1)	165.3 (20.886)
Snack Box (Kcal)	376.1 (48)[a]	369.9 (43)[a]	494.6 (66) [b]
PRO (g)	4.8 (0.7) [a]	4.6 (0.6) [a]	6.4 (0.9) [b]
PRO (%)	4.3 (0.3)	4.4 (0.3)	4.7 (0.3)
CHO (g)	63(8.3) [a]	60.9 (7.7) [a]	87.6 (13.4) [b]
CHO (%)	65.6 (5.1)	66.1 (6.4)	64 (5.3)
fat (g)	14.7 (2.5)	15.2 (2.1)	15.8 (2.1)
fat (%)	29.9 (3.8)	35.4 (5.4)	33.5 (5.6)
HFSV (g)	13.5 (4.6)	11.8 (3)	15.4 (4)
LFSV (g)	8 (1.8)	8.6 (1.8)	10.4 (1.8)
HFSW (g)	30.1 (3.9)	32.2 (4)	30.5 (4.1)
LFSW (g)	22.6 (8.8) [a]	17.2 (7.6) [a]	54.4 (14.8) [b]
Fruit (g)	67.3 (10) [ab]	74.9 (10.7) [a]	54.5 (9.5) [b]

Overall intake of the *ad-libitum* dinner and snack box is shown in Table 6.9. There was no effect of condition on overall *ad-libitum* calorie (ANOVA main effect: F [2, 70] = 0.148, P= 0.863) and gram (ANOVA main effect: F [2, 70]= 0.017, P= 0.983) intake.

Table 6.9 Overall means (±SEM) of energy intake (grams and kcal - calories) of dinner and snack box.

	Ratio		
	25CO:75SO	**50CO:50SO**	**75CO:25SO**
Overall grams	733.2 (43.1)	731.6 (37.7)	728 (44.8)
Overall kcal	2356.7 (156.3)	2327.3 (137.6)	2378.2 (160.2)

6.3.4 Rated appetite and associated questionnaires

There was no effect of condition on hunger (F [4, 140]= 0.510, p= 0.729), fullness (F [4, 140]= 1.633, p= 0.169), prospective consumption (F [4, 140]= 0.141, p= 0.966), satisfaction (F [4, 140]= 1.691, p= 0.155) or desire to eat (F [4, 140]= 2.232, p= 0.069 (Fig. 6.2) over the time lapse from pre-ice cream to pre-dinner**.** Similarly, AUC hunger ratings also showed no effect of condition (F [2, 70]= 1.292, p= 0.281). Retrospective questionnaires revealed no effect of condition on appetite, digestive experiences or mood suggesting that all the conditions were equally accepted by the participants and there were no unpleasant symptoms (see supplementary materials for detailed results).

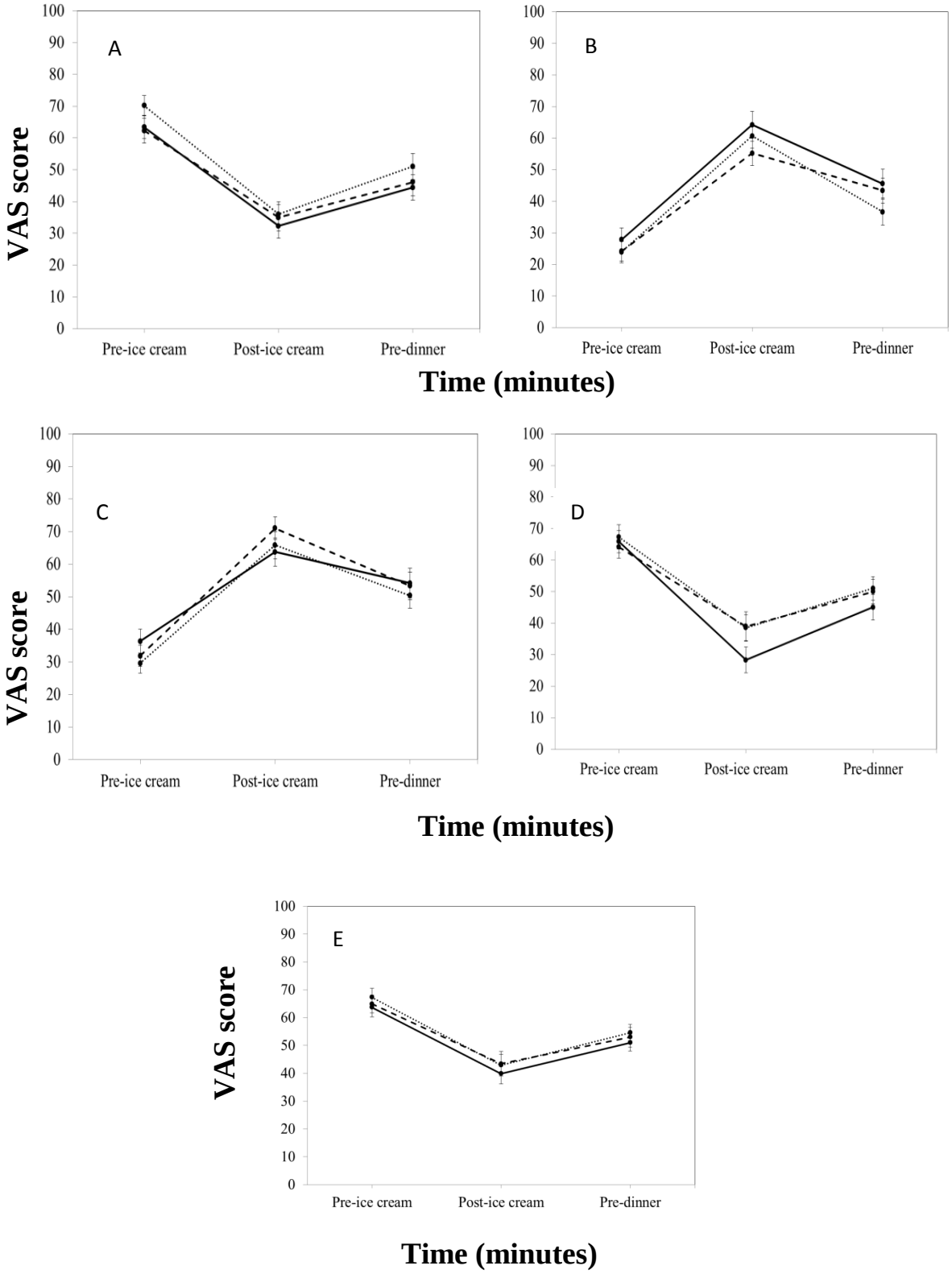

Fig.6.2 Appetite ratings over time from pre-ice cream to pre-dinner. A-hunger, B-fullness, C-satisfaction, D-desire to eat and E- prospective consumption. Dash lines represent ratio 25CO:75SO, round dot lines ratio 50CO:50SO and solid lines ratio 75CO:25SO. Error bars represent means ±SEM.

6.4 Discussion

This study aimed to elucidate the impact of a fixed quantity ice cream preload containing different ratios of MCTs and LCTs (mainly unsaturated) on subsequent *ad-libitum* energy intake and experience of appetite. Fat and HFSV food intake was significantly lower after ratio 75CO:25SO than all other conditions. Nevertheless, evening snack energy intake was significantly lower after 25CO:75SO and 50CO:50SO with less protein, carbohydrate, and LFSW food intake and higher fruit intake than that observed after 75CO:25SO. This indicates a potential earlier effect on macronutrient intake exerted by a high concentration of MCTs (fat and HFSV intake at *ad-libitum* dinner) and a delayed effect on food intake by a high concentration of LCTs (snack intake), which complements the differences found between MCTs and LCTs with respect to their absorption and metabolism by the body. However, it must be noted that while these differences were statistically significant and consistent across participants, the effects were small. No impact of condition on subjective appetite and desire was evident, indicating that participants were similarly satisfied irrespective of condition.

The bi-phasic effect found of MCTs suppressing fat intake earlier whilst LCTs reduced later snack intake may be explained by the differences in metabolism of these fats by the body. As previously stated, MCTs are absorbed by the enterocytes more rapidly (Bruce, 2010) and reach the liver faster than LCTs (Westergaard & Dietschy, 1976), directly entering the portal system. On the other hand, LCTs are incorporated into chylomicrons (structures with a lipid core of triglycerides, cholesterol, phospholipids, and fat-soluble vitamin esters coated by proteins), which are much larger and require time to reduce in size (releasing fatty acid) before they reach the liver. LCTs also require an additional carnitine transporter in order to

pass the mitochondrial hepatic wall (Barret & Raybould, 2010) whilst MCTs do not require a transporter, thus they are readily oxidised. This β-oxidisation process synthesises Ketone bodies, which have been related to decreases in food intake (Le Foll *et al.,* 2014; Davis *et al.,* 1981; Carpenter & Grossman, 1983) as well as the β-oxidisation process itself (Feltrin *et al.,* 2008; Friedman & Tordoff, 1986; Friedman *et al.,* 1990). Thus, MCTs are likely to generate satiation faster than LCTs because they are absorbed and oxidised faster than LCTs and lead to the production of Ketone bodies. LCTs, in turn, may have an effect on later satiety as a longer period of time elapses before LCTs become available for β-oxidation (as they are absorbed at a slower rate, reach the liver at a later point and have a rate-limiting step in oxidation). Similarly, the differences in fat and HFSV intake observed may also be influenced by the sensory experience of the ice creams as the 75CO:50SO ice cream was rated as creamier and thicker than 25CO:75SO and 50CO:50SO. This lends further support to previous research indicating that higher subjective creaminess ratings result in acute reduced intake and appetite (Bertenshaw *et al.,* 2009; Yeomans & Chambers, 2011; Bertenshaw *et al.,* 2013; McCrickerd *et al.,* 2012; McCrickerd *et al.,* 2014).

These results partially support previous findings showing that MCTs (intestinal infused, administered as a preload or added to a test meal) reduce acute food intake in comparison with LCTs (Feltrin *et al.,* 2008; Rolls *et al.,* 1988; Van Wymelbeke *et al.,* 1998, 2001) whilst LCTs can reduce subsequent intake at a delayed (240 minutes) eating occasion (Lawton *et al.,* 2000). Although there was no reduction in total *ad-libitum* intake, differences in fat and HFSV intake were apparent between conditions after the high MCT condition and reduced snack box intake after the high LCT conditions were also evident, despite being small. The discrepancies between the present work and previous literature in total *ad-libitum* dinner

energy intake may be due to the higher fat quantities used in the previously mentioned studies (30-40 g). Nevertheless, the present results suggest that consumers may be able to modestly reduce their fat intake after eating an ice cream portion containing a standard amount of fat. Without reducing the amount of fat there wouldn't be a decrease in the palatability of the product so that consumers wouldn't be discouraged to consumption.

To our knowledge this is the first time that this (albeit small) bi-phasic effect of MCTs and LCTs has been shown in the literature. Moreover, current trends suggest that the recommended fat intake of 30% energy per day is being exceeded in the UK with poor quality saturated fats such as butter (Harwood *et al.,* 2007) which has been reported to be harmful to health (O'Sullivan *et al.,* 2013). Despite the small effects on subsequent fat intake seen here, it is important to highlight the quality of the fats used in the present research. Although CO is a saturated fat, it also contains a high amount of MCTs which have received considerable attention for their potential health benefits (Nagao & Yanagita, 2010) and the unsaturated fat profile of SO has also been found to show health benefits (Li *el al.,* 2015).

There are a range of limitations to the present research which should be addressed. For instance, the potential for compensation should not be ignored. Indeed, it may instead be that the lower snack intake observed after 25CO:75SO and 50CO:50SO may be due to participants compensating for the lower energy intake at the *ad-libitum* dinner. Future research should aim to further elucidate the mechanism for action of the MCT/LCT ratio assessed here to comprehend these modifications in food intake. It must also be noted that an all-female sample was used and considerations regarding menstrual cycle stage were not taken into account as any potential variance in appetite seen here was expected to be

accounted for during the randomisation stage. The inclusion of a male sample would also improve understanding about the conclusions drawn but was not possible in the current research. This trial also utilized a single-blind design due to the nature of the study product making double blinding not possible. The research is also limited in the conclusions drawn due to the healthy sample assessed with further research with an overweight and obese sample required to appreciate the differences that may occur in this group. Similarly, extending the assessment period to further understand whether the small changes in fat intake and snack selection found here remain consistent, or are compensated for over time, would be efficacious to understand the clinical relevance of the present study.

6.5 Conclusion

Overall, the present research suggests that eating a standard portion of ice cream (150g, 10% of fat) containing different fat ratios of MCTs and LCTs can modestly affect fat intake and snack selection at subsequent *ad-libitum* eating occasions. High concentrations of MCTs (saturated) manifested their effects earlier, modestly but consistently decreasing fat intake, whereas high concentrations of LCTs (unsaturated) manifested their effects later, reducing subsequent snack intake. This may be due to differences in the absorption and metabolism of these fats. To our knowledge, this is the first study to report such a bi-phasic action of triglycerides. Nevertheless, the observed differences, being slight and only observed after an acute dose, require further research utilizing repeated dosing to understand whether this may be clinically meaningful.

6.6 Supplementary materials

Snack energy intake

Interaction: 25CO:75SO * 75CO:25SO t [35]= -2.423, p= 0.021;

Interaction: 50CO:50SO * 75CO:25SO t [35]= -2.261, p= 0.030.

Snack protein intake

Condition * protein intake main effect: F [2, 70]= 4.325, p= 0.017;

Interaction: 25CO:75SO * 75CO:25SO t [35]= -2.526, p= 0.016;

Interaction: 50CO:50SO * 75CO:25SO t [35]= -2.421, p= 0.021.

Snack carbohydrate intake

Condition * carbohydrate intake main effect: F[2, 70] = 5.002, p= 0.009;

Interaction: 25CO:75SO * 75CO:25SO t [35]= -2.514, p= 0.017;

Interaction: 50CO:50SO * 75CO:25SO t [35]= -2.345, p= 0.025.

Snack LFSW intake

Condition * LFSW intake main effect: F [1.238; 43.339]= 5.002, p= 0.007;

Interaction: 25CO:75SO * 75CO:25SO t [35]= -2.808, p= 0.024;

Interaction: 50CO:50SO * 75CO:25SO t [35]= -2.792 p= 0.025.

Snack fruit intake

Condition * fruit intake F [2, 70]= 4.149, p= 0.020;

Interaction: 25CO:75SO * 75CO:25SO t [35]= 1.921, p= 0.063;

Interaction: 50CO:50SO * 75CO:25SO t [35]= 2.872, p= 0.007.

Retrospective questionnaires

Condition * Hunger main effect: F [2, 70]= 0.304, p= 0.739;

Condition * nausea main effect: F [1.577; 55.192]= 0.950, p= 0.374;

Condition *abdominal main effect: discomfort F [2, 70]= 0.673, p= 0.514;

Condition *fullness main effect: F [2, 70]= 0.857, p= 0.429;

Condition *irritability main effect: F [2, 70]= 0.216, p= 0.807;

Condition *mental alertness main effect: F[2, 70]= 0.043, p= 0.958;

Condition *contentedness main effect: F [2, 70]= 0.735, p= 0.483;

Condition *food pleasantness main effect: F [2, 70]= 0.035, p= 0.966;

Condition *difficulty to consume the food main effect: F [2, 70]= 0.847, p= 0.433;

Condition *bloatedness main effect: F [2, 70]= 0.902, p= 0.410;

Condition *comfortableness main effect: F [2, 70]= 1.226, p= 0.300;

Condition *flatulence main effect: F [2, 70]= 1.226, p= 0.300;

Condition *stomach tightness main effect: F [2, 70]= 1.835, p= 0.167.

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CHAPTER 7

Conclusions and Future Work

7.1 Conclusions

Results of this work have been presented and fully discussed in the previous chapters of this thesis. In this section, the main findings of the investigations conducted will be summarised and some further work that might be considered for future research will be suggested based on the results obtained.

The objectives of this research were 1) to investigate the effect of formulation and processing parameters on microstructural and physical properties of ice cream; 2) to study the effect of formulation changes (in particular different solid to liquid fat ratios) on sensory perception as this could influence the eating behaviour; and 3) to conduct an appetite study in order to answer the research question of this work (*would it be possible to produce a more satiating ice cream by changing the formulation rather than the fat amount?*)

In general, results gathered in this research indicated that formulation and processing parameters have an impact on microstructural and physical properties of ice cream, sensory perception, palatability and food intake. In particular, it was possible to achieve different micro and macrostructures of ice cream by altering the fat blend utilised. However, results from consumers' study showed that 1) the sensory perception generally did not change among the developed ice creams and 2) it is the formulation, rather than the microstructure, the factor to influence the palatability of the product, whereas the microstructure could impact the storage conditions of ice cream. These results were unexpected due to of a variety of previous publications indicating a correlation between micro/macrostructure and sensory perception/palatability. Nevertheless, results could be influenced by the use of untrained panellists which anyway, might be useful after all as it reflects the actual consumers' response

and therefore could be more appealing from an industrial point of view. The results obtained in the last chapter are particularly relevant and novel, as they demonstrate that by changing the type of fat and not its amount, it was feasible to achieve a reduction in food intake. The reduction of fat within a food product usually leads to a considerable decrease in the palatability, which is likely to reduce consumption. Modifying the fat type instead could be a starting point for future investigations focussed on addressing obesity issues.

Specific conclusions of this research work are following discussed:

1) Tween 80 and MDG had a different influence the thermal behaviour of a fat blend with solid (CO) to liquid (SO) ratio of 75CO:25SO

MDGs acted as templates for the crystallisation of the bulk fat, whereas Tween 80 worked more as an impurity leading to less perfect crystals. These results suggested that MDGs could be employed to promote the formation of a fat network in ice cream as the fat droplets are likely to have well-structured protruding crystals. It is therefore believed that their use might be beneficial to improve certain physical and sensory properties of the ice cream (e.g. enhancing the shape retention, providing slow melting rates and leading to a possibly smoother product). On the other hand, it is thought that the use of Tween 80 would impair the fat destabilisation; the fat crystals protruding from the fat globules are expected to be less structured, limiting their interaction with the surrounding droplets and consequently impacting negatively the final product.

2) Surfactants with low and high HLB number favoured the development of a well-structured fat network in comparison with surfactants with intermediate HLB number.

In particular, surfactants with low and high HLB number favoured the development of a well-structured fat network by bringing fat droplets into closer proximity and allowing their interaction. Consequently, the structures obtained with these surfactants had higher hardness and slower melting rate in comparison with ice creams made with more intermediate HLB number surfactants.

3) The use of fat blends with different SFC had an effect on microstructural, physical properties, some sensory attributes and the palatability of ice cream; the aging step could be avoided, decreasing consequently ice cream production time.

As the SFC increased from 5% to 60.5%, the ice cream fat network became more structured. With an excessively high SFC (87%) the results suggested the organisation of the fat into discrete agglomerates rather than into a network. The aging step was proven to have a minimal effect on the final product, as it simply slowed down the meltdown of samples with a SFC of 39% and 60.5%. Key findings from the sensory study have shown that a reduction of about 40% in SFC in comparison with a standard ice cream (made with milk fat) might be possible without affecting consumers' sensory perception. The palatability of the ice cream was principally influenced by the formulation, rather than the microstructure developed.

4) Consuming a standard portion of ice cream (150g, 10% of fat) containing different fat ratios of CO (formed of mainly MCTs) and SO (formed of mainly LCTs), can mildly affect fat and snack intake at subsequent *ad-libitum* eating occasions.

High concentrations of MCTs manifested their effects earlier, modestly but consistently decreasing fat intake, whereas high concentrations of LCTs manifested their effects later, reducing subsequent snack intake. This may be due to differences in the absorption and metabolism of these fats.

7.2 Future work

The research work of this thesis has highlighted a number of relevant topics for future research in order to improve the understanding of upon present findings. Some suggestions in this respect may include:

- The investigation of the effect of Tween 80 an MDGs on 1) the thermal behaviour of an emulsified system (rather than only bulk fat) to determine the effect of additional ingredients (such as water) and 2) ice cream as final product, in terms of fat destabilisation, meltdown properties, texture and sensory perception;

- The study of how differences in the microstructure of ice cream given by surfactants with different HLB numbers affect the consumers' sensory perception and palatability to understand if the conclusions drawn could be relevant for an industry context;

- Although results generated from this work suggested that aging step, whose timing was carefully selected, could be omitted from the ice cream production (given the fact that unaged ice creams did not show structural differences in comparison to aged samples), it would be interesting to investigate the effect of different aging times and the impact of aging, if shown, on the consumers' sensory perception and palatability.

- Regarding the appetite study, future research should aim to 1) further elucidate the mechanism for action of the MCT/LCT ratio to comprehend the modifications in food intake observed, 2) include male participants as this would improve the understanding

on the conclusions drawn, 3) use double blinding design, 4) extending the assessment period to further comprehend whether the changes in food intake remain consistent or not.