Plant Fibres as Ice Cream Stabilisers

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ABSTRACT

Over recent decades, the push in consumer trends towards the production of clean label, functionalised foods has resulted in food manufacturers looking towards plant fibres as a source of alternative stabilisers. Not only can plant fibres be considered as sustainable ingredients but they are also able to, due to the functional properties that they possess, allow the food manufacturer to meet the functional foods demand. Plant fibres were assessed as an alternative source of ice cream stabilisers. Firstly, a selection of plant fibres of different plant origin where characterised and their functional properties in suspension were determined to identify which plant fibres provided the most potential as ice cream stabilisers. These fibres were then incorporated into ice cream formulations and it was found that their addition had no impact on the correct microstructure formation.

Heat shock is the process whereby ice cream is stored in inappropriate temperature conditions or experiences breaks in the frozen-chain. Such drastic changes in temperature can alter the microstructure of ice cream reducing the sensory quality. Hydrocolloids are added to ice cream formulations to prevent such sensory deteriorations and for plant fibres to be a suitable source of alternative stabilisers they too must be able to prevent such deteriorations. It was found that the ability to control ice recrystallisation was dependent on the water binding capacity of the fibre, which in turn was related to the total fibre content. However, under heat shock conditions, this ability to control the rate of ice recrystallisation was largely dependent on the freeze-thaw stability of the fibre. It was found, in comparison to a hydrocolloid stabilised sample, that plant fibres have no impact on the rate of ice cream meltdown, nor the final mass loss, but could inhibit the time to first drip due to the ability of plant fibres to behave as ‘sponges’, absorbing the water from melting ice and hindering the rate of drainage by the formation of a fibrous network with the matrix phase. Higher apparent viscosities were also associated with improved meltdown properties. However, when plant fibres were co-stabilised with guar gum at the

concentrations studied, improvements in the meltdown properties and control over microstructural deteriorations were observed but samples became undesirably over texturised.

Sensory analysis was performed to determine the impact of plant fibres on the sensory perception of ice cream as well as the ability of plant fibres to retain sensory quality under heat shock conditions. It was found that plant fibres impart a powdery and mouth drying sensation due to the presence of insoluble fibre particles. Plant fibres of a high enough total fibre content were also found to be able to impart the correct mouthfeel properties in ice cream. Under heat shock conditions, it was found that the more freeze-thaw stable fibres were able to control increases in the iciness perception. However, plant fibres were not found to be able to control the sensory changes that are related to the structure of air, as cryo-Scanning Electron Microscopy imaging identified that plant fibres behave as anti-foams in ice cream formulations under temperature cycling conditions.

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CHAPTER 1: INTRODUCTION

1.1 Introduction

Ice cream is a product well known to and popular in the western world. Although the history of ice cream is shrouded in myths and stories, it is thought that ice cream was introduced to the western world after Marco Polo returned from Far East Asia in the 13th century bringing with him a recipe for ice cream (Cook & Hartel, 2010). Over time these recipes evolved to the product we recognise today.

Ice cream is characterised as a semi-soft, sweetened and flavoured frozen dairy dessert intended to be consumed in the frozen state. In general, such products are also commonly consumed aerated. Ice cream generally contains seven categories of ingredients. These are fat, milk solids not-fat, sweeteners, stabilisers, emulsifiers, water, flavours and once frozen, air (Goff & Hartel, 2013). Thus, its ingredient constituents allow it be considered as an emulsion, a dispersion and a foam (Goff, 1997). Ice cream can be categorised as hard-frozen products, which are those that have been subjected to a second freezing stage after dynamic freezing and as soft-frozen products, which are consumed immediately after dynamic freezing without a further hardening step (Goff & Hartel, 2013). In the UK "ice cream" is legally defined as a pasteurised frozen product containing a minimum of 5% fat and 2.5% milk protein (Clarke, 2012).

Global production of ice cream was reported to be 15.3 billion litres in 2006 (Goff & Hartel, 2013). Unilever and Nestlé are the largest worldwide producers with about one third of the global market between them (Clarke, 2012). With rapidly changing consumer markets, the ice cream industry is very progressive with many new products introduced annually (Goff & Hartel, 2013).

Today's consumers are becoming increasingly more health conscious which has driven a wealth of development into healthier products, predominantly low fat and reduced sugar ice creams (El-

Nager et al., 2002), in addition to more natural products (Goff & Hartel, 2013). More recently, dietary fibre enhancement of ice cream has been receiving growing attention with the increase of trends in the development of functional foods. This interest in the use of dietary fibre has been sparked by their functional properties, acting as effective fat replacers without adversely affecting flavour or texture (El-Nager et al., 2002; Crizel et al., 2014) or for health promoting fortification purposes, attaining increases of dietary fibre in the diets of consumers (Dervisoglu and Yazici, 2006; Soukoulis et al., 2009; Yangilar, 2015b; Yangilar, 2016).

The physiological benefits associated with a high dietary fibre diet include; lower serum cholesterol levels, reduced risk of coronary heart disease, reduced blood pressure, enhanced weight control, better glycaemic control, reduced risk of some forms of cancer and improved gastrointestinal function (Anderson et al., 1994). Dietary fibres are described as the edible parts of plants that are resistant to digestion in the human small intestine with complete or partial fermentation in the large intestine (Tungland & Meyer, 2002). The Recommended Daily Intake of fibre for adults over the age of 18 is 30 g per day in the United Kingdom. For a product to legally be considered ‘high in dietary fibre’, it must contain 6 g of fibre per 100 g of food and for a label claim of ‘a source of fibre’ it must contain 3 g of fibre per 100 g of food (British Nutrition Foundation, 2010).

Such interest has led to the commercialisation of Fiber One ^{TM} fibre fortified ice cream products, a General Mills Inc. brand on sale in the US containing Inulin (Chicory root extract) (FiberOne, 2015). In addition, the development of cellulosic fibre ingredients for the use in frozen dairy applications are prominent in patents, especially for their use as fat mimetics, textural enhancement and providing structural stability (Lundberg & Scheffler, 2004; Lundberg, 2013; Homsma et al., 2013).

1.2 Ice cream manufacture

The processing steps of ice cream manufacture are shown in Figure 1.1. A typical ice cream pre-mix contains 10% fat from milk, 10% not-fat milk solids which are mainly proteins from milk or whey powders and lactose, 15% sweeteners, 0.1-0.3% stabilisers, 0.1-0.3 % emulsifiers and finally colourings and flavourings (Eisner et al., 2005). The ingredients are blended in vats followed by pasteurisation. Pasteurisation destroys any harmful bacteria or hydrolytic enzymes from the mix and consists generally of heating a mix to 70-80°C, holding it at this temperature for a period of time (typically 30 minutes) and then rapidly cooling to below 5°C. Pasteurisation also aids in the solubilisation of stabilisers. Colourings and flavourings are generally added to the mix post pasteurisation. During the pasteurisation step the fat is liquefied and subsequent homogenisation reduces the droplet size. Homogenisation aims to create stable and uniform fat globules of 2 μm or smaller. Two-stage homogenisation systems are commonly used, the first pressure stage reduces the droplet size and the second stage breaks up any aggregates of fat droplets (Stogo, 1998). If homogenised correctly, the fat will not form a cream layer at the top of the cooled mix and the finished product will be free of a greasy or buttery mouthfeel and flavour defects (Goff & Hartel, 2013). Following this is the aging step, where the mix is stored at 4°C for 4-24 hours. During aging the fat crystallises and the emulsifiers absorb to the newly formed interfaces of the fat globule surface (Tharp & Young, 2012). Once sufficiently aged, the mix can be concomitantly whipped and frozen by either batch or continuous processes in a scraped surface heat exchanger.

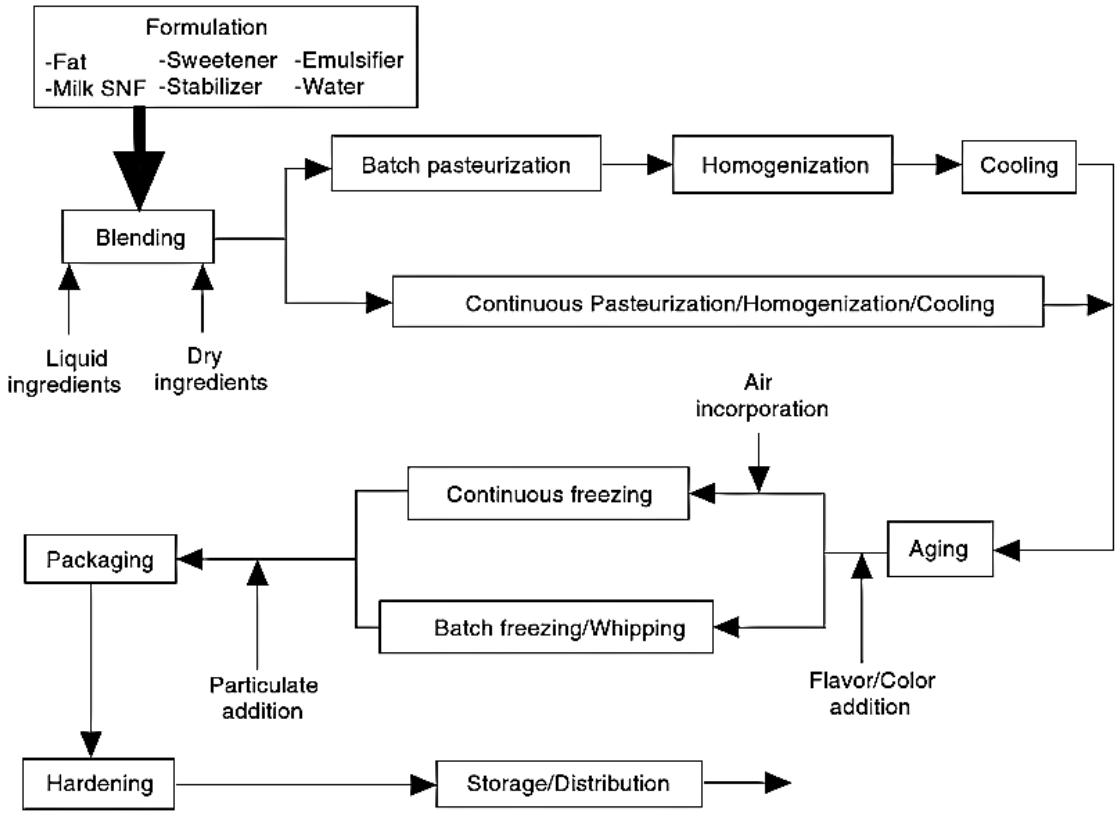


Figure 1.1 Ice cream manufacture processing steps (Hui, 2005).

Air is incorporated into the mix at the point of dynamic freezing. In continuous freezers, air injection is usually employed whilst in batch freezing the mix is commonly aerated by the whipping action of the scraper blades (Chang & Hartel, 2002). The partially frozen-aerated mix exits the freezer between -5°C and -8°C and is packaged (Eisner et al., 2005). For hard-serve ice cream, the packaged product is then subjected to further reductions in temperature in either hardening tunnels in large scale operations or blast freezers in small scale. During the hardening step the ice cream is statically cooled to around -40°C whereby the remaining ice is formed (Hui, 2005). More recently, low temperature extrusion has been receiving growing interest as it can

produce smaller air bubbles and ice crystals than conventional freezing. It also removes the requirement for a hardening step. In this process, the ice cream exits a conventional freezer at around -5°C and passes through a single screw extruder and is packaged at around -10°C. It is thought that both the shear forces and reduction in temperature whilst in the extruder combine to create the ideal microstructure (Clarke, 2012).

1.3 Stabilisers

Stabilisers are added to ice cream to impart specific and important functions such as increasing the viscosity of the mix, enhancing smoothness, preventing shrinkage, increased resistance to melting and reducing the rate of ice recrystallisation (Goff & Hartel, 2013). The most commonly used stabilisers are of hydrocolloid origin and include locust bean gum, xanthan gum, guar gum and carboxymethyl cellulose. Carrageenan may also be added to stabiliser blends to prevent phase-separation or 'wheying off' by interacting with proteins (Chandan et al., 2009).

Due to the thermodynamic instability of ice cream, ice recrystallisation occurs during storage, leading to the increase in ice crystal size and product deterioration (Soukoulis et al., 2009). Stabilisers play a key role in reducing the rate of ice recrystallisation, although the mechanisms by which they achieve this have received much debate. It has been suggested that stabilisers modify the crystal-serum interface by surface absorption, thus impacting the rate water can diffuse to the ice crystal surface during temperature fluctuations (Caldwell et al., 1992; Soukoulis et al., 2008). The cryogelation of hydrocolloids that can occur as a result of temperature fluctuations have also been identified as a mechanism of control (Goff et al., 1999; Patmore et al., 2003). Phase separation and an increase in phase concentration of the effective hydrocolloid caused by incompatibility

between hydrocolloid and milk proteins may also offer protection (Soukoulis et al., 2008). However, the current overriding mechanism of control is micro-viscosity enhancement and its ability to reduce the molecular mobility of water within the serum phase in between ice crystals and thus slowing down the rate that water can diffuse to the surface of a growing crystal during temperature fluctuations (Bolliger et al., 2000a; Goff & Hartel, 2013).

Despite the array of stabilisers available, there is still a place for new sources of stabilisers to meet the functional foods demand and the search for new sources of hydrocolloids has received growing attention in the last decade, due to a push from consumers to move products to the functional foods market (Farahnaky et al., 2009; Gharibzahedi et al., 2013). Food developers have begun utilising plant fibres for their functional properties such as enhancing viscosity, gelation ability, a high ability to bind oil, water and minerals in addition to providing health claims.

Due to the physiological and nutritional aspects of dietary fibre, it has led to their incorporation in a vast array of food products, including bakery, breakfast cereals, meat products, pasta, yogurts and ice cream (Soukoulis et al., 2009). Although hydrocolloid stabilisers may be defined as dietary fibre, generally, the low levels that they are used in frozen dairy desserts does not allow them to deliver the desired nutritional effect.

1.4 Plant fibres in ice cream

Plant fibres offer an alternative to conventionally used hydrocolloids as they can meet the functional foods demand, in some cases are of food waste origin as by-products from fruit and vegetable processing and thus can be considered as sustainable ingredients. They also have a natural ability to bind large amounts of water (Tungland & Meyer, 2002). Several advantages of

using plant fibres in ice cream have been reported. These include improvement of body and texture due to the formation of a fibrous framework, which also aids in enhanced melting properties, reducing ice recrystallisation thus extending the shelf life and viscosity enhancement allowing higher overruns (Anonymous, 2000).

The use of citrus fibre in ice cream has been reported in the literature. Dervisoglu & Yazici (2006) evaluated the effect of citrus fibre on the physical, chemical and sensory properties of ice cream. Three types of ice creams were assessed; ice creams with citrus fibre (0.4%, 0.8% and 1.2%), ice creams with 0.4% stabiliser and finally, citrus fibre stabiliser blends (total stabiliser content 0.8 – 1.6%). Citrus fibre was found to have a positive impact on melt resistance at 0.8%, but optimum melting properties were provided by fibre/stabiliser blending at 1.2% fibre and 0.4% stabiliser. Sensory analysis was performed by a semi-trained panel to assess flavour, texture and appearance. Panelists gave significantly lower scores to samples containing only citrus fibre but could not differentiate between samples containing stabiliser and fibre/stabiliser. The most preferred samples by the sensory panel were fibre/stabiliser samples containing 0.4% or 0.8% fibre. Higher fibre concentrations had a negative impact on flavour. It was suggested that a combination of citrus fibre and stabiliser produced desirable physical and sensory characteristics. Orange fibre and citrus fibre have also been suggested to behave as effective fat replacers in ice cream (Homsma et al., 2013; Crizel et al., 2014).

The ability to control meltdown rates and ice recrystallisation is suggested to vary between soluble and insoluble fibre sources as the ability to bind water is reported to vary between the two. Soukoulis et al. (2009) investigated the impact of dietary fibre (soluble and insoluble) from four different sources (oat, wheat, apple and inulin) on the rheological and thermal properties of model sucrose solutions and ice cream mixes. Differential Scanning Calorimetry (DSC) assessment found

that an increase in soluble fibre limited the freezing point depression and elevated the glass transition temperatures (T_g), indicating a potential cryoprotective action by limiting the molecular mobility of water within the bulk phase. The presence of apple fibre or inulin significantly decreased the percentage of freezeable water in both sucrose and ice cream systems as higher soluble fibre contents increased the proportion of bound water. In the case of apple fibre, this was due to the water binding ability of the gel network formed by pectin. The addition of inulin to yog-ice cream has also been found to reduce melting rates in low fat formulations (El-Nagar et al., 2002).

Freeze drying experiments have suggested that the addition of cellulose increases the solid content and thus could enhance the number of ice crystal nucleation sites (Winkworth-Smith, 2014). This can improve ice cream smoothness as numerous nucleation sites aid in the production of lots of small ice crystals during dynamic freezing (Russel, et al., 1999a).

Mucilage gums have recently been receiving growing interest for their use as novel stabilisers. Mucilage gums can be obtained from several sources including basil seeds, chia seeds, flaxseed and psyllium husk. Their incorporation in ice cream has previously focused on gums from Basil seeds (BahramParvar et al., 2012; BahramParvar & Goff, 2013a; Bahramparvar et al., 2013b; Javidi et al., 2016), Chia (Campos et al., 2016) and Psyllium (Upadhyay et al., 1978). Upadhyay et al. (1978) compared the use of Psyllium husk to sodium alginate in its ability to stabilise ice cream formulations. The addition of Psyllium fibre did not significantly effect product flavour and gave results comparable to that of sodium alginate for freezing temperature, melting resistance and organoleptic properties pre and post heat shock, but imparted a lower mix viscosity.

Assessing the efficiency of plant fibres in controlling microstructural changes as a result of inappropriate storage, otherwise known as 'heat shock' is key to assessing the suitability of plant fibres as alternative stabilisers. Preventing such deteriorations is vital to maintaining the shelf life quality of ice cream. Despite this, the work of Upadhyay et al. (1978) is the only investigation identified from the literature that has assessed fibre stabilised ice creams pre and post heat shock. Thus, there is little to no knowledge of if plant fibres are able to control heat shock deteriorations such as air cell disproportionation, shrinkage, serum drainage and ice recrystallisation.

In other studies assessing the suitability of waste by-product plant fibres, Yangilar (2015a) assessed the impact of green banana peel flour at 1% and 2% on the physical and sensory properties of ice cream. It was found that flour addition significantly reduced overrun, had little impact on melting rate but increased creaminess. In further studies the impact of fortification with lab prepared peach fibre (Yangilar, 2016) and date fibre (Yangilar, 2015b) on the physical and sensory properties of ice cream were also assessed. Peach fibre improved meltdown characteristics, enhanced viscosity, reduced overrun and had little impact on sensory properties. Date fibre also enhanced melt resistance and had little impact on the sensory properties.

Considering the other sources of plant fibres as sustainable ingredients, a large waste by-product of the manufacture of soy based products such as tofu and soy milk is okara, a fibrous bean curd residue. Chen et al. (2010) extracted water soluble soybean polysaccharide (SSPS) from okara and assessed its impact on the physical properties and consumer acceptability of low-fat ice creams. Ice cream formulations were investigated with 1-4% SSPS and a 3% blend of SSPS, guar gum and κ-carrageenan. It was found that viscosity increased with increasing SSPS concentration and meltdown was only affected at 4% SSPS, although this was found not to be significantly different from the control sample. Sensory analysis revealed ice creams containing 2% SSPS received the

highest mean acceptability score at 6.2 (“like slightly”) and 4% the lowest with a mean acceptability of 5 (“neither like nor dislike”). Consumers also indicated that they were “moderately likely” to consume fibre-fortified low-fat ice creams.

The use of plant fibres in ice cream formulations is a patented technology. Bartkowska et al. (2011) patented an invention which relates to a frozen aerated product, such as ice cream, wherein the frozen product is stabilised by plant derived ingredients in their unrefined state. These plant derived ingredients can be either soluble or insoluble fibre and can be derived from the inclusion of fruit and/or vegetable puree. The use of plant derived stabilisers has allowed the development of all natural ice creams, which do not contain any emulsifiers or stabilisers.

1.5 Objectives and structure of the thesis

1.5.1 Objectives

The main aim of this work is to investigate the potential use of plant fibres as novel ice cream stabilisers. Long or abusive storage conditions known as 'heat shock' which can occur during ice cream transportation, storage, in selling cabinets or at home with the consumer can reduce the shelf life quality of ice cream due to changes in ice cream microstructure which can impact on a products sensory experience. The ability to control such changes in microstructure is key to an effective stabiliser. Potential lies with plant fibres to control such sensory deteriorations due to their functional properties. The literature provides us with a large understanding of how plant fibres impact the physical and sensory properties of ice cream. However, given the little understanding from the literature of if plant fibres are effective at controlling structure and quality deteriorations with heat shock, assessment of their suitability under such conditions needs conducting. Fibre-hydrocolloid blends may also provide optimum functionality (Dervioglu & Yazici, 2006). An understanding of how different fibres behave in suspension will enable the identification of suitable plant fibres. Assessing the impact of plant fibres on ice cream microstructure and the changes that occur with abusive storage conditions will allow assessment of their effectiveness as stabilisers as well as a better understanding and interpretation of sensory data. Within this framework, the objectives of the work are:

- To investigate the use of plant fibres as ice cream stabilisers.
- To assess commercially available plant fibres for their suitability through screening techniques.
- To optimise fibre functionality using processing.

- To investigate the impact of fibres on ice cream microstructure and the changes in microstructure after abusive storage conditions.
- To assess the sensory impact and their ability to retain sensory quality.

One method that can be used to enhance the functionality of fibrous materials is to expand their internal surface area by processing, which increases the water binding capacity and apparent viscosity of the material (Lundberg et al., 2014). Thus, in the present study, specific interest lies with the creation of 'swellable hairy cellulose particles' whereby cellulose can be defibrillated using homogenisation, thus increasing the volume occupied by fibres in suspension and increasing the number of water binding sites. By 'swellable' it is intended that these fibres can absorb free water from melting ice crystals during increased temperature periods, and then release them upon decreases in temperature to reform ice. However, retaining such structures in freeze compression and thaw-hydrate conditions is key to fibre functionality.

1.5.2 Structure of the thesis

The introductory chapter, Chapter 1, will present a general literature review and identify the relevant research to date within the topic area. Chapter 2 will comprise a techniques chapter. A variety of research techniques have been used as part of this study and a description of the main techniques used throughout the thesis will be presented.

The work in Chapter 3 focuses on the selection of suitable fibres to stabilise ice cream formulations. Within this chapter, the results of screening of a range of fibres for their suitability will be presented in addition to the optimisation of fibre functionality using processing. The reasoning for the removal of unsuitable fibres from the study will be justified.

The plant fibres that proved to have the most potential as ice cream stabilisers from screening tests were incorporated into ice cream formulations. Chapter 4 will present the impact of plant fibre inclusion on the microstructure of ice cream and its ability to control quality deteriorations with abusive temperature storage conditions. Subsections of this chapter and its research results will focus on the impact on the different phases within ice cream: ice, air, fat, serum and finally the behaviour of the fibres themselves within an ice cream formulation.

In Chapter 5 the outcomes of the sensory assessment of ice cream formulations in addition to temperature abused samples using Qualitative Descriptive Analysis (QDA) techniques will be presented.

Each results chapter will contain its own specific introduction, methods and materials, results and discussion and conclusion sections.

In Chapter 6 a general conclusion of the work will be presented along with some suggestions for future work.

In the Appendix, unrelated to the work undertaken into the use of plant fibres as ice cream stabilisers, is the published work undertaken during the first year of this PhD. Sponsored by Quorn Foods Ltd., this work was undertaken to create products which allow consumers to conveniently reduce their meat consumption by the use of Hybrid meat products whereby a proportion of the meat has been replaced with healthier and more sustainable protein sources. A consumer-orientated approach was used to drive the development of products which were tested in a consumer study. Check-all-that-apply (CATA) questioning using consumers offers an alternative methodology to the conventionally used descriptive analysis techniques using trained panels. The CATA data when paired with consumer hedonic data can provide a wealth of information and direction for further product development to maximise consumer acceptability.

CHAPTER 2: TECHNIQUES

2.1 Nuclear Magnetic Resonance (NMR) spectroscopy

NMR spectroscopy is a research technique that exploits the magnetic properties of certain nuclei that contain an uneven number of protons to neutrons. All subatomic particles (protons, neutrons and electrons) spin on their axis either clockwise or counter clockwise. In a nucleus where protons are equal to the number of neutrons, the spins are paired and there is no overall spin. However, if there are more protons than neutrons, the nucleus possesses a spin of ½, such is in ^{1}H. This spin allows the atom to behave like a small magnet (Keeler, 2011).

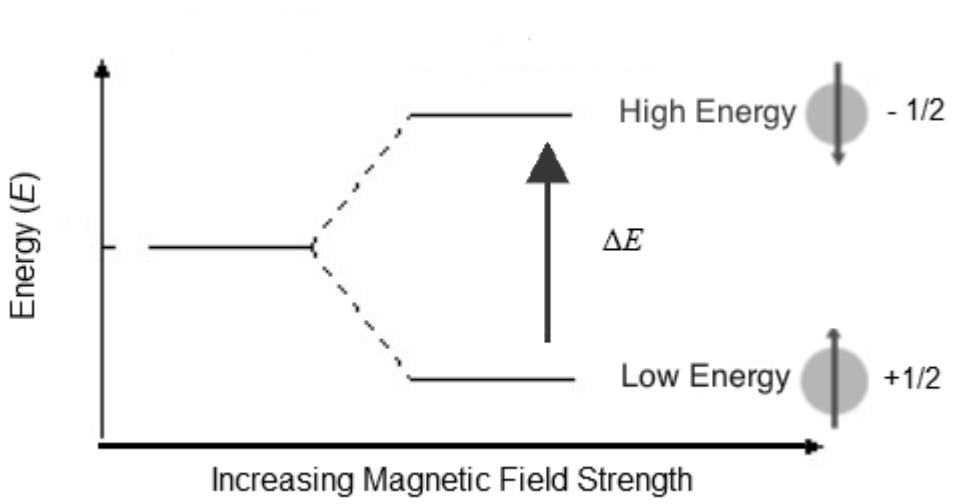


Figure 2.1 The energy levels of nucleus in magnetic fields (Keeler, 2011).

If these nuclei are placed in a magnetic field they will align with or against the magnetic field in either the low energy state or the higher energy state (Figure 2.1), respectively, as depicted by the Bolzmann distribution (B_o). The energy difference between the two energy levels is ΔE, this is the energy required to get the nuclei from the lower energy state to the higher state, which is known as the resonance frequency. Increasing the field strength increases the energy difference between the two states resulting in an improved signal to noise ratio as it is made easier to detect movements between the two states. The energy difference between the higher

and lower state corresponds to the energy of the electromagnetic field (magnet strength). When these nuclei are excited by a pulse of electromagnetic energy, nuclei in the lower energy state will jump to the higher state. On returning to their ground state a photon of energy is released which can be quantified (Keeler, 2011).

Spin-spin relaxation NMR also known as the transverse relaxation (T_2) is the study of the relaxation behaviour of protons after excitement to the higher energy level from a magnetic pulse as they return to their equilibrium state on the x, y plane (Figure 2.2), which is perpendicular to the magnetic field.

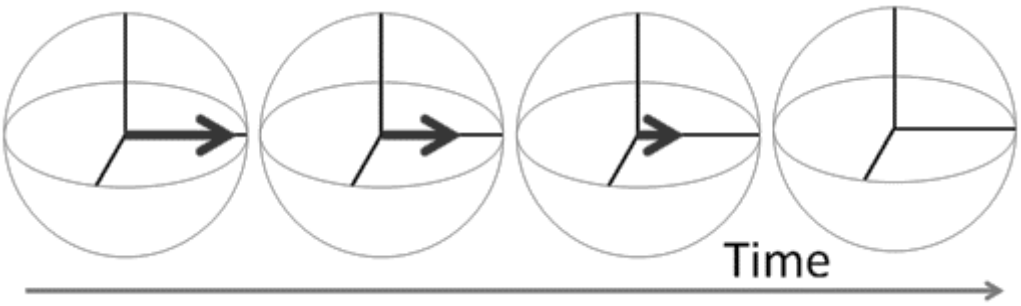


Figure 2.2 The process of transverse relaxation (Keeler, 2011).

The way in which these protons relax is suggested to be related to the environment in which they are located. Protons which are free to move have long relaxation times whereas protons which are bound or close to protein or polysaccharides (exchangeable proteins) have shorter relaxation times (Belton, 1997).

2.2 Rheology

Rheology is the study of the deformation and flow of matter under applied forces (Mezger, 2006).

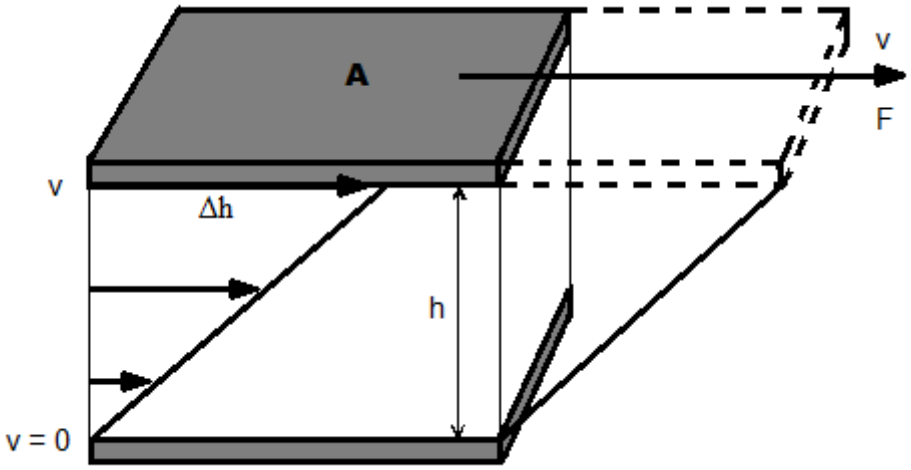


Figure 2.3 The flow of a fluid using the Two-plate Model (Mezger, 2006).

The two-plate model can be used to define some fundamental rheological parameters (Figure 2.3). For two plates with a distance of h, which is the shear gap, the lower plate is stationary (v = 0) and the upper plate with the shear area (A) is set in motion by the shear force (F), the resulting velocity (v) can be measured. This is only the case with the following two assumptions:

1. The sample adheres to both plates whereby there is no slip.
2. The flow is laminar and therefore there is an absence of turbulent flow.

The shear stress τ (Pa) is the external force acting on an objects:

Equation 2.1

$$\tau = \frac{F}{A}$$

The shear strain γ (unitless) is the relative deformation resulting from the stress:

$$\gamma = \frac{\Delta h}{h} \qquad \textit{Equation 2.2}$$

Where Δh is the change in height and h is the original height.

The shear rate $\dot{\gamma}$ (s^{-1}) is the speed of deformation:

$$\dot{\gamma} = \frac{v}{h} \qquad \textit{Equation 2.3}$$

Generally fluids exhibit three main behaviours when under flow. These are shown in Figure 2.4.

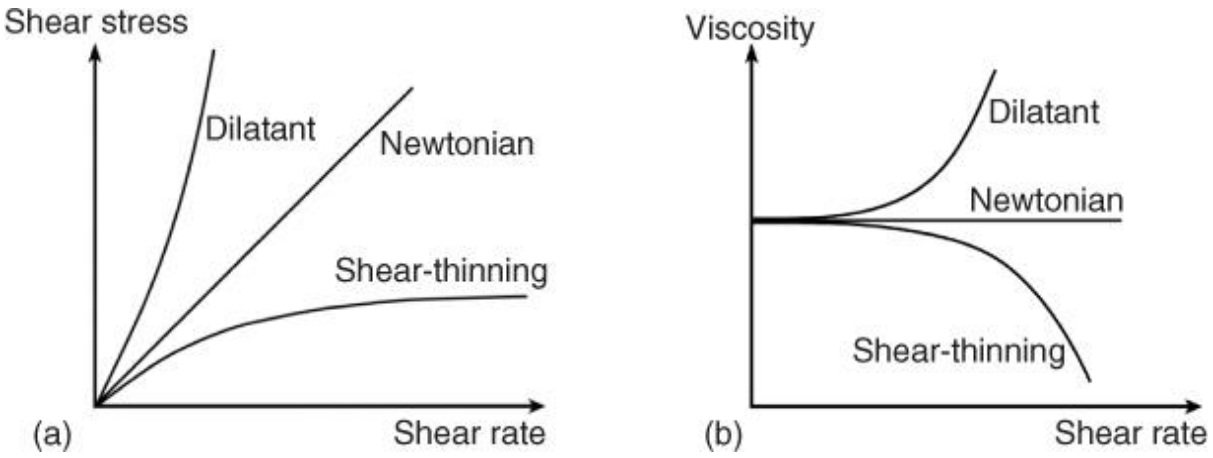


Figure 2.4 Flow curves (a) and viscosity curves (b) exhibiting the three flow behaviours of fluids (Willenbacher & Georgieva, 2013).

Under shear, for Newtonian fluids, the shear stress is proportional to the shear rate and the viscosity remains constant independent of the applied shear rate e.g. water. In a shear thinning

or pseudoplastic behaviour the viscosity decreases with increasing shear rate e.g. hydrocolloid solutions. For shear thickening behaviour, viscosity increases with increasing shear rate e.g. concentrated starch suspensions. Often shear thickening behaviour is associated with an increase in sample volume which is known as dilatency.

Viscosity η (Pa.s) is the resistance of a fluid to flow and is defined using Equation 2.4 and is performed using rotational measurements:

$$\eta = \frac{\tau}{\dot{\gamma}} \qquad \textit{Equation 2.4}$$

Three types of geometry are generally used to measure the viscosity of a fluid; cone and plate, parallel plate and double gap geometry. Cone and plate geometry allows uniform shear rates across the sample whereas with parallel plate geometry the shear rate is not homogeneous. For samples of low viscosity, generally less than 10 mPa.s, a double gap geometry can be used which has a much greater sensitivity due to a larger surface area.

Shear-thinning behaviour is the most common behaviour of non-Newtonian materials. At rest or in low shear rates, molecules are randomly organised. As the shear rate increases, the molecules align with the direction of flow. For entangled polymer systems, with increasing shear rate the polymers become disentangled. For some samples higher shear rates are required to cause flow. This referred to as the yield stress which is shear stress that needs to be applied to the solution before it begins to flow. Some samples also exhibit thixotropy. This is a time-dependent behaviour. Some materials flow when submitted to shear and take a fixed time to return to their original state. For example for xanthan which exhibits thixotropic behaviour, different viscosity curves can observed when the shear rate goes from low to high and from high back to low.

The flow behaviour properties of fluids can be described using several models. For non-Newtonian fluids, three models can be used and information of their rheological parameters can be obtained from plots of the shear stress against the shear strain (Rao, 2014):

1. Power-law model or Ostwald-de Waale model can be applied to flow behaviour that does not exhibit a yield stress.
2. Bingham model can be used in the case where samples exhibit a yield stress that is then followed by Newtonian behaviour.
3. Hurschel Bulkley model can be applied when the flow behaviour exhibits a yield stress which is proceeded by non-Newtonian behaviour.

2.2.1 Oscillatory tests

Dynamic or oscillatory tests can be performed in order to describe the viscoelastic behaviour of materials, which are samples that are able to exhibit both elastic and viscous properties simultaneously. Information on the viscous and elastic properties of materials can be generated using either a strain sweep or a frequency sweep.

The storage or elastic modulus, G', is the ability of the sample to store energy during deformation while the G" is the loss or viscous modulus and is a measure of the energy lost during deformation.

The loss factor or damping factor (tan δ) can be determined using Equation 2.5. And reveals the ratio of the viscous and elastic portion of the viscoelastic deformation behaviour:

$$\tan \delta = \frac{G''}{G'} \qquad \textit{Equation 2.5}$$

For a fluid-like behaviour tanδ > 1, while tanδ < 1 is an indication of gel-like behaviour.

A strain sweep (or Amplitude sweep) is carried out at a constant frequency with a change in strain as shown in Figure 2.5.

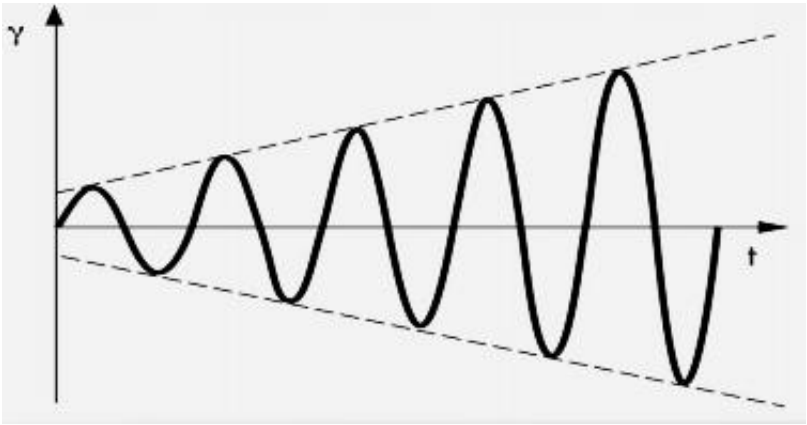


Figure 2.5 Amplitude sweep (Mezger, 2006).

This measurement is generally used to determine the linear viscoelastic (LVE) region (Figure 2.6), whereby the curves of G' and G" display a constant plateau. The yield point γ_L is the limit of the LVE. Beyond the yield point the structure of the sample is considered to be irreversible altered or destroyed. For G' > G" the crossover where by G' and G" are equal is described as the flow point where the fracture or internal structure occurs and the sample begins to flow.

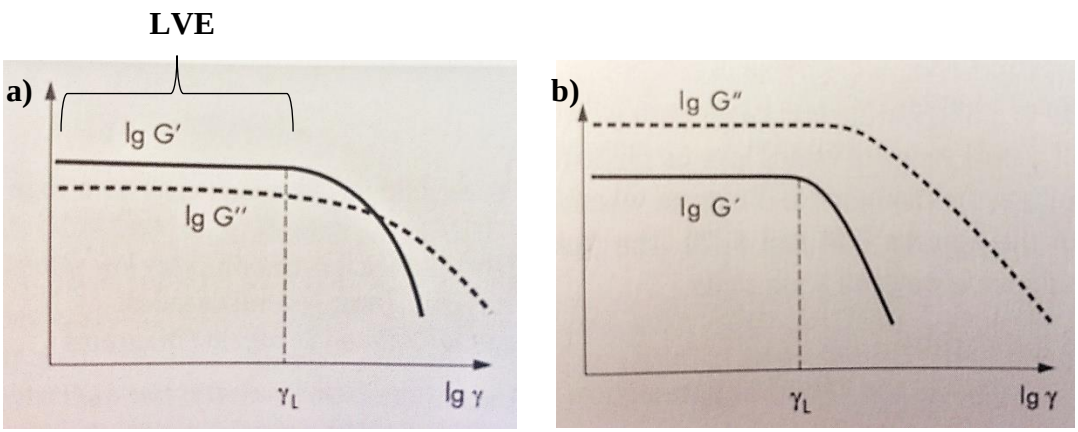


Figure 2.6 Strain sweep curves of samples showing (a) gel character and (b) liquid character (Mezger, 2006).

If within the LVE region G' > G", the material can be described as showing a gel character as the elastic behaviour dominates the viscous one. If G" > G', the sample exhibits a liquid character as the viscous behaviour dominates the elastic one.

Once the LVE region has been determined a frequency sweep is generally performed. A frequency sweep (Figure 2.7) is an oscillation test performed at variable frequencies but at a constant strain (the strain must be within the LVE).

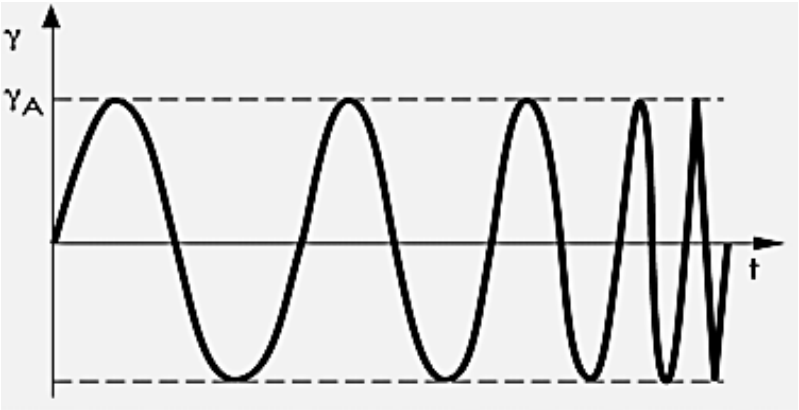


Figure 2.7 Frequency sweep (Mezger, 2006).

Three characteristic mechanical spectra can be determined using frequency sweep analysis as shown in Figure 2.8.

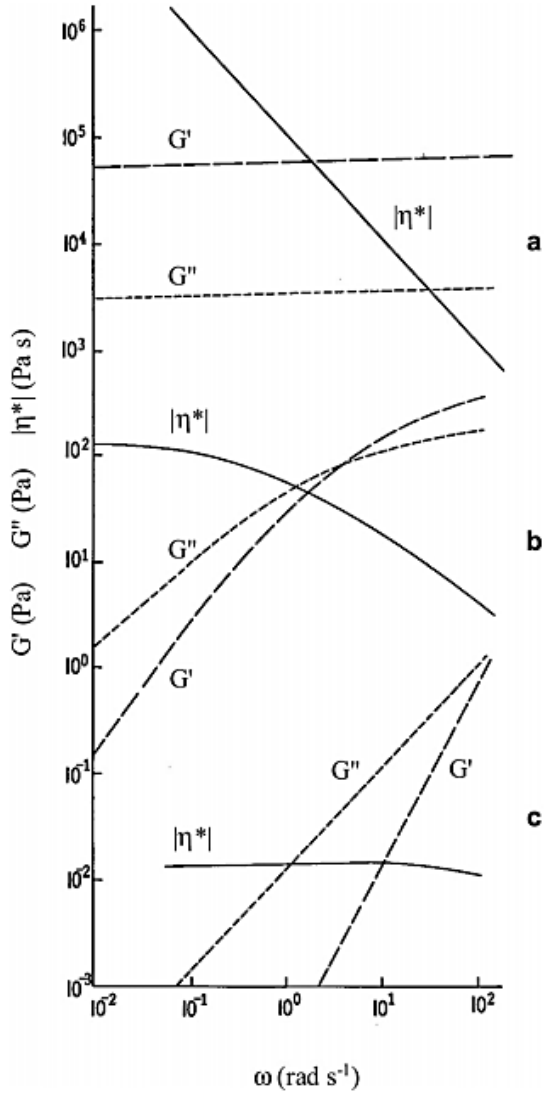


Figure 2.8 Mechanical spectra of (a) a gel (b) an entangled solution and (c) a dilute solution (Morris et al., 2012).

For a spectra exhibiting a gel character (Figure 2.8 a), G' and G'' are almost independent of the frequency and G' is higher than G''. The complex viscosity also decreases with increasing frequency and the slope is about -1. The complex viscosity (η*) is the frequency dependent viscosity measured under oscillatory tests. For the entangled (concentrated or semi-dilute)

regime (Figure 2.8 b), at high frequency, the entanglements have less time to disentangle and therefore G' > G". However, at lower frequencies G" > G' as there is sufficient time for the polymers to disentangle and flow. In dilute systems (Figure 2.8 c) the polymers are free to move independently.

2.3 Microscopy

Microscopy is the technical field of using microscopes to view samples and objects that cannot be seen by the human eye.

2.3.1 Light microscopy

Light microscopy or optical microscopy is a type of microscopy technique that uses the transmission of visible light through a sample to generate a magnified image. The sample restricts the transmission of light and appears as a ‘shadow’ against a bright background.

As shown in Figure 2.9 light coming from an internal or external source passes through a condenser lens which concentrates the light focusing it onto the specimen. After the light passes through the specimen it goes through an objective lens which magnifies the image. This image is then magnified again as it passes through an ocular lens where the image can be viewed. Compound microscopes like the one shown in Figure 2.9 will use several lenses to magnify the sample. The total magnification can be calculated by multiplying the magnification of the objective lens by that of the ocular lens.

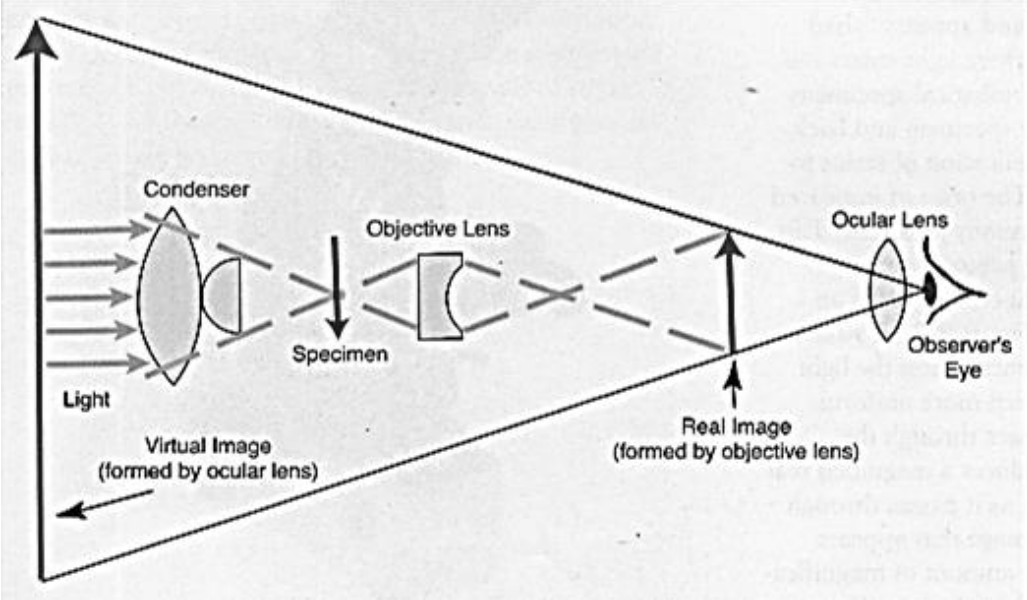


Figure 2.9 Image production in a compound light microscope (Peres, 2017).

A practical limit of resolution exists with light microscopy. Resolution refers to the clarity of an image i.e. the ability of a microscope to detect two points as separate entities and not one blurred image. Generally the best limit of resolution achieved by a light microscope is 0.2 µm but requires sophisticated equipment and lenses with high magnification.

Light microscopy can be Brightfield microscopy which is the 'common' optical microscopy technique and is the simplest of all techniques whereby the sample is illuminated by white light. Limitations to the use of Brightfield microscopy exist for the amount of contrast that can be seen to observe both the specimen from the background and any internal structures. In the case whereby an improved contrast is required, dark-field, fluorescence, polarised-light microscopy and phase-contrast microscopy can be used. In dark-field microscopy a special condenser is used so that only light reflected off the specimen enters the objective. This results in a brightly lit specimen against a dark background. Phase contrast microscopy works on the principal that light slows slightly when passing through a biological specimen. The specimen is illuminated by a hollow cone of light coming through a condenser. The degree of retardation

of the light results in light and dark areas on the image allowing the identification of biological structures. Florescence microscopy allows the identification of structures to been seen that would otherwise not be visible in light microscopy by causing fluorescent molecules to fluoresce. These molecules can either be auto-fluorescent, such as some biological structures like chlorophyll or tagged with fluorescent markers. Polarised-light microscopy uses polarised light to assess the location of structures that are birefringent; these are structures that have two different refractive indices at right angles to each other (e.g. cellulose microfibrils). A polarised light microscope is equipped with both a polariser, positioned in the light path before the samples and a second polariser just after the objective (Murphy, 2002).

2.3.2 Confocal laser scanning microscopy (CLSM)

Fluorescence is the ability of some molecules to absorb the light from a particular wavelength. The absorption of a photon of energy causes the molecule to excite and on returning to the ground state will re-emit a photon of energy at a different wavelength, known as the stokes shift. As different flurochromes become excited at different wavelengths and will re-emit different wavelengths of light this allows the spatial arrangement of these molecules to be identified by labelling them with different fluorescent molecules (Lorén et al., 2017).

In traditional fluorescence microscopy, this results in a fluorescent image from the entire sample. A primary problem with fluorescent imaging is that out of focus fluorescence appears as ‘flare’ or ‘blur’ reducing the quality of the image. However, in Confocal microscopy it works using focal planes. The laser can penetrate into a sample generating information only from that focal plane producing a sharp image without the interference of fluorescent molecules in the surrounding layers. By adding sacks of images together from different planes, a three-dimensional image of the microstructure can be generated.

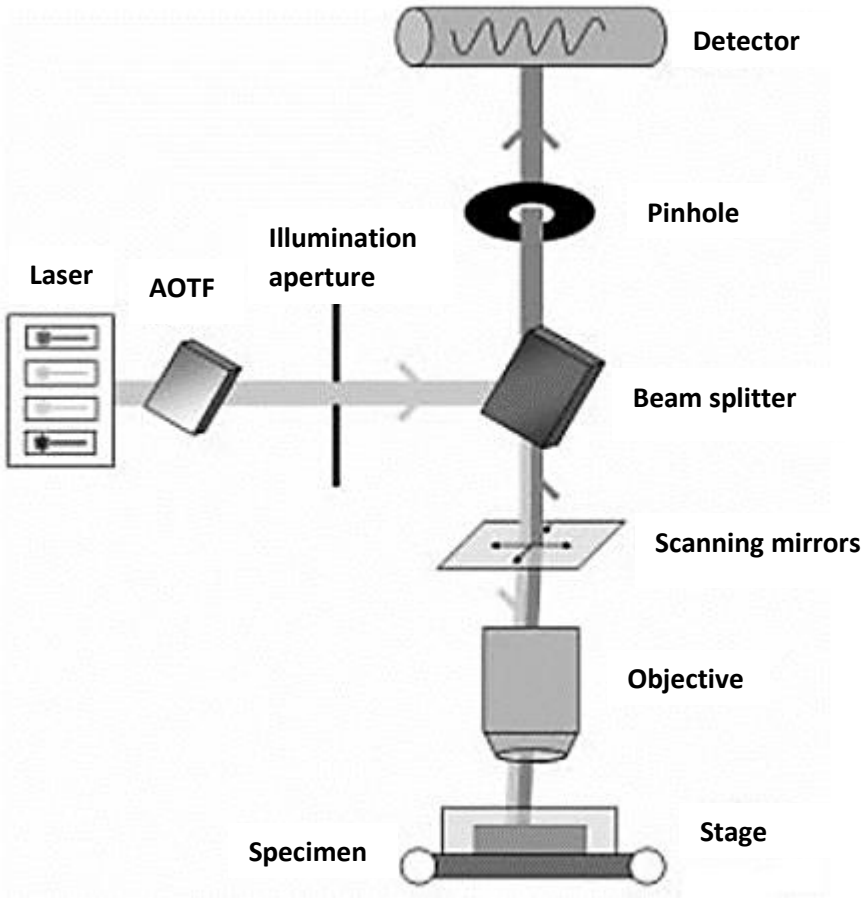


Figure 2.10 Components of a Confocal Microscope (Lorén et al., 2007).

The components of a confocal microscope are shown in Figure 2.10. A laser beam is emitted from a laser box and passes through an acousta-optical tunable filter (AOTF) which mixes the proportion of light coming from the laser to that needed of the different wavelengths needed to excite the different flurochromes. The beam splitter acts as a three way valve; directing incoming laser light to the sample and re-emitting fluorescent light towards to detector. The laser passes through the objective and scans the focal plane laterally. The photons then travel back through the beam splitter and through a pinhole. The pin hole prevents the fluorescence from the out of focus light from other planes to pass through it and therefore only light from the selected focal plane reaches the detector. The primary component of the detector is the

photomultiplier tube (PMT), which counts the number of photons at different wavelengths, building an emission fingerprint (Lorén et al., 2017).

2.3.3 Scanning electron microscopy (SEM)

SEM uses electrons to image specimens. The components of a typical SEM are shown in Figure 2.11. An electron gun sends a beam of electrons down into the vacuum chamber. The vacuum chamber prevents the electrons from colliding with air particles as they travel. This electron beam is focused using a series of electro magnets (condenser lens) which create a magnetic field and finely focus the electron beam onto the sample. The anode, with a positive charge, attracts the negatively charged electrons towards the chamber where the specimen to be examined is located.

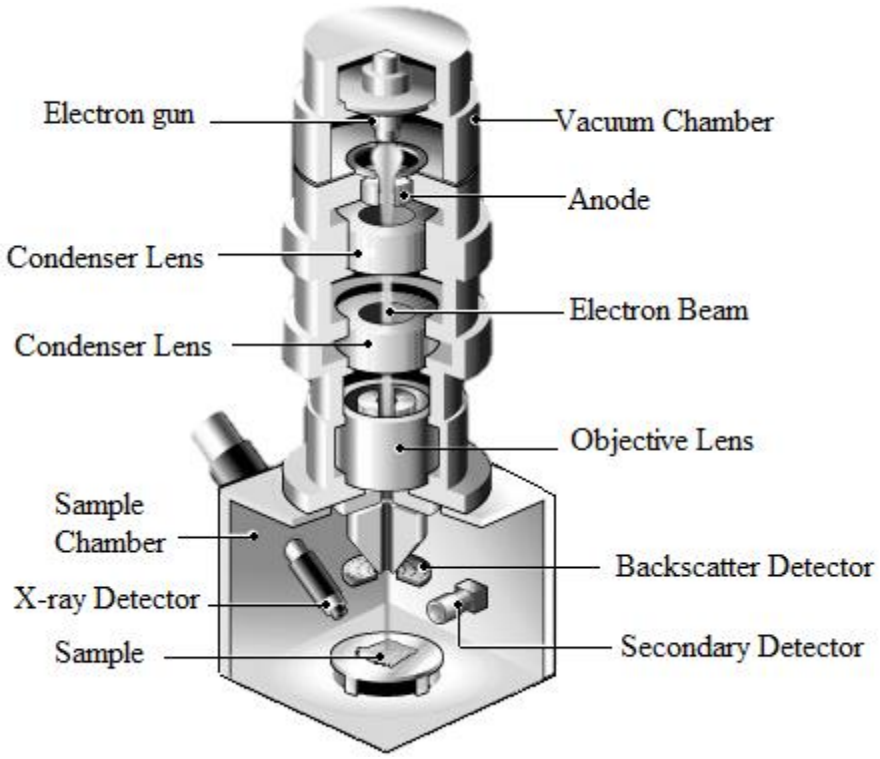


Figure 2.11 Scanning Electron Microscope (Attenberry, 2017).

When the electron beam reaches the specimen, some electrons are reflected (backscatter electrons) and other electrons (secondary electrons) are emitted from the metallic stain used from sputter coating whereby specimens are coated with a thin layer of a conductive metal such as Gold or Platinum. The way in which the electron beam interacts with the samples is captured by the detector (either backscatter, secondary electrons or x-ray detectors) which uses them to produce a three-dimensional image.

2.3.4 Transmission electron microscopy (TEM)

TEM works on a similar principal to Light Microscopy but an electron beam instead of a beam of visible light is used. It also addresses the resolving issue of light microscopy as the resolution can be improved by a factor of 1000 and objects in the nano scale range can be imaged (typically down to 0.1-2 nm). In TEM (Figure 2.12) a beam of electrons is generated by an electron gun and travel through a vacuum chamber. These electrons pass through an ultrathin section of specimen. The degree of interaction between the electrons and the heavy metal stains (e.g. osmium tetroxide) affects the kinetic energy of the electrons. Such stains absorb the electron beam or scatter it affecting the end image. The image is magnified and focused onto an imaging device such as a fluorescent screen or to be detected by a sensor linked to a camera. The light of varying intensity produced is directly proportional to the electrons kinetic energy on the image. Fixatives are used in sample preparation to react with the macromolecules in the sample to preserve the structure. In addition, embedding samples in resin enables the cutting of ultrathin sections (Williams & Carter, 1996).

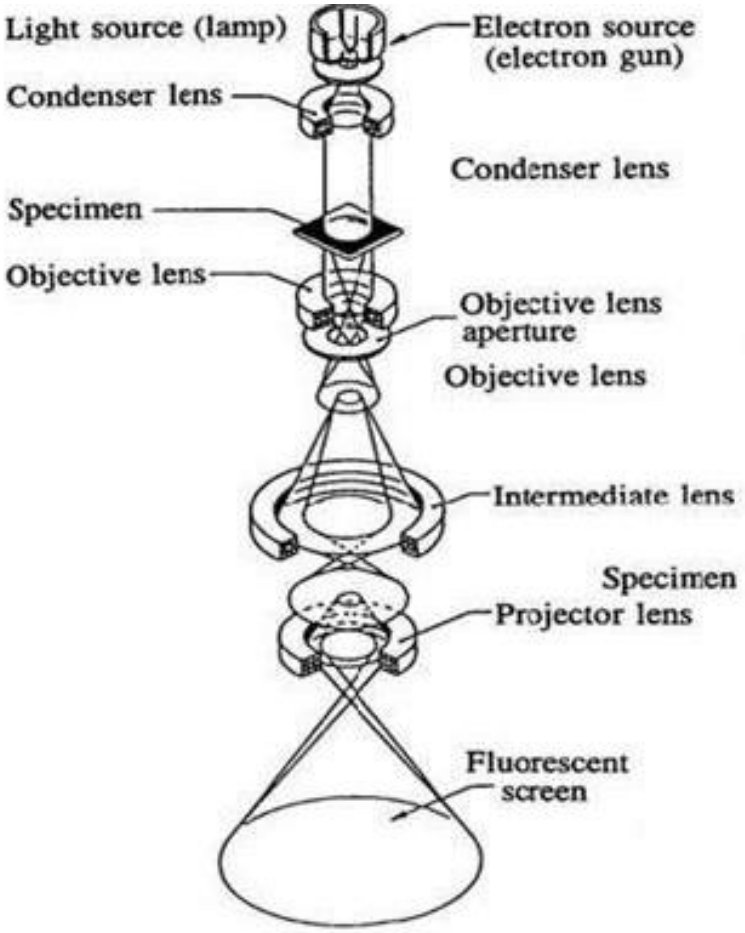


Figure 2.12 Transmission Electron Microscope (Williams & Carter, 1996).

2.4 Differential scanning calorimetry (DSC)

DSC is a thermoanalytical technique in which the difference in energy required to heat a sample in comparison to a reference is measured. Both the sample and reference are maintained at the same temperature throughout the experiment. Any differences between the energy required to heat the sample and reference will indicate some sort of physical transition, such as the glass transition. The event will either be exothermic or endothermic depending on the physical transition undertaken and resultantly the process will require less or more heat to maintain the same temperature between the sample and reference. As solids melt to a liquid, more heat will be required as this is an endothermic process. Likewise as the sample undergoes the exothermic

process of crystallisation, less heat is required to maintain the temperature. Measurements of the amount of heat absorbed or released can be observed on the DSC traces and information on the temperatures at which phase transitions occur can be determined. One important phase transition in ice cream is the glass transition temperature (T_g) (Figure 2.13). The T_g is the temperature range at which a material is converted from a viscoelastic liquid (rubber) to an amorphous solid (glass) with an associated increase in viscosity. For ice cream this is the temperature of the maximally free-concentrated solution.

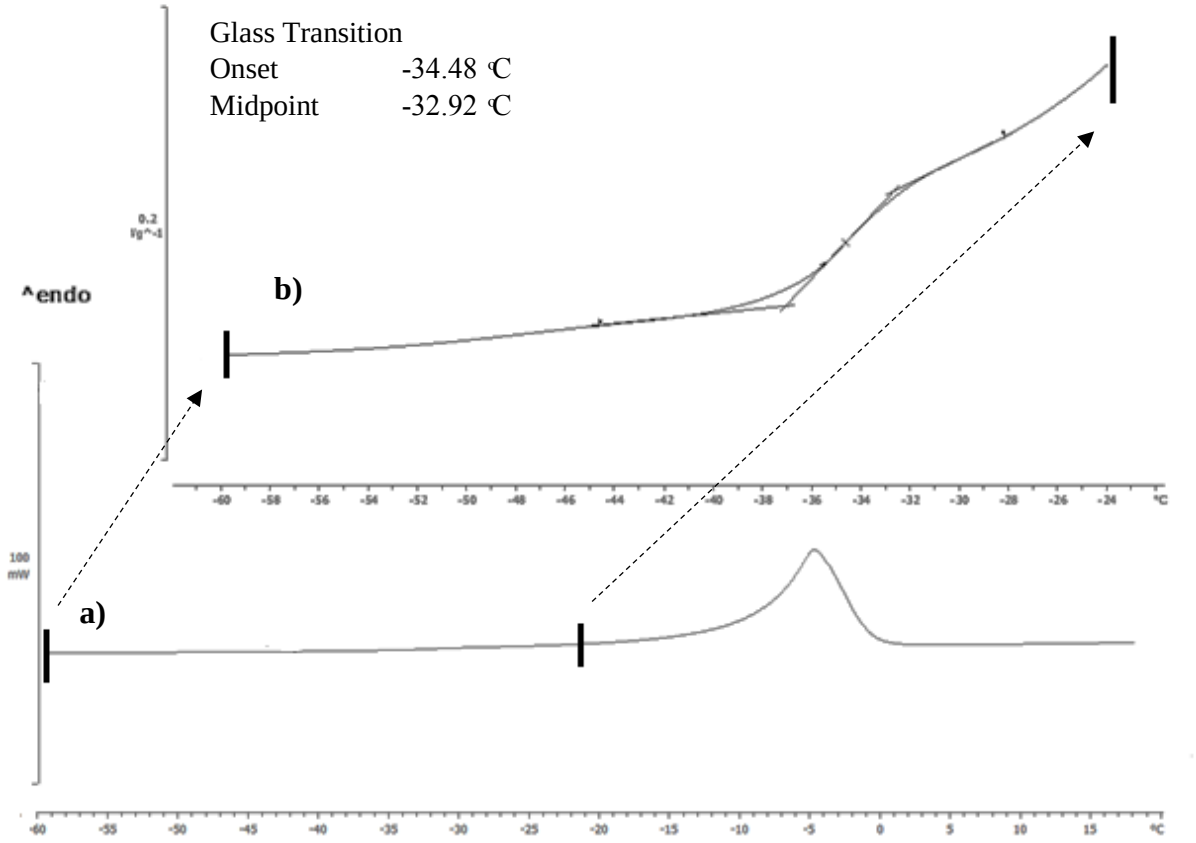


Figure 2.13 Differential scanning calorimetry (DSC) traces showing (a) the cooling curve and (b) an enlarged section of the cooling curve indicating the tangents constructed to determine the glass transition temperature (T_g).

The T_g on DSC traces can be calculated by constructing a series of tangents on the base line before and after the glass transition. The intersection of these tangents to the inflection point gives the extrapolated onset, midpoint and endpoint temperatures (Soukoulis et al., 2009).

2.5 Sensory Evaluation

Sensory evaluation is a scientific discipline used to evoke, measure, analyse and interpret those responses to products that are perceived by the senses of sight, smell, touch, taste and hearing (Stone & Sidel, 1993). When products are consumed, there is an interplay between all of the five senses and therefore only the use of human panels as analytical instruments can provide information on such interplay. However, the attributes of a food item are typically perceived in the following order: appearance, odor, texture and finally flavour and taste. However, humans generally receive an amalgamation of the attributes and therefore trained panelists are required to decipher each stimulus.

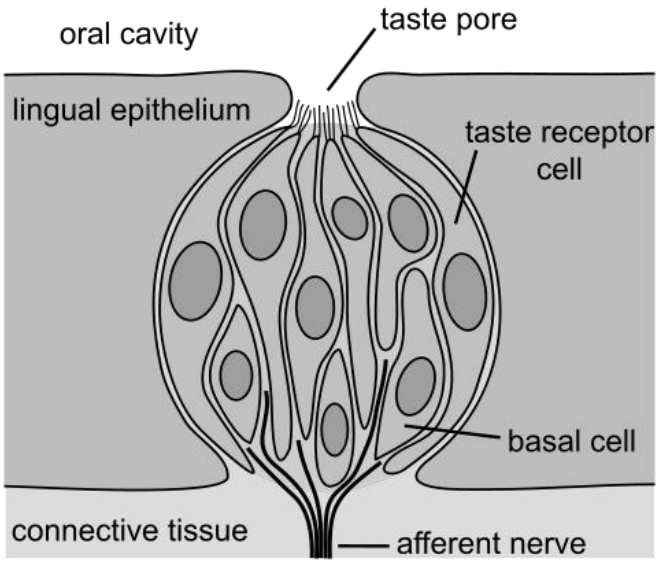


Figure 2.14 Taste bud on the human tongue (Meilgaard et al., 2006).

There are five basic tastes; sweet, salty, sour, bitter and umami, all of which are detected by taste buds that are primarily located on the surface of the tongue as well as in the mucosa of the palate. Taste buds (Figure 2.14) on the tongue are located on papillae. Each taste bud contains 30-50 taste receptor cells which project microvillae to form a taste pore. It is here where it is believed that taste molecules from food bind resulting in a taste response. Once the tastants bind to the cell, transduction occurs. For chemicals that produce salty or sour tastes, transduction occurs through the ion channels directly. For chemicals responsible for sweet, umami and bitter tastes, these bind to the surface receptors that triggers a series of signals and the release of neurotransmitters that results in the generation of an electrical signal that is sent to the brain along affective nerves. Irritant stimulus such as the burn of chili, the sting from mustard and the numbing of menthol stimulate the trigeminal nerve endings that are located on the papillae.

The perception of touch can be divided into somesthesis (tactile sense, skin feel) and kinesthesis (mechanical movements of the muscles). Tactile receptors on the tongue and mouth cavity are mainly responsible to the perception of texture and mouthfeel. However, the perception of some stimulus such as hardness, stickiness or softness will be perceived via kinesthetic perception.

Much of flavour is actually a result of the volatile compounds in foods that stimulate the olfactory cells in the nasal cavity responsible for smell. Therefore, flavour is used to describe the perception of taste and smell together. Orthonasal olfaction and Retronasal olfaction are the two mechanisms by which a smell is detected (Figure 2.15). Orthonasal smelling is the detection of an odor through the nostrils by sniffing or inhalation. Retronasal olfaction is the detection of odorants released from food in the mouth. These odorants pass through the posterior nares of the nasopharynx where they bind to olfactory receptors and where each

odorant binds to a unique combination of receptors. This is then converted to an electrical signal that is carried to the brain (Meilgaard et al., 2006).

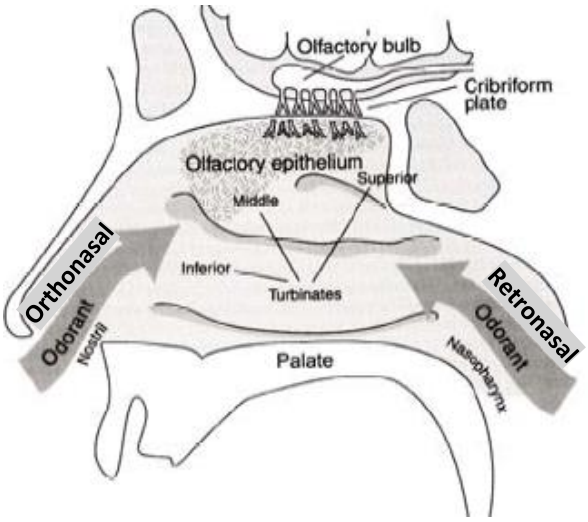


Figure 2.15 Orthonasal and Retronasal olfaction (Meilgaard et al., 2006).

Sensory evaluation of food products can be carried out by consumers or trained panellists depending on the objectives of the test. There are two types of sensory tests: objective and subjective. Objective tests provide objective data on the sensory properties of products and are carried out by trained assessors. Objective tests can be split into two classes: (1) discrimination tests determine whether there are sensory differences between samples such as in triangle tests (2) descriptive tests identify the nature of the sensory difference and the magnitude of difference such as in descriptive analysis. Subjective tests are known as affective or consumer tests and can provide data on the acceptability, liking or preference of food products and is carried out using untrained panellists (Kemp et al., 2011).

CHAPTER 3: FIBRE SCREENING AND OPTIMISATION

3.1 Introduction

It has been suggested that the molecular mobility of water within ice cream plays a key part in ice recrystallisation. Stabilisers are added to ice cream to control such processes (Goff & Hartel, 2013). Although opinions vary, it is thought this ability to control ice crystal growth is due to their ability to reduce the molecular mobility of water within the serum phase, thus reducing the rate of ice recrystallisation (BahramParvar & Mazaheri Tehrani, 2011; Goff & Hartel, 2013). Further to this, studies have found that cellulose has the ability to restrict the molecular motion of water and as such the use of cellulose as an alternative stabiliser is growing in interest (Magne et al., 1947; Preston & Nimkar, 1953).

It is generally understood that there are three types of water that can be associated with biopolymers in solution; non-freezable bound water, freezable bound water and freezable water. Freezable bound water has a reduced freezing temperature, significantly lower than that of bulk water and thus large reductions in sub-zero temperatures are required to release it from its bound state. Therefore, it has been suggested that increasing the proportions of bound water in a polymer solution can provide protection over ice recrystallisation as the water is 'trapped' and is no longer available to be involved in mobility related degradations (Nakamura et al., 1981). Hydroxyl, carboxyl and carbonyl groups all have the ability to form either strong or weak interactions with water (Hatakeyama & Hatakeyama, 1998). Therefore, assessment of the amount of water within each of these three states gives an indication of the potential impact of the polymer over control. However, difficultly arises in ice cream formulations as even at sub-zero temperatures a significant proportion of unfrozen water exists due to the freeze-concentration of sugars. This unfrozen phase is available to participate in the deteriorating processes of ice coarsening and matrix drainage (Sahagian & Goff, 1995). However, it has been suggested that sufficient enhancements in both micro and macro viscosity of the unfrozen phase using hydrocolloids impedes mobility at sub-zero temperatures (Goff & Hartel, 2013),

although attempts to correlate viscosity to action has been inconclusive, as results differ among hydrocolloid solutions (Budiaman & Fennema, 1987).

Differential scanning calorimetry (DSC) measurements can be used to determine the three types of water within polymer solutions. Soukoulis et al. (2009) investigated the impact of inulin, wheat, oat and apple fibre on the percentage of freezable and non-freezable (bound) water in sucrose model solutions and ice cream mixes using DSC. They found that the addition of wheat or oat fibre led to a significant increase in the percentage of un-bound freezable water in both sucrose and ice cream systems. On the contrary, both inulin and apple fibre lead to an increase in the percentage of bound water. It was suggested that this influence is related to the ratio of insoluble to soluble fibre. Oat and wheat fibre are highly cellulosic and thus are primarily insoluble fibre, whereas inulin and apple fibre contain high soluble matter contents. Consequently, the water binding capacity varies between soluble and insoluble fibre and their impact on control over water mobility may vary accordingly. Nakamura et al. (1981) investigated the relationship between the degree of cellulose crystallinity and the amount of bound non-freezable water using DSC. Their investigations found that bound non-freezable water content decreased with increasing degree of crystallinity of the cellulose. This would suggest that only cellulose molecules in the amorphous state can be regarded as the absorption site of water molecules. It is suggested that bound water attaches the hydroxyl groups in the amorphous region of cellulose.

Alternatively, NMR spin-spin relation times (T_2) can provide an indication of how a stabiliser can impact water (proton) mobility (Herrera et al., 2007). Assessment of the mobility of water has been performed at both ambient (Hills et al., 1990; Pudus & Schmidt, 1992) or in sub-zero conditions (Weisser & Harz, 1984; Sahagian & Goff, 1995). Higher T_2 values are generally related to an increase in the rotational motion of a molecule and thus increased mobility (Belton, 1997), although measurements made at ambient temperatures can be difficult to

correlate to the state of protons in sub-zero temperatures due to the effect of freeze-concentration. Therefore, knowledge of the glass transition temperature of a mix provides information on the temperature that water molecules become kinetically immobilised and thus unable to participate in ice recrystallisation (Sahagian & Goff, 1995). Positive correlations between recrystallisation rates in stabilised frozen sugar solutions and molecular mobility of ambient solutions using spin-spin relaxation NMR have been reported (Belton et al., 2003).

The extent of functionality of a fibre is undoubtedly related to plant origin, the insoluble to soluble fibre ratio, its water holding and binding capacity and its interactions with other food components (Soukoulis et al., 2009) and thus a variety of considerations should be made when selecting a fibre for its application. In addition, in terms of the current application as an ice cream stabiliser, plant materials are notoriously susceptible to damage upon freezing. As plant materials freeze, ice forms either inside or outside cell wall materials causing dehydration of the cell which can result in damage. In addition, the growth of large ice crystals within cells can result in rupture of cell wall material leading to a loss of structure (Burke et al., 1976). The resistance of plant cell walls to freeze-dehydration induced damage is a major factor in the freeze tolerance of plant materials and is reported to be more apparent in non-acclimated plants (Pearce, 2001) although some plants can produce antifreeze factors that can inhibit the growth of ice crystals and the extent of freeze induced damage. Cereal plant cell walls have been identified to contain such factors (Olien & Smith, 1981).

As commercial plant fibres will contain a proportion of cell wall material, assessment of their freeze-thaw stability will examine their suitability as ice cream stabilisers.

3.1.1 Plant fibres

Figure 3.1 shows a schematic illustration of the construction of the plant cell wall. Plant cell walls are composite materials of cellulose, hemicellulose, lignin, pectin and proteins. Cellulose, hemicellulose and lignin are considered the insoluble fraction of the plant cell wall, whilst pectin is the soluble fraction. Although the ratios of composition vary between plant origin, cellulose is generally the main constituent. The cell wall provides tensile strength to plant cells allowing cells to develop turgor pressure; the pressure of cell contents against the cell wall (Sticklen, 2008).

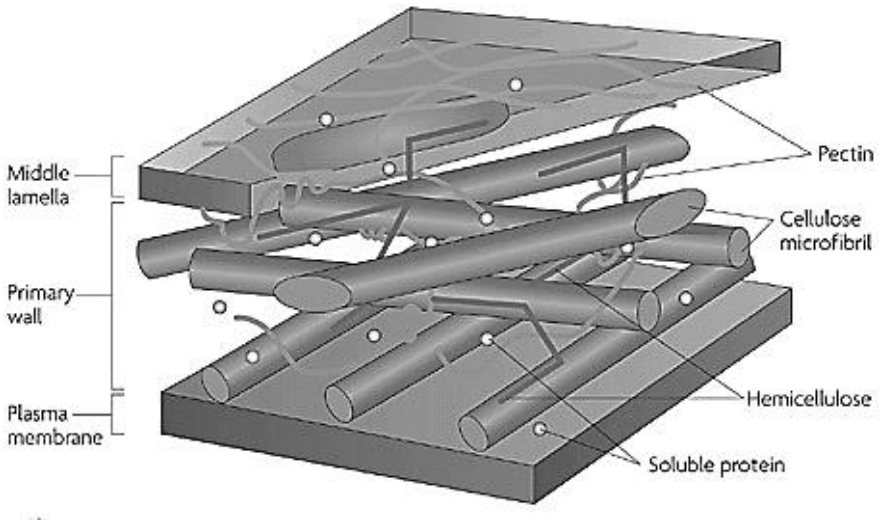


Figure 3.1 Schematic illustration of the structure of a plant cell wall (Sticklen, 2008).

3.1.1.1 Cellulose

The arrangement of the structure of cellulose is shown in Figure 3.2. Cellulose is an unbranched polymer chain of β- 1,4 glycosidic bond linked glucose molecules which is insoluble in water. Cellobiose is the repeating unit and the degree of polymerisation (DP) refers to the number of glucose units (Chen, 2014). The glucose chains are aggregated into bundles of microfibrils by hydrogen bonds (Thakur et al., 2014). These chains are arranged parallel to each other and can

either be tightly packed and bound via hydrogen bonds (crystalline regions) or loosely packed and lacking hydrogen bonds between adjacent chains (amorphous regions). It is these inter-microfibrilar hydrogen bonds that give cellulose little solubility in water (Dhingra et al., 2012). The microfibre bundles are encased in hemicellulose and lignin and arranged into macrofibrils which form a lattice structure within the plant cell wall (Kaushik & Singh, 2011).

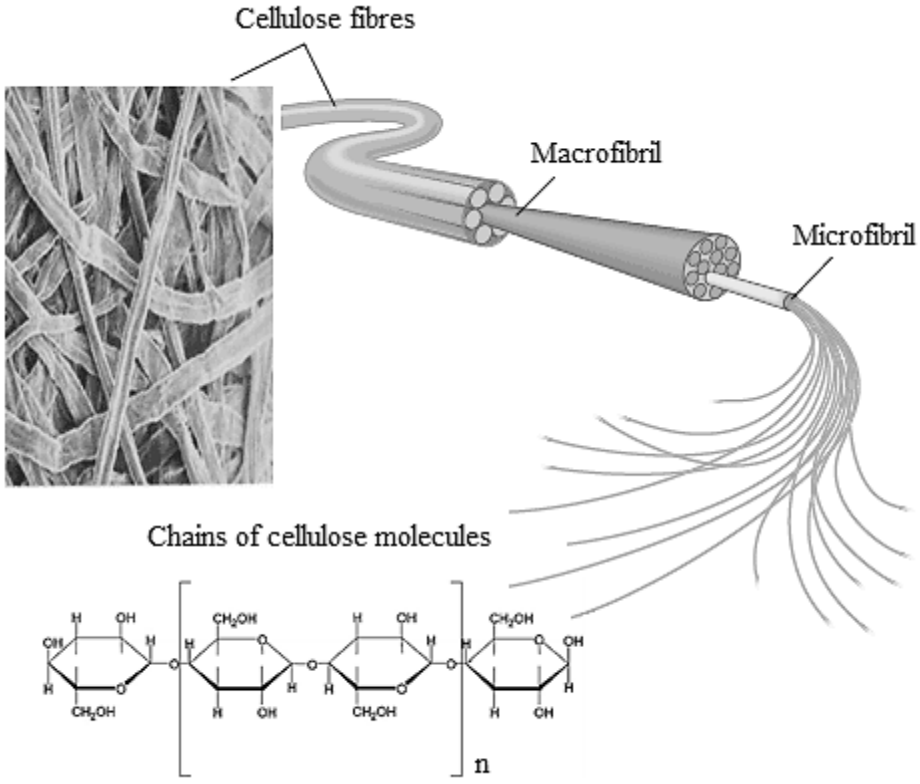


Figure 3.2 The structure of cellulose (Adam-day, 2016).

3.1.1.2 Hemicelluloses

Hemicelluloses are a heterogeneous group of polysaccharides which can be extracted with alkaline treatment (Scheller & Ulvskov, 2010). They are branched polymers of glycans consisting of a main backbone of a mixture of sugar monomers of xylose, mannose, galactose and glucose all liked via β- 1,4 glycosidic bonds. The main backbone varies in its degree of

branching with side chains of arabinose, galactose and galacturonic acid (Kay, 1982). Xylose is generally present in large amounts in hemicellulose forming xyoglucans and are the most abundant hemicellulose type in the plant kingdom. It is generally thought that the main function of hemicellulose is to aid in cell wall structure and the cell growth process (Chen, 2014). However, the type of hemicellulose present vary as a function of plant origin. Hemicelluloses are usually grouped into five structurally different polysaccharide types: xylans, xylogulcans, mannans, glucomannans and mixed-linkage β-glucans (Scheller & Ulvskov, 2010).

Xylans can be grouped into several structural subclasses: homoxylans, glucuronoxylans, arabinoxylans and heteroxylans. Homoxylans are commonly found in seaweeds. Glucuronoxylans are a component of the secondary cell wall of woody material. Arabinoxylans are typically found within the cell wall of cereals. Heteroxylans are structures isolated from cereal bran, seeds, gum exudates and mucilages. The β- 1,4 backbone of heteroxylans is heavily substituted with either single sugars or oligosaccharide side chains that form highly viscous solutions (Ebringerová, 2006).

Mannans sub-divide into two classes: (i) galactomannans which have a β- 1,4 mannose backbone substituted at position 6 by a single galactose unit and are commonly present in the cell wall of storage tissues such as seeds and (ii) glucomannans which have a backbone of both β- 1,4 linked glucose and mannose residues and are the main hemicellulose component of soft woods, herbal plants and grasses (Ebringerová, 2006).

While xyloglucans can be found in most plant species, β-glucans are unique to cereal plants, in particular oat and barley (Scheller & Ulvskov, 2010).

3.1.1.3 Lignin

Lignin encloses and links the cellulose bundles in the cell wall providing structural rigidity and mechanical support (Chen, 2014). Lignin is a non-carbohydrate structure. It is a complex three-dimensional structure of aromatic compounds linked with ester and covalent bonds (Sticklen, 2008).

3.1.1.4 Pectin

Pectins are traditionally characterised by being relatively easily extracted with hot acid and by containing a large number of galacturonic acid residues (Scheller & Ulvskov, 2010). The structure of Pectin is shown in Figure 3.3. Pectin is the soluble polysaccharide present in plants and is mainly located between the cellulose microfibres. It also aids in connecting adjacent plant cells as well as linking the components within the cell wall. Pectin is a long polymer chain consisting mainly of α- 1,4 glycosidic linked galacturonic acid with each chain containing more than 200 galacturonic acid residues (Davison et al., 2013; Chen, 2014). These chains of galacturonic acid residues (smooth regions) can be interrupted by rhamnose units which form 'hairy' non-gelling regions (Phillips & Williams, 2009).

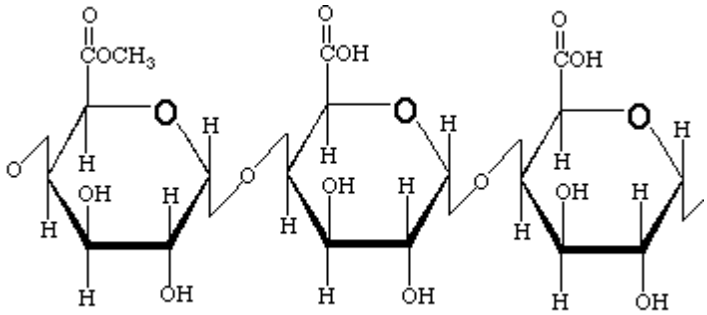


Figure 3.3 Structure of pectin (Kay, 1982).

In its natural state, acid groups along the main chain are largely esterified with methoxyl groups. Many pectins have covalently linked side chains of arabinose and galactose and to a lesser extent xylose, rhamnose and glucose (Kay, 1982).

Pectin, as an ingredient, are classified according to the degree of esterification which results in different properties. High methoxy pectin (>50% esterified) forms a gel at low pH and in the presence of a high concentrations of sugar and therefore are commonly used as gelling agents in fruit jams. Low methoxy pectin (<50% esterified) requires the presence of calcium ions to form a gel making them more suitable in ice cream formulations (Saha & Bhattacharya, 2010). In these systems gelation is due to the formation of intermolecular junction zones between galacturonic smooth regions of different chains. The structure of these junction zones are ascribed to the 'egg box' binding process (Phillips & Williams, 2009).

3.1.2 Commercial sources of plant fibres

Commercial processes for the preparation of powdered plant fibres generally consist of wet milling, washing, pressing to remove excess water, drying, followed by dry milling to obtain the correct particle size (Figuerola, et al., 2005).Washing is performed in either cold or hot water, depending on the requirements to retain soluble fibre and aids to remove microbiological organisms and free sugars which would cause browning of the powder during drying. Fibres are also dried at a temperature that reduces changes in structure and colour (Larrauri, 1999). Whilst these processes are mostly prominent for the production of powdered fibres from plant and vegetable origin containing high proportions of soluble fibre, other processes to functionalise insoluble fibre are performed including delignification by acid treatment as it reduces the amount of crystalline cellulose regions, improving hydrogen bonding with water (Kumar et al., 2013). More recently, the use of extrusion processing to obtain high quality

dietary fibre powders has been investigated, as extrusion processing has been found to increase the soluble fibre proportion in oat bran by 14% (Zhang et al., 2011). Spray drying plant fibres and the impact on structure have also been assessed using cellulose isolated from banana fibre (Elanthikkal et al., 2010). It was found that spherical fibre particles could be produced instead of the needle like fibres with high aspect ratios predominantly produced using conventional drying technologies. During spray drying, the droplets of cellulose-water dispersions are sprayed through a small orifice into a drying chamber. These small droplets rapidly lose their aqueous component resulting in the production of spherical particles.

3.1.2.1 Citrus fibres

Citrus fruits including oranges, lemons, grapefruits and mandarins are some of the most abundant crops in the world. A major by-product of processing citrus fruits are the peels and pulp which contain high levels of both cellulose and pectin (Sharma et al., 2016). It has been estimated that worldwide industrial citrus waste is more than 15 million tonnes, thus large interest has grown into their utilisation (Marín et al., 2007). The high proportion of soluble fibre within citrus fruits make them highly desirable as functional ingredients in food and therefore their incorporation has focused on meat products (Viuda-Martos et al., 2010; Fernández-López et al., 2004), baked goods (Larrea et al., 2005; Romero-Lopez et al., 2011) and ice cream (Dervisoglu & Yazici, 2006). However, it has been identified that the source of waste streams has a large impact on the functional properties, as citrus fibres as by-products of the food industry (e.g. juicing) have been found to have 10 fold higher the amount of pectin than citrus fibres from the chemical industry (e.g. flavonoid extraction) due to the previous industrial processing undertaken (Marín et al., 2007). It has been suggested that the peels of these fruits contain a higher proportion of both soluble and insoluble dietary fibre in

comparison to the pulp (Gorinstein et al., 2001). Nonetheless, due to the natural requirement of plant materials of citrus origin to hold large amounts of juice or oils, their water holding and water binding capacities are considered to be higher than fibres obtained from cereal plants which have rigid cellulosic coats that are designed to protect the germ and not to bind water (Figuerola et al., 2005). Fischer (2008) has reported that it is the cell wall architecture of fruit fibres that provide the beneficial water binding capacity that swell upon hydration forming a sponge like network. It has been proposed that plant fibres of fruit origin, in particular citrus, provide great opportunities for the production of reduced energy or fat products. Two possibilities exist for the reduction of a foods calorific content: enhance the water content and the replacement of fat, carbohydrates or protein by low calorific fillers. Either of these can change the consistency of a food making it unacceptable to consumers. However, the structuring material of plant fibres, with a low calorific content and high water binding capacity, can aid in structuring the water to help develop highly acceptable products to consumers.

3.1.2.2 Psyllium husk

Psyllium husk is obtained from the *Plantago* species and contains around 70% soluble fibre (Cui, 2000). When hydrated in water, it produces a mucilage gum from its husk which provides high water uptake properties (Anand et al., 2010). Mucilage is secreted from specialised cells on the epidermis of plant seeds to prevent dehydration or to aid in seed germination (Dhingra et al., 2012). Fischer et al. (2004) identified that the gum obtained by extraction from the husk is a highly branched arabinoxylan, the structure of which is shown in Figure 3.4 b. The xylan backbone having both β- 1,4 and β- 1,3 linkages and the majority of the residues in the xylan backbone are variously substituted at 0-2 and 0-3 with L-arabinose (20%), D-xylose (65%) and

D-galacturonic acid (9%). The epidermis of the husk is composed of large cells filled with mucilage and it is from here where the mucilage swells rapidly in mainly the radial direction when in aqueous suspension (Figure 3.4 a). The hemicellulose arabinoxylan has also been found to contain traces of other sugars like mannose and glucose (Qaisrani et al., 2016).

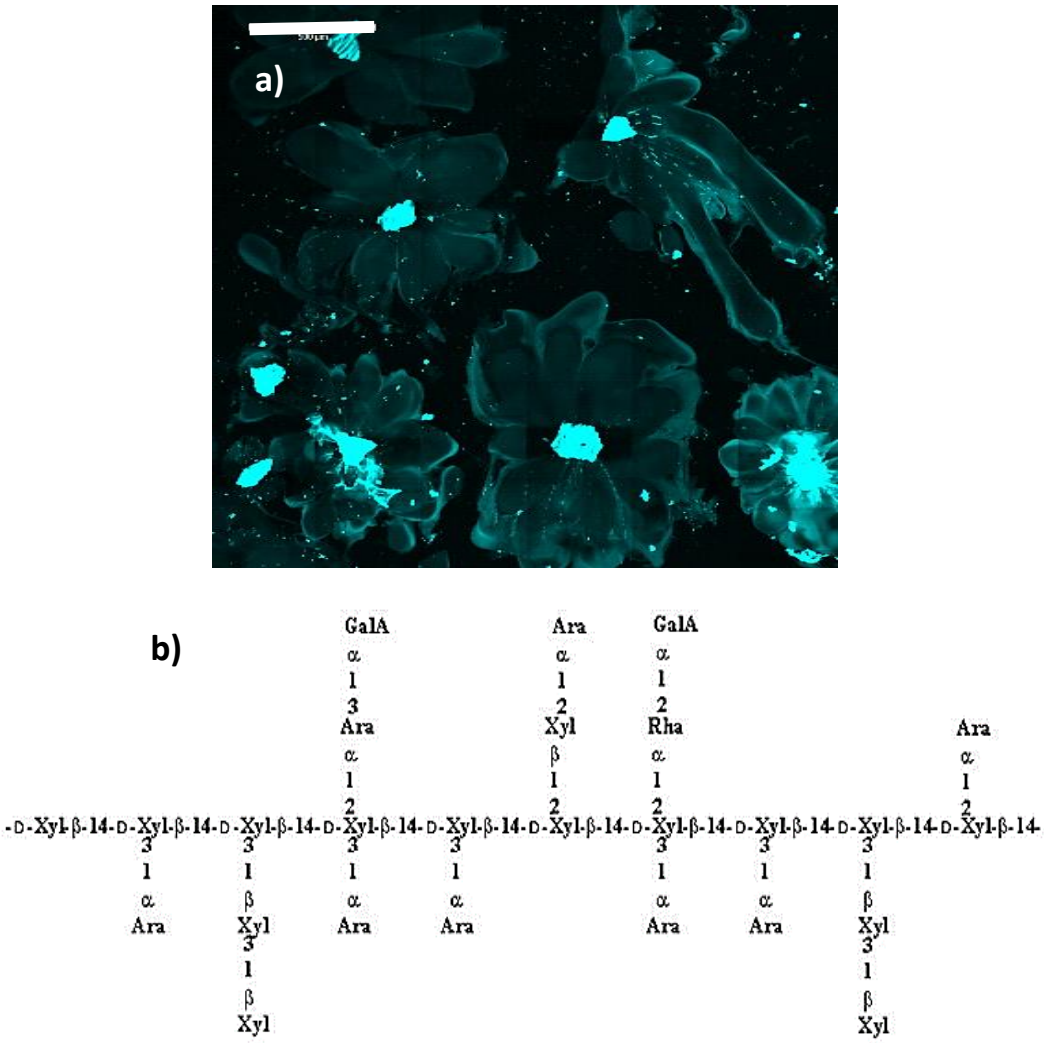


Figure 3.4 The structure of (a) hydrated husk in water, scale bar shown represents 500 µm (b) arabinoxylan from Psyllium husk (Edwards et al., 2003).

3.1.2.3 Okara

Okara or soy fibre, is the insoluble residue of soy milk and tofu production. The fibres are irregular in shape and consist of cellulose, hemicellulose, lignin, proteins and pectic substances. It has been thought that this pectic fraction is mainly galacturonic acid (Yoshii et al., 1996). It is suggested that 1.1 kg of okara is produced for every kilo of tofu (Khare et al., 1995a). Traditionally this by-product is fed to silkworms or disposed in landfills in Asian countries (O'Toole, 1999). Khare et al. (1995b) investigated the consumer acceptability of fibre fortified biscuits and found that biscuits with 60% okara supplementation were the most accepted among consumers. In addition, the water soluble polysaccharide that can be extracted from okara, as a result of the pectic fraction, have received further interest in food fortification (Chen et al., 2010).

3.1.2.4 Sugar Cane Fibre

Sugar Cane bagasse is the fibrous matter that remains after sugar cane stalks are crushed to extract the juice. It is suggested that for every 10 tonnes of sugar cane crushed, a sugar factory produces around 3 tonnes of wet bagasse, although it is often burnt as a fuel source in sugar factories in Latin American countries (Lavarack et al., 2002). The produced fibre is highly cellulosic with little to no soluble fibre content with a high lignin content ~25% (Pandey et al., 2000). Little research into the fortification of food products with sugar cane fibre could be identified from the literature. Martínez-Bustos et al. (2011) assessed the physicochemical characteristics of extruded sugar cane bagasse as well as fibre blends with whey protein and corn starch to determine whether it is a feasible method to formulate new snacks. Their investigations identified that suitably expanded snack products could be produced with the

correct microstructure as the fibre contributed to the formation of a fibrous network within the product.

3.1.2.5 Apple fibre

Apple pomace, a waste product of juicing is primarily composed of cellulose, hemicellulose and high levels of pectin ~20% (Sharma et al., 2016). Sudha et al. (2007) investigated the use of apple pomace as a wheat flour replacer in cakes. The total dietary fibre from apple pomace was around 51% with a soluble fibre content of 14% and a water binding capacity of 8.39 g/g. However, increasing pomace addition reduced dough characteristics and sensory quality. It was thought this reduction in quality is due to the high water binding capacity of apple pomace resulting in an increase in water absorption by the fibres and a decreased hydration and development of gluten. This high water binding may not be a suitable property in dough development but on the contrary has been found to be more suitable in ice cream formulations (Soukoulis et al., 2009).

3.1.3 Working hypothesis

Although the studies identified in the literature have not directly assessed soluble versus insoluble fibre on their ability to control the rate of ice recrystallisation and have only measured their impact indirectly using DSC measurements, based on the literature, according to Soukoulis et al. (2009), it may be expected in the present study that the fibres that contain a higher proportion of soluble fibre (pectin), due to their ability to bind water, will have a larger ability to reduce the molecular mobility of water and therefore provide optimum control over ice recrystallisation. Although cellulose too has been found to reduce the molecular mobility

of water (Nakamura et al., 1981) the degree of crystallinity will impact the amount of bound water and consequently the control over ice coarsening. Given this, it may be suggested that a fibre with a high water binding capacity may provide optimum control over ice recrystallisation and therefore this is a key measurement that should be assessed. As identified from the literature (Figuerola et al., 2005), given the natural requirement of fibres from fruit origin to bind large amounts of water, it may be predicted that fibres from fruit origin will have a higher ability to bind large amounts of water. However, as plant fibres exhibit some susceptibility to freeze-thaw conditions, the stability of the fibres and their functional properties under temperature cycling conditions will identify the most suitable fibre for ice cream formulations.

3.1.4 Aims of the chapter

The key aims of this chapter are to identify the most suitable plant fibres to be used as ice cream stabilisers. Based on the literature, five key requirements were proposed to be necessary for a plant fibre to be a suitable stabiliser. With these requirements outlined, a series of screening methods were used to identify the most suitable fibres. These are as follows:

1. The ability to bind large amounts of water.
    a. Water Binding Capacity (WBC).
2. Be space filling.
    a. Water Holding Capacity (WHC) to assess the volume occupied by hydrated fibres in suspension under atmospheric pressure conditions.
    b. Confocal imaging to assess homogeneity of dispersions.
3. Can reduce the molecular mobility of water.
    a. Spin-spin relaxation NMR (T_2).
4. Enhance both macro and micro viscosity.
    a. Rheological measurements.
5. Be freeze-thaw stable.

    The functionality of fibres should not be altered by the freezing process or fluctuating temperatures. A number of experiments were performed under freeze-thaw conditions:

    a. Confocal microscopy.
    b. T_2 NMR.
    c. WHC & WBC.
    d. Apparent viscosity

Based on the outcomes of screening, the fibres offering the most promising potential will then be taken forward for further assessment.

3.2 Materials and methods

3.2.1 Plant fibres

A selection of plant fibres were assessed for the evaluation of their properties. These fibres were selected based on their varying soluble fibre content, total fibre content, particle size and other information gained from the literature. An overview of the properties of these fibres are shown in Table 3.1, which have been obtained through supplier specifications. Herbacel AQ+ products were procured from Herbafoods Ingredients GmbH (Werder, Germany). AQ+ Citrus Fibre is produced from harvested-fresh dejuiced, de-oiled, dried citrus fruits. AQ+ CA-U is produced form citrus fruits and apple pomace. FibreGel Citrus Fibre was obtained from Florida Food Products Inc. (Florida, USA) and has been standardised with dextrose (concentration unknown). Ultracel Sugar Cane Fibre was obtained from Watson Inc. (Connecticut, USA). Vitacel Psyllium Fibre, Vitacel Apple Fibre and Vitacel Soy Fibre were all obtained from J. Rettenmaier & Söhne GmbH (Rosenberg, Germany).

3.2.2 Characterisation of fibres

3.2.2.1 Scanning electron microscopy

An electron microscope (7 600 FESEM, Joel, Massachusetts, USA) was used to image the dry fibre samples under appropriate magnification.

1 **Table 3.1** Overview of fibre properties

	Abbreviation	Total fibre content (TFC %)*	Total soluble (%)*	Average dry particle size (μm)*	pH in solution**	WHC (%)**	WBC (g/g)**
Herbacel AQ+ Citrus Fibre	AQ+ CF	88 – 93	20	<100	4.33	27 ± 0.56	14 ± 0.99
Herbacel AQ+ Citrus & Apple Blend	AQ+ CA-U	85	15	<300	3.88	30 ± 1.01	15± 0.72
FibreGel Citrus Fibre	FibreGel CF	61	32	100	4.87	20 ± 0.92	8 ± 0.43
Ultracel Sugar Cane Fibre	Sugar Cane	95	10	30	6.14	35 ± 0.74	27 ± 1.26
Vitacel Psyllium Fibre	Psyllium	80	66	250	5.63	35 ± 0.88	30 ± 0.98
Vitacel AF401-30 Apple Fibre	Apple Fibre	55	10	30	4.26	8 ± 1.73	4 ± 1.07
Vitacel Soy Fibre	Soy Fibre	52	3	<50	7.31	8 ± 1.24	5 ± 1.23

2

* Information obtained from supplier specification.

** Values obtained by dispersing 1 g (w/w) of dry fibre in water and measured using the methods outlined in this chapter.

± indicates the standard deviation.

3.2.2.2 Sugar analysis

Analysis was performed using the acid hydrolysis method of (Saeman, 1945) with some modification. 30 mg of sample were placed into heat resistant screw cap tubes and 1 mL of 12 M H_2SO_4 added, mixed and then placed into a water bath at 37°C for 60 minutes. The samples were then removed and 11 mL of demineralised water added. Samples were then placed in a water bath at 100°C for 120 minutes. Samples were then removed and placed into cold water to cool and halt the reaction. When cool, 0.1 mL of sample was then placed into 15 mL Falcon tubes and 9.9 mL of 10 mM NaOH added to make a 1:100 dilution. A 2 g/L stock solution of sugar standards (arabinose, galactose, glucose and xylose) was produced and a series of dilutions were performed in order to obtain a calibration curve. The monosaccharide content of the fibres was obtained using ion chromatography (AS-1 Dionex, Dionex Corporation, Sunnyville, CA).

3.2.2.3 Protein analysis

Protein content was determined using a nitrogen analyser (Flash EA1112, Thermo Scientific, Massachusetts, USA). The nitrogen content was converted to the protein content using the global factor of 6.25. A factor of 5.71 was used for Soy Fibre (Lundberg et al., 2014).

3.2.2.4 Total lipid

Total lipid content of the plant fibres was determined using the method of Bligh & Dyer (1959) with some modification. Stock solutions of 0.9% Sodium Chloride solution were prepared using purified water. A stock solution of chloroform 2:1 methanol was also prepared. Dried Fibre (1 g) was weighed into 50 mL centrifuge tubes and 12 mL of chloroform/methanol

solution added followed by vortexing for 1 minute. After vortexing, 5 mL of Sodium Chloride solution was added followed by vortexing for 1 minute. After centrifuging (3000 rpm for 10 minutes) three discrete phases formed and the lower phase was transferred to a new centrifuge tube. A further 12 mL of chloroform/methanol mix was added to the remaining two phases, vortexed for 1 minute and centrifuged again (3000 rpm 10 minutes). The lower phase was then removed and added to the centrifuge tube containing the previous lower phase. The centrifuge tube containing the lower phase was then centrifuged (3000 rpm 10 minutes). The lower phase was removed and transferred to a syringe connected with a 0.45 µm PTFE filter before being placed into pre-weighed glass bijoux bottles. The lipid extract was then dried under nitrogen (30 bar for 1 hour). The remaining lipid was weighed and the total lipid content was calculated using the following equation:

$$\text{Total Lipid (\%)} = \frac{\text{weight of dried lipid extract}}{\text{weight of dried powder}} \text{ X } 100 \qquad \textit{Equation 3.1}$$

3.2.2.5 Moisture content

Fibre samples of 1 g were placed into pre-weighed crucibles and dried at 105°C for 12 hours. Samples were removed from the drying oven and were cooled for 1 hour in a desiccator containing silica beads. Once cool, samples were weighed and the moisture content was calculated using Equation 3.2:

$$\text{Moisture (\%) Content} = \frac{\text{original sample weight} - \text{dried weight}}{\text{original sample weight}} \text{ X } 100 \qquad \textit{Equation 3.2}$$

3.2.2.6 Ash content

Dried samples in crucibles from moisture content assessment were ashed in a muffle furnace at 550°C for 8 hours. The ash content was calculated using Equation 3.3:

$$\text{Ash (\%) Content} = \frac{\text{ash weight}}{\text{original sample weight}} \times 100 \qquad \textit{Equation 3.3}$$

The moisture content was then subtracted from the value to yield the ash content (%).

3.2.3 Fibre Screening

Screening of the properties of the fibres to assess their suitability and functionality was performed using both the dry powders and homogenised dispersions. For homogenised dispersions, fibres were dispersed in water (1% w/w) and allowed to hydrate overnight (4°C). Dispersion were homogenised in a single stage homogeniser at varying pressures (APV2000, SPX Flow Technology, UK). Where the effect of heating was used to assess the impact on fibre functionality, fibres were dispersed in water at 80°C ± 2°C and allowed to cool and hydrate overnight in refrigerated conditions (4°C) prior to homogenisation.

3.2.3.1 Water holding capacity (WHC)

Dispersions were placed into 50 mL centrifuge tubes and left over night at room temperature for the fibres to settle. Where settling was seen the water layer was removed and the remaining settle was weighed. The WHC was calculated using Equation 3.4:

$$\text{WHC (\%)} = \frac{\text{settle weight} - \text{dry pellet weight}}{\text{original sample weight} - \text{dry pellet weight}} \text{ X } 100 \qquad \textit{Equation 3.4}$$

The dry weight was generated by drying the settling fibre solution at 60°C for 8 hours, cooling in a desiccator for 2 hours and then finally weighed.

3.2.3.2 Water binding capacity (WBC)

Dispersions were placed into 50 mL centrifuge tubes and centrifuged at 2000 g for 20 minutes (Jouan CR3imultifunction centrifuge, ThermoElectron Corporation). After removal of the supernatant, the remaining pellet was weighed, dried at 60°C for 8 hours, allowed to cool in a desiccator for 2 hours and then weighed. All samples were assessed in triplicate. The weight of the hydrated pellet and dried pellet was used to calculate the WBC using the following formula:

$$\underset{\text{(g of water / g of dry fibre)}}{\text{WBC}} = \frac{\text{hydrated pellet weight} - \text{dried pellet weight}}{\text{dried pellet weight}} \qquad \textit{Equation 3.5}$$

3.2.3.3 Bulk and serum viscosities

Rheological measurements were performed on a MCR 301 Rheometer using double gap geometry (Anton-paar GmbH, Germany). Samples were loaded, allowed to equilibrate (10 min) before being subjected to shear rates of 1 to 100 s^{-1}. Serum solutions were prepared by centrifuging (Jouan CR3imultifunction centrifuge, ThermoElectron Corporation, France) samples at 2000 g for 20 minutes, centrifuge tubes were inverted and allowed to drain for 10 minutes. The supernatant was collected as the serum. All bulk and serum apparent viscosities

are reported at 10 s^{-1}. All measurements were performed in triplicate at 20°C. Where the impact of freeze-thaw cycling was used to assesses the impact on apparent viscosity, solutions were frozen to -20°C, held for 1 week and then thawed in a fridge overnight.

3.2.3.4 Freeze-thaw T_2 relaxation nuclear magnetic resonance (NMR) spectroscopy

Spin-spin relaxation times (T_2) were obtained on a R4 Benchtop NMR System (Advanced Magnetic Resonance Ltd., UK) operating at 21 MHz using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. The decay was then fitted to a distributed exponential function using Deeper softwear (Advanced Magnetic Resonance Ltd., UK). To assess the effect of freeze-thaw cycling on T_2 values, samples were assessed at 20°C, cooled to -20°C in a water bath containing glycol (1:1 glycol:water) for 20 minutes and defrosted in a 20°C water bath for 20 minutes. Samples were subjected to two freeze-thaw cycles and tested in triplicate. All measurements were performed at 20°C.

3.2.3.5 Freeze-thaw confocal laser scanning microscopy (CLSM)

Images were obtained using a Ziess LSM780 Microscope System (Ziess, Germany). Samples were stained with 0.1% (v/v) Calcofluor-white solution (Sigma-Aldrich, Germany), loaded onto glass slides with 50 μm spacers, covered with a glass cover slip and excited using a 405 nm diode laser (emission range 395-493 nm). To assess freeze-thaw stability, samples were placed in a cold stage at 5°C (Linkam THMS 600, Linkam Scientific Instruments, United Kingdom), cooled to -20°C at 1°C/min before being warmed to 5°C at 10°C/min.

3.3 Results and discussion

3.3.1 Characterisation of fibres

Characterisation of the plant fibres was undertaken in order to better understand the differences amongst samples and to relate this to their properties. The SEM micrographs of the plant fibres under investigation are shown in Figure 3.5. All fibres presented exhibit heterogeneous particle sizes. In general, Apple Fibre and FibreGel CF were identified to have the smallest fibre particles whilst Sugar Cane was identified to have the largest and most plate like fibre particles. AQ+ CF, Psyllium, AQ+ CA-U and Soy Fibre all also have small irregular shaped particles of heterogeneous size.

These fibres, when hydrated in water will form particulate dispersions due to their insoluble fibre components. The size, shape and aspect ratio, interactions between particles, the spatial arrangement and packing of particles will all impact the rheological properties and phase volume of the particulate dispersions (Brouwers, 2006; Hemar et al., 2011). The aspect ratio refers to the differences in length to width of a particle (Rosa et al., 2010). Mueller et al. (2010) identified that particles with a high aspect ratio (prolate particles) had a more pronounced impact on apparent viscosity than spherical particles. Shearing prolate particles caused them to tumble end over end in the flow whilst spherical particles rotate on their axis. Lundberg et al. (2014) found that as the particle size of citrus fibre dispersions increased the apparent viscosity increased. Rayment et al. (1995) have investigated the impact of particulate inclusions of rice starch on the rheological properties of entangled polysaccharide solutions. The presence of particulates (6-10 μm) in guar gum solutions resulted in the system becoming more rate-dependent at low shear rates and modified the viscosity – shear rate flow curve as the Newtonian flow properties of guar systems at low shear were replaced by a more shear thinning behavior.

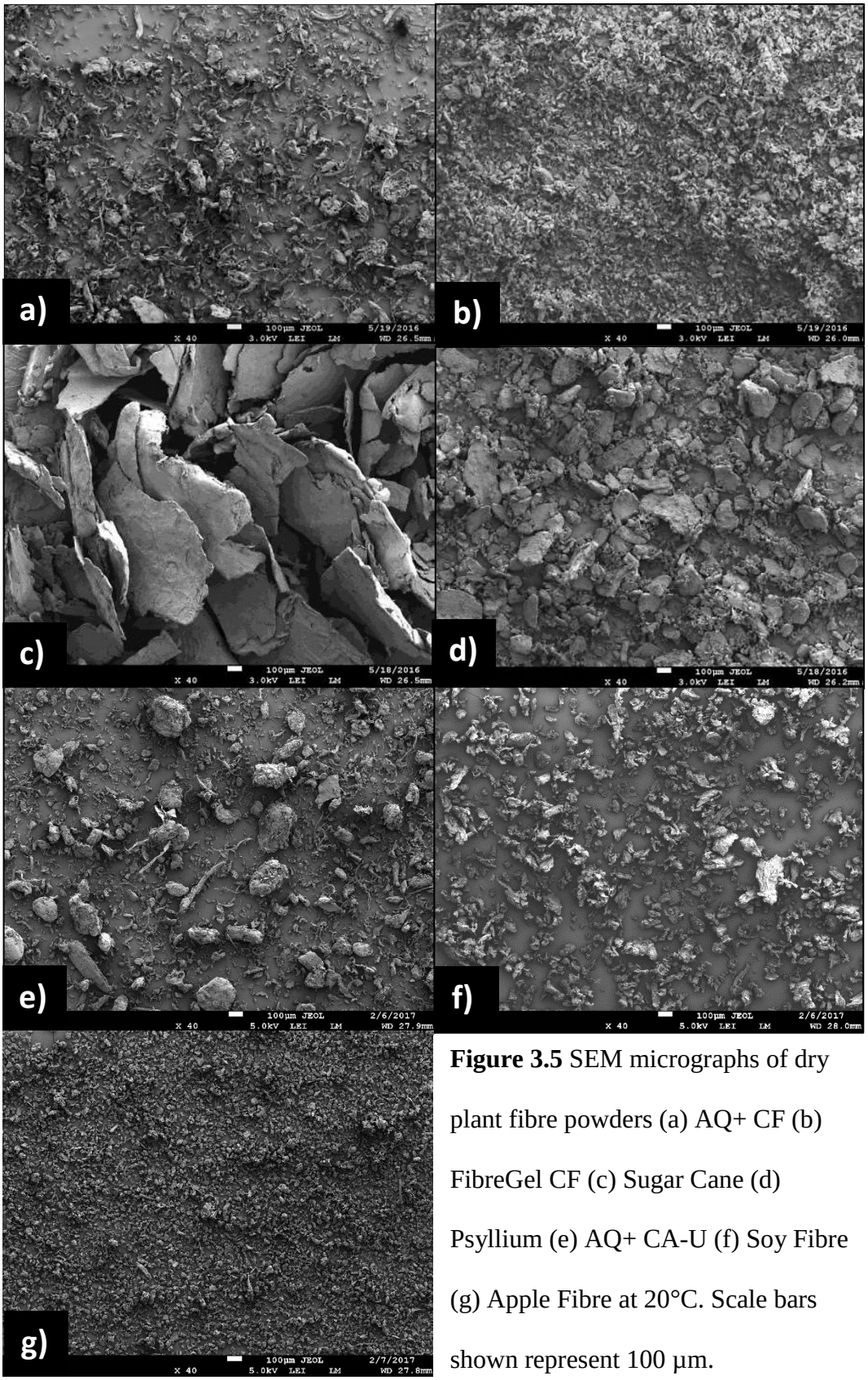


Figure 3.5 SEM micrographs of dry plant fibre powders (a) AQ+ CF (b) FibreGel CF (c) Sugar Cane (d) Psyllium (e) AQ+ CA-U (f) Soy Fibre (g) Apple Fibre at 20°C. Scale bars shown represent 100 µm.

Subsequent homogenisation of fibre suspensions will also have an impact on the rheological properties by modifying the particle shape or increasing the surface area of the fibres (Lundberg et al., 2014).

3.3.1.1 Compositional analysis

Acid hydrolysis of the plant fibre powders was performed to determine their sugar monomer composition. Fibres high in glucose can be considered to be highly cellulosic. Fibres with a high percentage of xylose and arabinose as well as glucose will contain proportions of hemicellulose. A large content of galactose will indicate the presence of pectin (Chen, 2014). The sugar monomer makeup of the plant fibres are presented in Table 3.2. Assessment of protein, lipid and moisture content of the powdered fibres was also performed. AQ+ CF and AQ+ CA-U, both from the same supplier, presented similar compositions. The results suggest that these two fibres contain a large amount of dietary fibre, little ash, and a proportion of protein and lipids. Given that these fibres are produced from de-oiled fruits, they still contain a high comparative amount of lipids. Sugar monomer analysis suggests that these fibres are mostly cellulose but also contain a large proportion of pectin and a little hemicellulose. FibreGel CF was identified to contain the highest proportion of galactose at 20.61% suggesting the highest amount of pectin, with almost equal amounts of glucose (cellulose) at 28.08%. It was also found to have the highest ash content of 10.32%. Sugar Cane Fibre is reported in the literature to be predominantly cellulose and hemicellulose with negligible amounts of pectin (Pandey et al., 2000). This is reflected in the compositional analysis as it was found to contain the most glucose at 53.72% with no galactose monomers. Psyllium is described as an arabinoxylan, with a β- 1,4 linked xylose backbone and branched at various positions with L-arabinose, D-xylose and D-galacturonic acid units (Edwards et al., 2003). It is the xylan

fraction of Psyllium that is responsible for the hydrophilic nature of the mucilage (Israr et al., 2016). Sugar analysis identified Psyllium to have the largest hemicellulose content with high levels of arabinose and xylose residues at 16.48% and 45.30%, respectively. However, Apple Fibre and Soy Fibre were found to be the least pure fibres as Apple Fibre was found to contain the second lowest amount of total sugars and Soy Fibre the lowest amount. Given that apple pomace is reported to contain high amounts of pectin (Sharma et al., 2016) the galactose content was found to be low at 6.45%. This might be suggestive of a high loss of soluble fraction during fibre production through the washing steps undertaken or due to the origin of waste streams (Martín et al., 2007). Apple Fibre also has one of the smallest particles sizes at 30 µm. It is suggested that the washing step is more likely to remove soluble fibre in fibres of small particle size (Larrauri, 1999). Soy Fibre was found to contain the highest amount of protein at 29.83% which is in agreement with the literature (Yoshii et al., 1996) and small amounts of cellulose, hemicellulose and pectin.

Table 3.2 Compositional analysis of plant fibres.

	AQ+ CF	AQ+ CA-U	FibreGel CF	Sugar Cane	Psyllium	Apple Fibre	Soy Fibre
Total Sugars %	68.44	70.05	57.07	72.19	69.15	34.97	22.89
% Glucose	*42.55*	*43.93*	*28.08*	*53.72*	*4.05*	*22.06*	*9.01*
% Galactose	*18.65*	*18.86*	*20.61*	*0.00*	*3.32*	*6.45*	*4.62*
% Arabinose	*0.00*	*0.00*	*4.80*	*0.00*	*16.48*	*4.24*	*6.77*
% Xylose	*7.24*	*7.25*	*3.58*	*18.49*	*45.30*	*2.23*	*2.49*
Ash %	1.72	1.23	10.32	5.11	2.69	3.70	4.23
Protein %	5.18	5.75	2.86	0.63	3.20	5.99	27.25
Total Lipid %	10.04	7.43	7.53	13.53	4.90	5.70	13.77
Moisture %	7.01	8.32	9.86	8.60	7.26	7.22	7.83

N.B Some under calculations of sugar monomers may arise from acid hydrolysis as sugar monomers can be susceptible to thermal degradation. Glucose and xylose are considered as relatively stable but arabinose and galactose are moderately susceptible to thermal degradation (Saeman, 1945)

Because fibres contain different amounts of total fibre, the specific volume will vary accordingly. That given, at say 1% (w/w) dry fibre powder, there will be more total fibre of AQ+ CF per gram than total fibre of Soy Fibre per gram. Thus, complete comparison of functional properties is difficult and differences in total fibre should be taken into consideration.

3.3.2 Screening and optimisation using homogenisation

3.3.2.1 Effect of homogenisation pressure on water holding capacity

The water holding capacity relates to the volume occupied by the fibre on swelling and hydration and is a reflection on how space filling a fibre is in a dispersion (Bengtsson & Tornberg, 2011). It is hypothesised that a fibre that occupies a large amount space in dispersion will have a larger impact on apparent viscosity and will contribute more to the formation of a fibrous network within the ice cream matrix phase. The results of the WHC assessment of the plant fibres are presented in Figure 3.6. At all three homogenisation pressures used Psyllium, AQ+ CF, AQ+ CA-U, Sugar Cane and FibreGel CF all show no settling behaviour in dispersions and therefore demonstrate a large volume occupancy. Therefore, it might be suggested that these fibres will have a higher impact on apparent viscosity. However, both Apple and Soy Fibre settle in dispersions and thus have a poor ability to occupy space. Increasing the homogenisation pressure caused an increase in WHC for the two settling fibres. It can be suggested that homogenisation causes the cell wall structure to open up and therefore an increase in the volume occupancy.

However, Larrauri (1999) has suggested that excessive changes in particle size can result in a decrease in WHC, as the WHC decreases with decreasing particle size as smaller particles are

able to pack together more closely. This is reflected in the present results as homogenisation at 800 bar could be reducing the particle size and thus WHC will be reduced due to changes in spatial arrangement and the packing of particles.

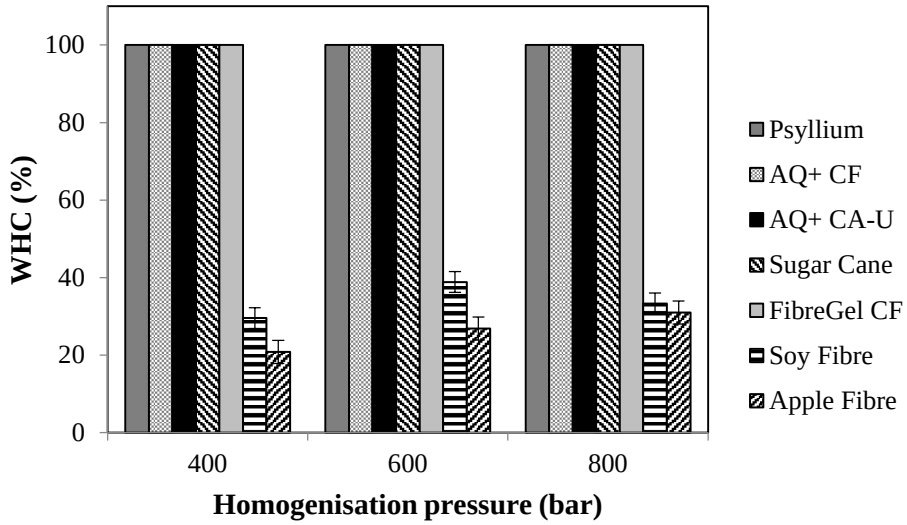


Figure 3.6 Effect of homogenisation pressure on the water holding capacity (WHC %) of different plant fibres. Error bars shown represent the standard deviation (1% w/w fibre 20°C).

It is suggested that the functionality of fibres can be improved by heating. Larrauri (1999) reported that the WHC of apple fibre could be increased from 5.1 to 6.2 mL/g (10% to 12%, respectively if related to the present study) when fibres were boiled. This increase in WHC was due to the solubilsation of pectic compounds. However, at high temperatures thermal degradation and depolymerisation of pectin can cause reductions in viscosity. Heating can cause the leaching of fibre into the water and the solubilisation of insoluble dietary fibre components which will cause a reduction in both water binding and holding properties.

3.3.2.2 Effect of homogenisation pressure on water binding capacity

The ability of an ice cream stabiliser to bind large amounts of water has been suggested to contribute to reducing the molecular mobility of water which aids in controlling ice recrystallisation and other mobility related quality deteriorations (Goff & Hartel, 2013). Therefore, optimising the WBC of plant fibres may offer improved functionality. The effect of homogenisation pressure on the WBC is reported in Figure 3.7. In most cases, a peak in WBC can be seen at a homogenisation pressure of 600 bar. However, at higher pressures the WBC is reduced with the exception of Psyllium where a near linear relationship between increasing homogenisation pressure and increasing WBC was observed. Previous research (Saghir et al., 2008) reported that the D-xylose residues substituted on the xylan backbone bears three hydroxyl groups. Homogenisation will increase the surface area exposed to solution and by opening up mucilage cells, the exposure of further xylose units to solution will increase the number of binding sites able to form hydrogen bonds. However, for AQ+ CF, AQ+ CA-U and FibreGel CF, WBC is reduced after homogenisation at 800 bar. Fischer (2008) has proposed that it is the cell wall architecture of fibres from citrus origin that provides the beneficial water binding capacity which swell upon hydration acting as a sponge. Perhaps the homogenisation at 600 bar aids in functionalising the fibres by increasing the number of binding sites. However, with further increases in homogenisation pressure the cell wall architecture is lost and the ability to bind and hold water is reduced.

A relationship between total fibre content and WBC was observed. Sugar Cane Fibre had the highest WBC at all homogenisation pressures and contains the highest total fibre content (TFC) at 95%. AQ+ CF had the second highest TFC of 88-93% and the second highest WBC. Soy and Apple Fibre had half the TFC in comparison to the other fibres of 52% and 55%, respectively and therefore had the lowest WBC. Interestingly, AQ+ CF and FibreGel CF, two

fibres both of citrus origin, exhibited similar trends in WBC behaviour as an effect of homogenisation pressure, thus suggesting that fibre origin has an impact on functionality.

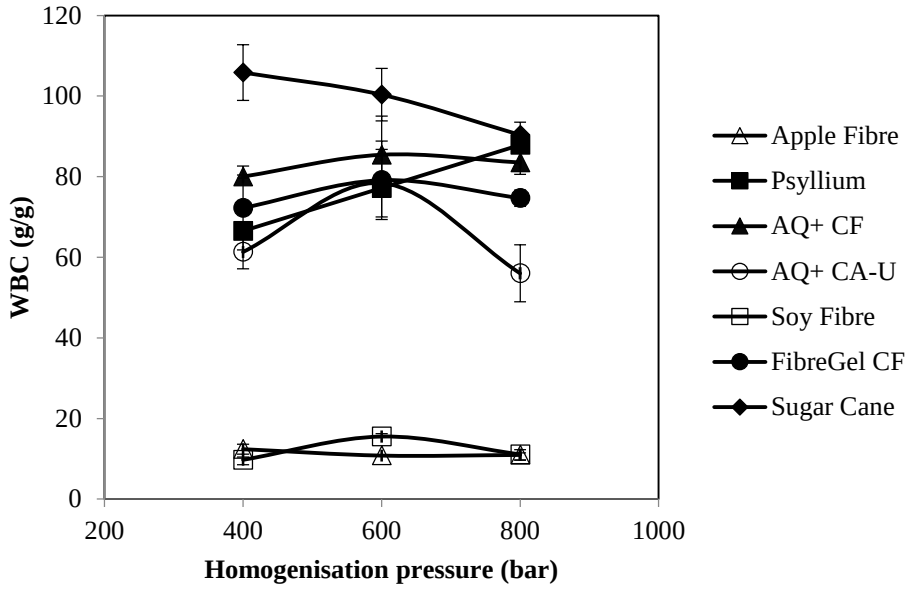


Figure 3.7 Effect of homogenisation pressure on the water binding capacity (WBC g/g) of different plant fibres. Error bars shown represent the standard deviation (1% w/w fibre 20°C).

Soukoulis et al. (2009) reported that fibres with a high level of soluble fibre have a higher WBC and thus were more likely to have greater control over ice recrystallisation. However, given that Sugar Cane Fibre has little soluble fibre content, it would suggest that insoluble fibre also has a high ability to form hydrogen bonds with water, although the work of Nakamura et al. (1981) suggests for this to be the case, cellulose chains must be highly amorphous in structure as glucose units in crystalline regions are unable to form hydrogen bonds with water. This suggests that Sugar Cane Fibre will provide the best control over the rate of ice recrystallisation. However, a limitation of the WBC measurement used, is that soluble fibre will be discarded

with the supernatant after centrifugation and thus the contribution towards WBC will be predominantly from the insoluble fraction.

3.3.2.3 Effect of homogenisation pressure on rotational viscosity

Measurements of the ability of plant fibres to enhance both micro and macro viscosity can provide information on their ability to control microstructural deteriorations. Micro viscosity is associated with the movement or diffusion of molecules at a molecular level (environments in the micrometer scale). Macro viscosity relates to the bulk fluid movement associated with the flow of fluid on a macroscopic scale.

Enhancement of the macro viscosity by insoluble fibre within ice cream has been reported to impact on bulk fluid mobility and aid in preventing serum drainage with fluctuating freezer temperatures by enhancing the melt resistance (Alvarez et al., 2005). Enhancement of the micro viscosity through soluble components is said to be linked to molecular mobility and control over ice recrystallisation and the T_g (Goff & Hartel, 2013). Thus assessments of both bulk and serum viscosities were performed to identify which fibres would enhance both the macro and micro viscosity.

The impact of homogenisation pressure on bulk apparent viscosity is shown in Figure 3.8. An increase in apparent viscosity was identified with increasing homogenisation pressure for some samples. The increase in surface area as a result of homogenisation resulted in an increase in hydrodynamic volume and thus increasing viscosity (Mueller et al., 2010). It was predicted during WHC measurements that AQ+ CF, AQ+ CA-U, Psyllium, Sugar Cane and FibreGel CF due to their high WHC would result in a higher apparent viscosity. This is reflected here as these fibres have the highest impact on enhancing apparent viscosity. AQ+ CF and AQ+ CA-

U had the highest apparent viscosities at all homogenisation pressures. Therefore it may be hypothesised that AQ+ CF in an ice cream formulation would have the best control over serum drainage and the rate of ice cream meltdown. Apple and Soy Fibre had the lowest bulk viscosities at all homogenisation pressures, which is probably a manifestation of both a lower hydrodynamic volume and specific volume. However, due to the settling nature of these fibres in suspension, accurate measurement of the apparent viscosity became difficult.

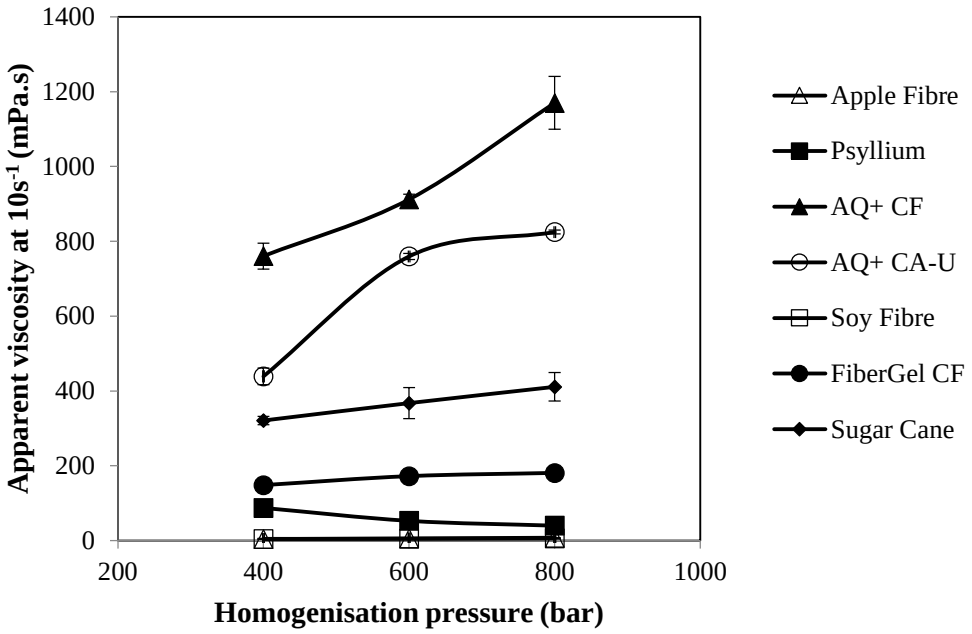


Figure 3.8 Effect of homogenisation pressure on bulk apparent viscosity. Error bars shown represent the standard deviation (1% w/w fibre 20°C).

The serum viscosity will reflect the amount of soluble fibre in solution. An increase in serum viscosity is due to water retention by the soluble fibre components (Moelants et al., 2013). Soukoulis et al. (2009) have suggested that a higher amount of soluble fibre provides optimum protection over ice recrystallisation. At higher soluble fibre contents, an elevation of the glass

transition temperature (T_g) was reported which suggests a reduction in the molecular mobility of water.

The effect of homogenisation pressure on serum viscosity is reported in Figure 3.9. FibreGel CF had the highest serum apparent viscosity which is in line with the compositional analysis as it was identified to contain the highest percentage of galactose units. However, supplier specifications stated that FibreGel CF is standardised with dextrose, which is added to aid in the spray drying process which would contribute to increasing the serum viscosity. The supplier specification of Psyllium Fibre, reported it to have the highest total soluble fibre content in comparison to all the other fibres at 66% with high levels of hemicellulose. For Psyllium, an increase in serum apparent viscosity was seen at a homogenisation pressure of 800 bar. However, for bulk viscosity (Figure 3.8), apparent viscosity steadily decreased with increasing homogenisation pressure suggesting a decrease in particle size as the mucilage cells are destroyed. This might suggest that the increase in homogenisation pressure begins to break the structure of Psyllium down so extremely that it begins to become a solution of soluble polymer. Soy Fibre and Apple Fibre have the lowest soluble fibre content of 3% and 10%, respectively, and therefore produced low apparent serum viscosities, accordingly their control over ice recrystallisation may be minimal in ice cream formulations. Based on this the results suggest that while AQ+ CF might provide the best control over the rate of ice cream meltdown due to a higher bulk viscosity, FibreGel CF due to its higher serum viscosity may have a better control over the rate of ice recrystallisation.

With the exception of Psyllium, the results so far suggest that increasing the homogenisation pressure to 600 bar increases the WBC and WHC due to an increase in volume occupancy and the exposure of more water binding sites to solution, which increases the apparent viscosity. However if the homogenisation pressure is further increased to 800 bar the cell wall

architecture begins to be completely broken down resulting in a decrease in WBC and WHC and therefore apparent viscosity.

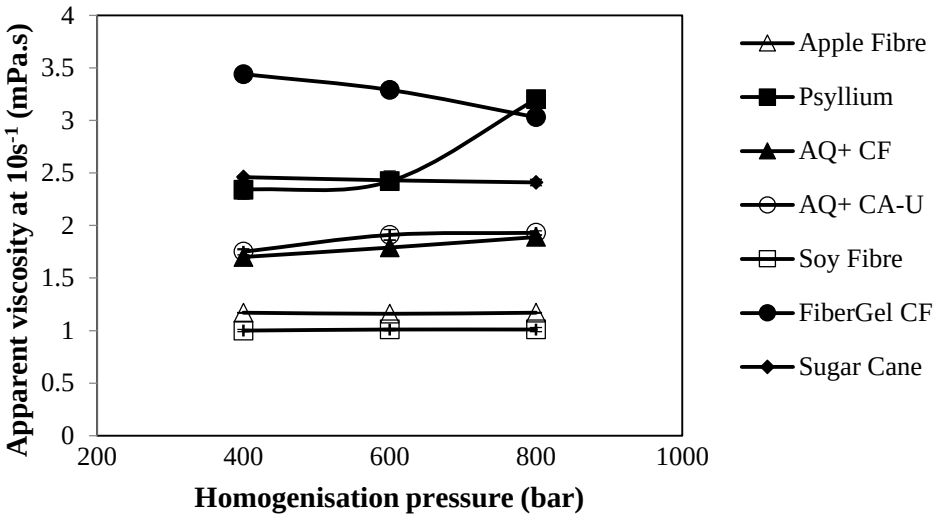


Figure 3.9 Effect of homogenisation pressure on serum apparent viscosity. Error bars are shown but are smaller than the markers and represent the standard deviation (1% w/w fibre 20°C).

3.3.2.4 Freeze-thaw confocal laser scanning microscopy of homogenised fibres

Confocal microscopy was used to investigate the structures of fibre dispersions. In addition, freeze-thaw cycling was used to assess the freeze-thaw stability of these structures. For plant fibres to be suitable ice cream stabilisers they must be freeze-thaw stable. Fluctuations in freezer temperatures either at home with the consumer or during distribution can result in repeated freeze-thaw cycling of ice cream. During freezing all freezable water is removed to form ice, when plant materials freeze, the growth of ice crystals can cause compression and structural damage that can prevent structures from reforming and rehydrating upon thawing

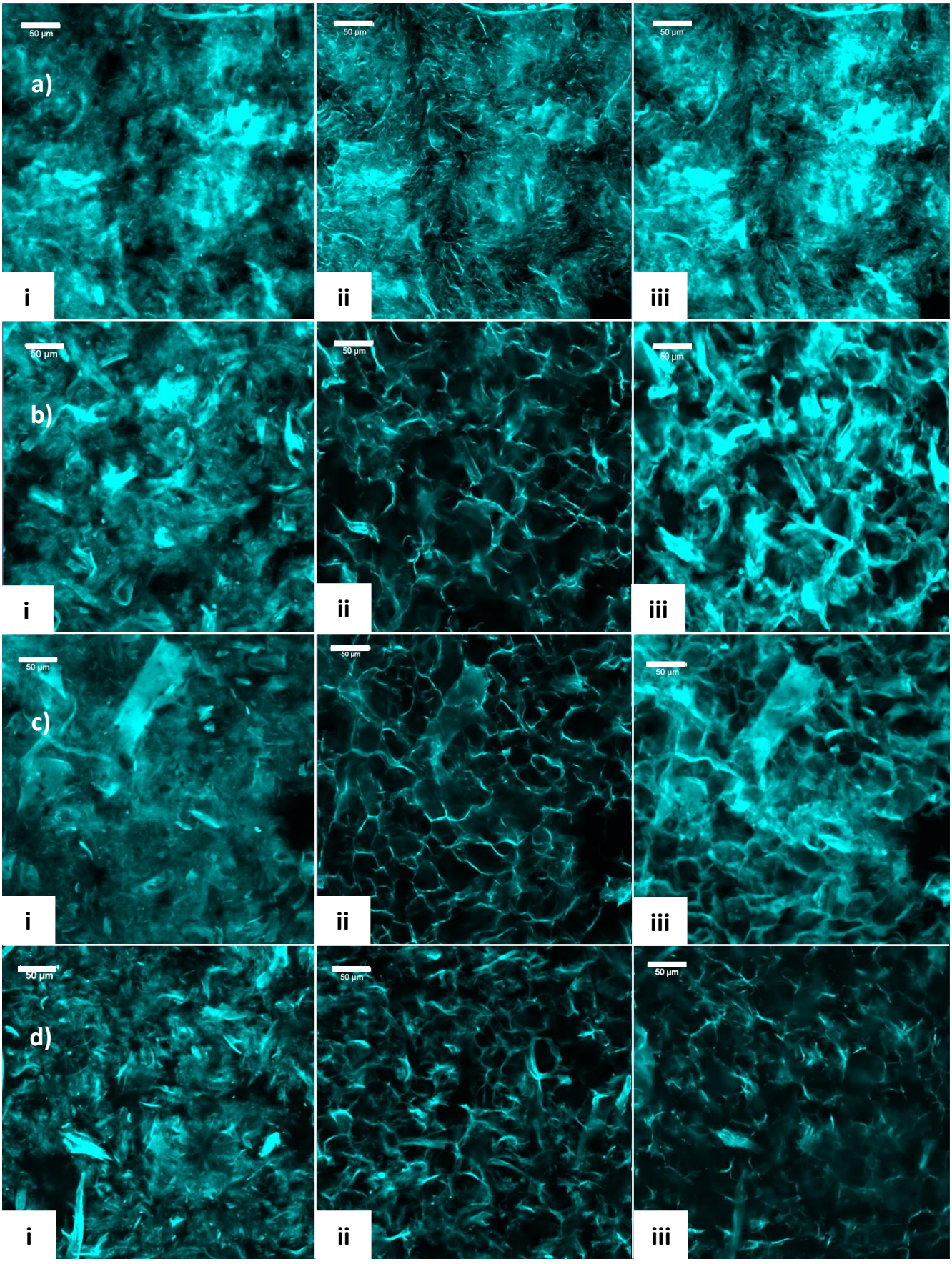
(Burke et al., 1976). For plant materials to be suitable stabilisers they are required to re-swell on thawing, retrieving their original volume, viscosity and other functional properties.

The ability of the plant fibres to rehydrate after thawing is presented in Figure 3.10. As can be seen, upon freezing, the growth of ice crystals causes compression of the fibres as all available water is frozen to form ice. FibreGel CF (Figure 3.10 a) produced heterogeneous dispersions but with a good amount of space occupancy. In addition, it also possesses good freeze-thaw stability as a large proportion of the structure reforms and rehydrates upon thawing. However, as FibreGel CF is standardised with dextrose (the concentration is unknown) the amount of freezable water in comparison to the other fibres will be different and thus the impact of freezing may not be so destructive as clearly smaller ice crystals have formed in comparison to the other fibres. AQ+ CF exhibits some larger clumps of fibre in comparison to FibreGel CF (Figure 3.10 b) and also exhibits relatively good freeze-thaw recovery but indicates some susceptibility to damage in comparison to FibreGel CF. AQ+ CA-U (Figure 3.10 c) was shown to have a poor ability to rehydrate after thawing as did Sugar Cane Fibre (Figure 3.10 d) with evidence of clumping and voids remaining in the structure where ice had grown. Such a poor ability to rehydrate and re-occupy space on thawing could be indicative of a drop in functionality such as the water binding capacity and therefore its impact on water mobility may be reduced.

Water holding capacity assessment identified that both Soy and Apple Fibre have poor space occupancy in solution, whilst FibreGel CF, AQ+CF, AQ+ CA-U, Sugar Cane and Psyllium all occupied 100% of the volume in suspensions. This is reflected in confocal assessment of the fibres. Both Soy and Apple Fibre (Figures 3.10 e and 3.10 f, respectively) show poor space filling properties, with the presence of large spaces in solution where the fibres are not interacting with the water in solution. This may suggest that there are areas in dispersions where water mobility is not being impacted and reduced by the presence of fibre. However, the robust,

dense structure of Soy Fibre resulted in good recovery upon thawing after compression. In contrast, the images suggest Apple Fibre has poor freeze-thaw stability.

Psyllium Fibre (Figure 3.10 g) exhibited excellent space filling properties as well as a good freeze-thaw stability. The growth of ice crystals caused the compression of fibres and upon thawing there was little damage as structures reform and hydrate well, illustrating good freeze-thaw stability suggesting that this fibre is most likely to retain its functionality in freeze-thaw conditions. However, some voids were found to remain in the structure after freeze-thaw cycling. Hui (2005) reported that the gels of Psyllium gum upon aging begin to contract over long storage periods, a process that can be accelerated by freezing and thawing cycles. Perhaps this offers an explanation to the changes seen here as Psyllium gum gels may possess some cryo-gelation properties.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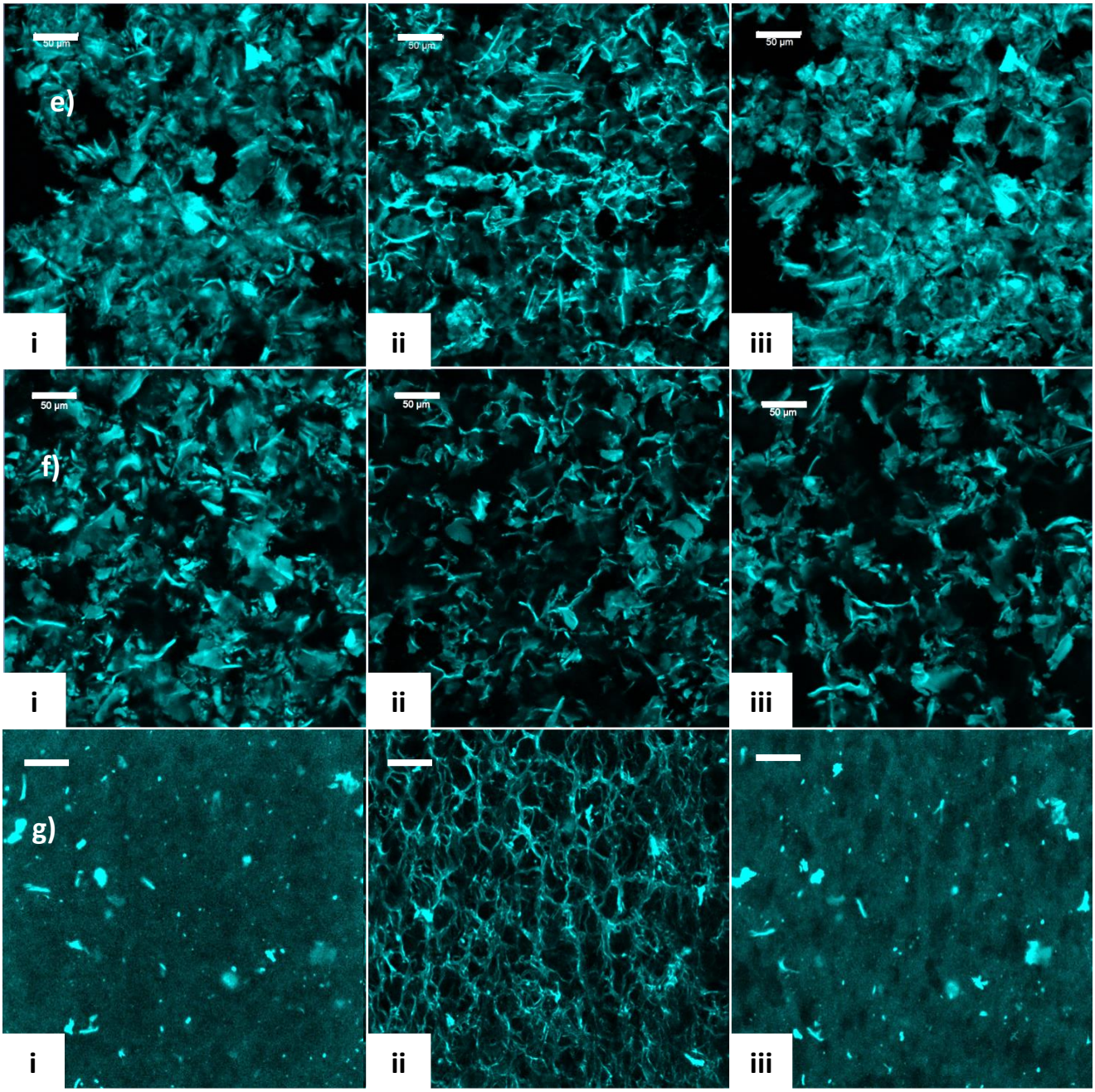

Figure 3.10 Confocal microscopy images of the ability of fibres homogenised at 800 bar to recover from freeze-thaw temperature cycling (a) FibreGel CF (b) AQ+ CF (c) AQ+ CA-U (d) Sugar Cane (e) Soy Fibre (f) Apple Fibre (g) Psyllium (i) initial (ii) frozen at -20°C (iii) defrosted. Scale bars shown represent 50 µm. Staining performed using Calcofluor-white.

3.3.2.5 Effect of homogenisation pressure on spin-spin relaxation times

Ice recrystallisation can diminish the sensory quality of ice cream. Stabilisers are added to ice cream to reduce the rate of recrystallisation and it is thought that their mechanism of control is due to their ability to reduce the molecular mobility of water within the serum phase (Cook & Hartel, 2010; BahramParvar & Mazaheri Tehrani, 2011). NMR spin-spin relaxation times (T_2) can provide an indication of how a stabiliser can impact molecular mobility. A decrease in relaxation time can be associated with a decrease in water (proton) mobility (Herrera et al., 2007).

The effect of homogenisation pressure on NMR relaxation curves are presented in Figures 3.11, 3.12 and 3.13. Freeze-thaw cycling was also used to further assess the freeze-thaw stability of fibre functionality. These curves indicate the three types of water associated with biopolymers in solution; free, bound and exchangeable (Nakamura et al., 1981). Bound water is the water (protons) that are chemically bound to the macromolecule. These are the non-freezable protons that are not able to partake in mobility related deteriorations such as microstructural coarsening. In contrast, free water is the water that is not chemically bound to the macromolecules and is considered the freezable water that is able to partake in ice recrystallisation. Exchangeable water is water that can be interchanged between loosely bound and strongly bound, usually associated with water that can form hydrogen bonds with either the macromolecule or neighboring water molecules and are considered to have a reduced freezing temperature in comparison to free water. However, this type of exchange behaviour can be removed by replacement with D_2O to yield the bound water proton shift. Therefore it is considered that a macromolecule that has a higher proportion of bound water is more likely to provide better control over ice cream microstructural coarsening (Nakamura et al., 1981; Le Botlan et al., 1998).

The large peak shown on these curves at slower relaxation times can be attributed to bulk water and correlates with the free and exchangeable water. The water molecules in exchange will have a slower relaxation time than bound water but relaxes faster than free water. The smaller peaks at faster relaxation times can be attributed to the protons of the fibre and bound water. Pure water has been reported to have a peak relaxation time of around 2000 ms (Belton, 1997). All fibres assessed were able to reduce the peak relaxation time in comparison to pure water suggesting that they are able to reduce the molecular mobility of water due to their ability to bind water. FibreGel CF (Figure 3.11 a) was found to have the largest impact on reducing T_2. In most cases, increasing the homogenisation pressure also resulted in a slight shift of bulk peaks to faster relaxation times suggesting a reduction in water mobility with increasing homogenisation pressure. This may be as a result of the exposure of additional hydroxyl groups on the glucose molecules in cellulose as well as the exposure of galactose units in pectin from the cell wall architecture with increasing homogenisation, which are able to from hydrogen bonds with the water molecules in solution and thus reducing the molecular mobility of water. AQ+ CF (Figure 3.11 b) had a broader distribution of T_2 times in comparison to FibreGel CF which is an indication of a less homogeneous distribution of T_2. A broadening of the peaks with repeated freeze-thaw cycling was also observed. This may be suggestive of fibre clumping and poor freeze-thaw stability as the compression of fibres and poor hydration on thawing would result in a drop in functionality. Confocal assessment identified the structure of AQ+ CA-U to be largely susceptible to freeze-thaw microstructural rearrangement. This is reflected in the relaxation times as AQ+ CA-U (Figure 3.11 c) indicated a very poor freeze-thaw stability with a large broadening of bulk relaxation curves with repeated freeze-cycling. In contrast, Psyllium (Figure 3.12 b) showed good freeze-thaw stability across all homogenisation pressures and a very homogenous distribution of relaxation times.

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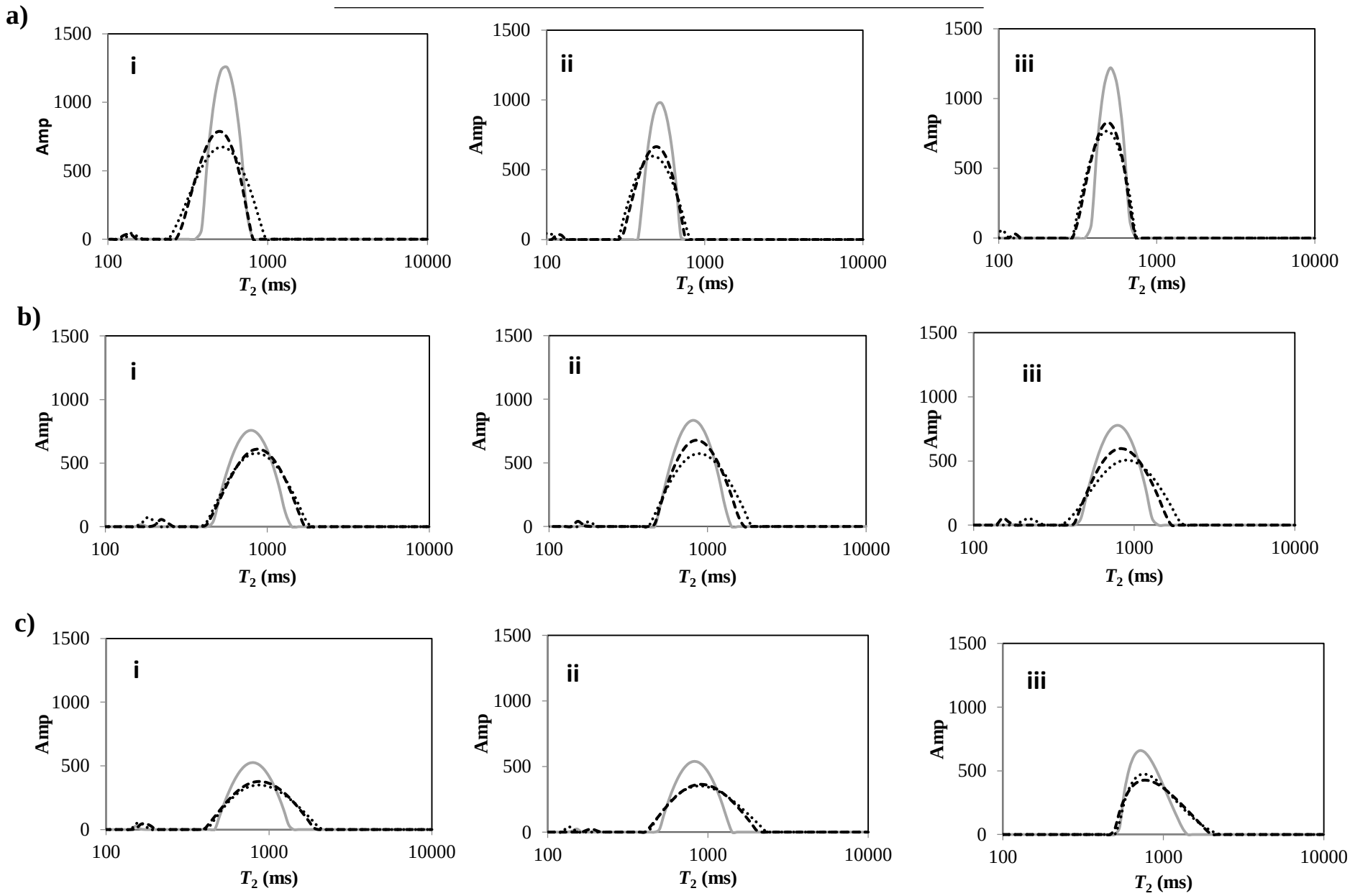

Figure 3.11 Effect of homogenisation pressure on freeze-thaw spin-spin relaxation NMR spectra (a) FibreGel CF (b) AQ+ CF (c) AQ+ CA-U (i) 400 bar (ii) 600 bar (iii) 800 bar. ——— Initial - - - First Freeze • • • Second Freeze (1% w/w fibre 20°C).

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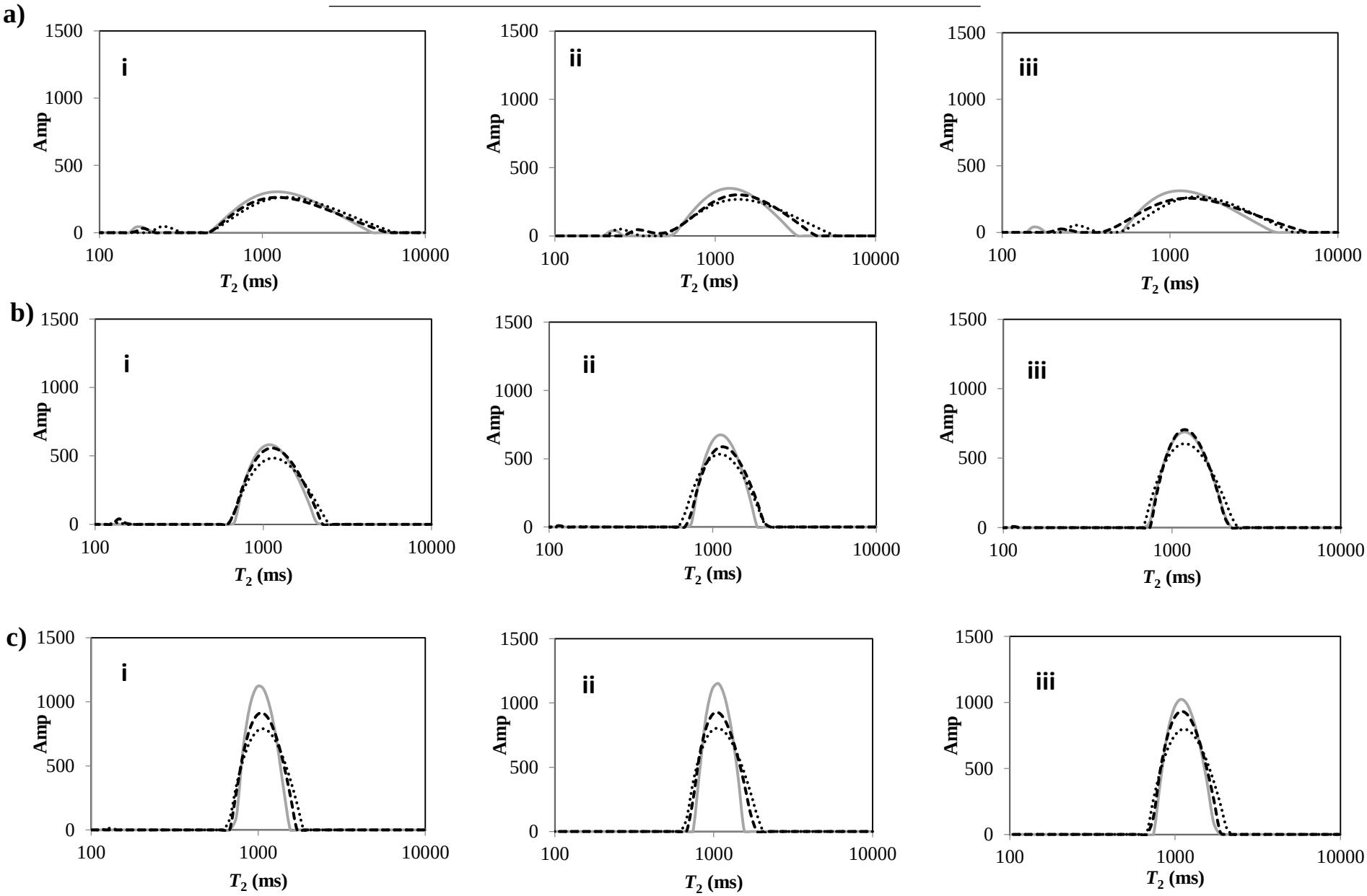

Figure 3.12 Effect of homogenisation pressure on freeze-thaw spin-spin relaxation NMR spectra (a) Soy Fibre (b) Psyllium (c) Sugar Cane (i) 400 bar (ii) 600 bar (iii) 800 bar. ——— Initial - - - First Freeze . . . Second Freeze (1% w/w fibre 20°C).

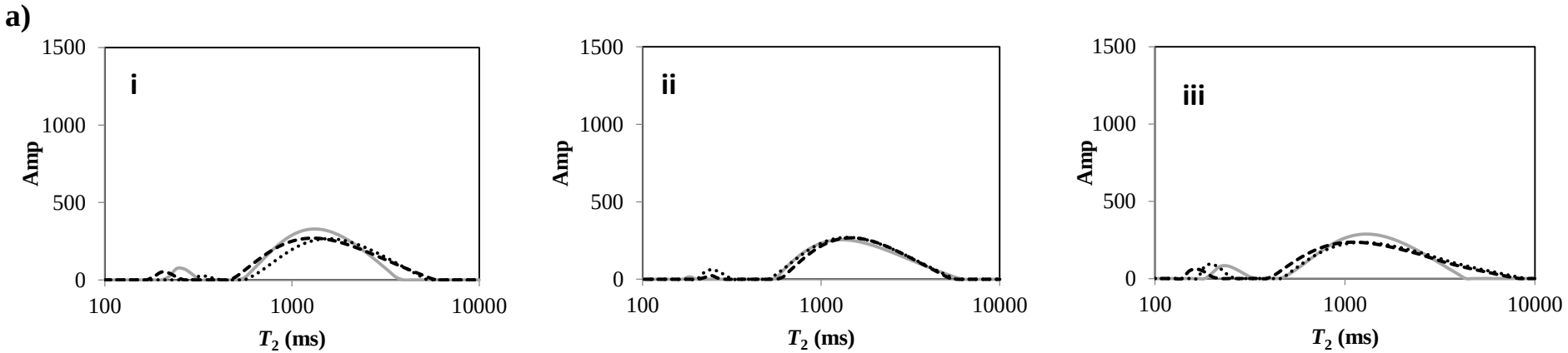

Figure 3.13 Effect of homogenisation pressure on freeze-thaw spin-spin relaxation NMR spectra (a) Apple Fibre (i) 400 bar (ii) 600 bar (iii) 800 bar. ——— Initial - - - First Freeze • • • Second Freeze (1% w/w fibre 20°C).

Confocal assessment of freeze-thaw stability identified that Sugar Cane had a poor ability to rehydrate on thawing, which would indicate a drop in functionality. However, this is not reflected in T_2 measurements (Figure 3.12 c) as little change in bulk peaks were seen after repeated freeze-thaw cycling at all homogenisation pressures. Suspensions of Sugar Cane Fibre also had a homogeneous distribution of relaxation times with a narrow bulk water peak.

As mentioned, the width of the bulk peaks can prove us with an idea on the homogeneity of the relaxation times. Soy and Apple Fibre (Figures 3.12 a and 3.13 a, respectively) were both found to have very broad T_2 distributions and therefore heterogeneous relaxation times. This can be attributed to their settling behaviour in solution and poor space filling properties as identified via confocal microscopy and water holding capacity assessment. If minimal space is occupied by the fibre, there are areas within dispersions where water is free and not interacting with the fibre. Areas where there is fibre interaction and other areas where there is no interaction will result in a large distribution (heterogeneous) of relaxation times.

A high water binding capacity has been suggested to substantially reduce water mobility (Soukoulis et al., 2009; Goff & Hartel, 2013). Sugar Cane Fibre and AQ+ CF were found to have the highest WBC. However, this is not reflected in T_2 assessment as FibreGel CF had the largest contribution to reducing the T_2. The impact of FibreGel CF on T_2 however may be as a result of its content of dextrose. Previous studies have found a relationship between sugar molecular weight and impact on T_2, as small molecular sugars reduced T_2 more than larger sugar molecules. Thus there is a hierarchy of dextrose > Fructose > Sucrose in terms of impact on T_2 (Hagiwara & Hartel, 1996). Therefore the presence of dextrose would have a larger impact of the T_2 of the fibres in solution.

So far the presence of dextrose in FibreGel CF has contributed to a higher serum apparent viscosity, good freeze-thaw recovery and higher impact on T_2 reduction. Higher serum

viscosities and higher reductions in T_2 are associate with a biopolymer that can provide good control over the rate of ice recrystallisation. However, it has been found that sugars provide little to no control over the rate of ice recrystallisation (Hagiwara & Hartel, 1996) as so the predictions made about FibreGel CF may not actually be seen during ice recrystallisation assessment, which will be assessed in Chapter 4.

3.3.2.6 The impact of fibre phase volume

As it has been identified that both Soy and Apple Fibre contain comparatively half the total fibre content of the other fibres being assessed, contributing to different phase volumes, measurements of their WHC and spin-spin relaxation times (T_2) were undertaken at double the concentration to assess the impact of increasing the phase volume (Figures 3.14).

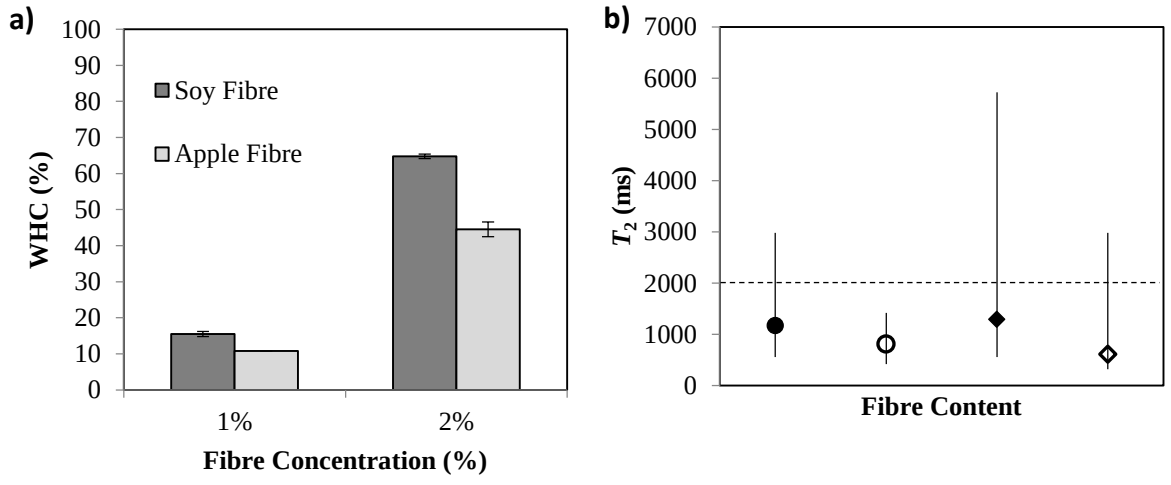

Figure 3.14 The impact of doubling the fibre concentration of Soy fibre and Apple fibre on (a) water holding capacities and (b) spin-spin relaxation times of bulk peaks of (•) Soy Fibre (♦) Apple Fibre (closed symbol) 1% (w/w) fibre (open symbol) 2% (w/w), (- - -) represent the peak T_2 of pure water (20°C). Error bars shown represent the standard deviation.

The other fibres being assessed all showed 100% space occupancy in suspensions in WHC assessment. However, doubling the fibre concentration in both Apple and Soy Fibre did not result in a 100% space occupancy but did result in the WHC increasing by more than double the original WHC. Soy Fibre increased from 15.51% to 64.79% and Apple Fibre from 10.79% to 44.52%. In addition, doubling the concentration of fibre resulted in a narrowing of T_2 distributions as a result of the increase of fibre and therefore an increase in the number of hydroxyl groups available to form hydrogen bonds with water molecules. However, given that pure water has a peak relaxation time of 2000 ms (Belton, 1997), it would suggest that there is still a large proportion of water molecules in these dispersions with undisturbed mobility due to lower volume occupancy of these fibres even at higher concentrations.

3.3.2.7 Removal of fibres of poor suitability as ice cream stabilisers

Apple Fibre was removed from this point of the investigations due to its low WBC and WHC, poor micro and macro viscosity enhancement, settling behaviour in solution and poor ability to rehydrate in freeze-thaw conditions. Although Soy Fibre also possessed some of these issues, it was taken forward in further studies due to its ability to recover in freeze-thaw conditions.

AQ+ CA-U was also removed from the investigations from this point forward. AQ+ CA-U is comparable to AQ+ CF in WHC, WBC, both macro and micro apparent viscosity and its impact on T_2 relaxation times. However, freeze-thaw assessment of T_2 and confocal images suggest it is largely more susceptible to damage upon freezing than AQ+ CF with little ability to rehydrate and retain functionality upon thawing.

It was also identified that the homogenisation pressure has a large influence on fibre functionality. A pressure of 600 bar was identified to optimise the WBC, WHC and T_2 in the majority of cases. Therefore, further assessment of the functionality of fibres was performed using a 600 bar homogenisation pressure from this point forward.

3.3.3 Screening and optimisation using heating

Compositional analysis of sugar monomers identified that AQ+ CF and FibreGel CF have a large content of galactose. This is an indication of a high proportion of pectin. To increase the solubility of the pectin in plant materials heating is required (Bengtsson & Tornberg, 2011; Moelants et al., 2013). Thus, assessment of the functional properties of the fibres after heating was undertaken to assess if this improves fibre functionality. In addition, as ice cream pre-mixes are subjected to a pasteurisation step, it is required to asses if this hinders or improves functionality.

3.3.3.1 Effect of heating on water holding capacity

The effect of heating fibre suspensions on the WHC is shown in Figure 3.15. It shows that heating has little impact on the WHC of FibreGel CF, AQ+ CF, Psyllium and Sugar Cane and thus no loss in functionality with pasteurisation of the mix is expected. However, for Soy Fibre, a slight drop in WHC with heating was observed although this decrease was not found to be significant. Bengtsson & Tornberg (2011) reported that the WHC of apple and carrot fibre decreased when fibre suspensions where heated. This is due to a reduction in particle size due to degradation of the fibre as sugar polymer chains can be broken down into their sugar monomer constituents. Heating fibres at 90 - 95°C for 5 minutes resulted in more significant reductions in WHC than when heated at 65°C for 40 minutes, suggesting the higher the temperature the more the degradation.

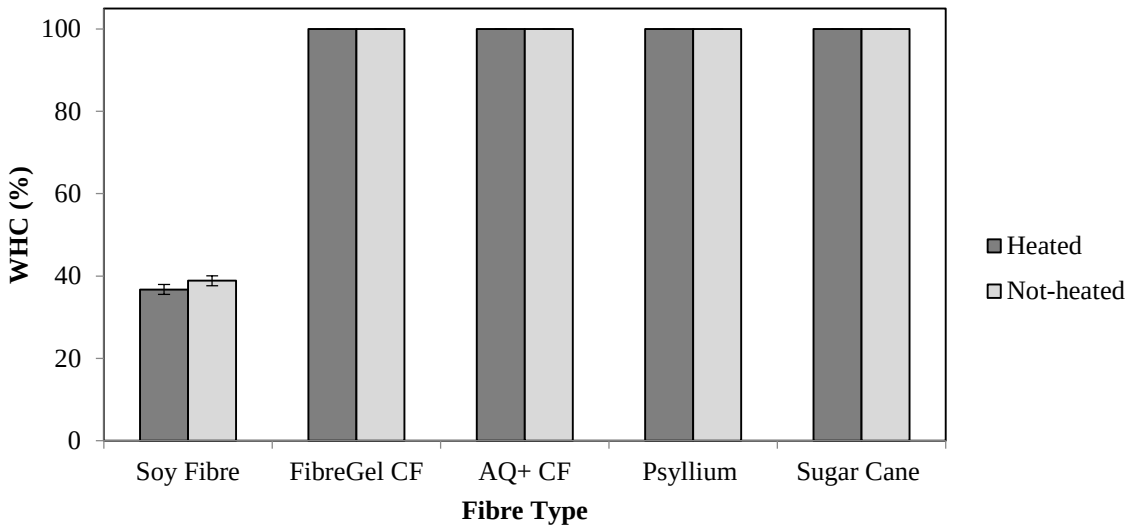

Figure 3.15 Effect of heating on the water holding capacity (WHC %) of plant fibres. Error bars shown represent the standard deviation (1% w/w fibre 20°C)

3.3.3.2 Effect of heating on water binding capacity

The effect of heating fibres on WBC is shown in Figure 3.16. It shows that the WBC of Soy, Sugar Cane and Psyllium Fibre is reduced with heating. This could mean that heating during ice cream pre-mix pasteurisation could compromise the functional properties of these fibres. However, heating increased the WBC of both FibreGel CF from 79.12 ± 1.38 to 96.98 ± 2.01 grams of water per gram of dry fibre and AQ+ CF from 85.43 ± 0.98 to 96.22 ± 1.56 g/g. This is thought to be due to the solubilisation of pectin which has a large ability to bind water (Soukoulis et al., 2009). The solubilisation of pectin should result in an increase of serum viscosity and a decrease in NMR relaxation times as a result of an increase in the amount of bound water, which is discussed in the next sections.

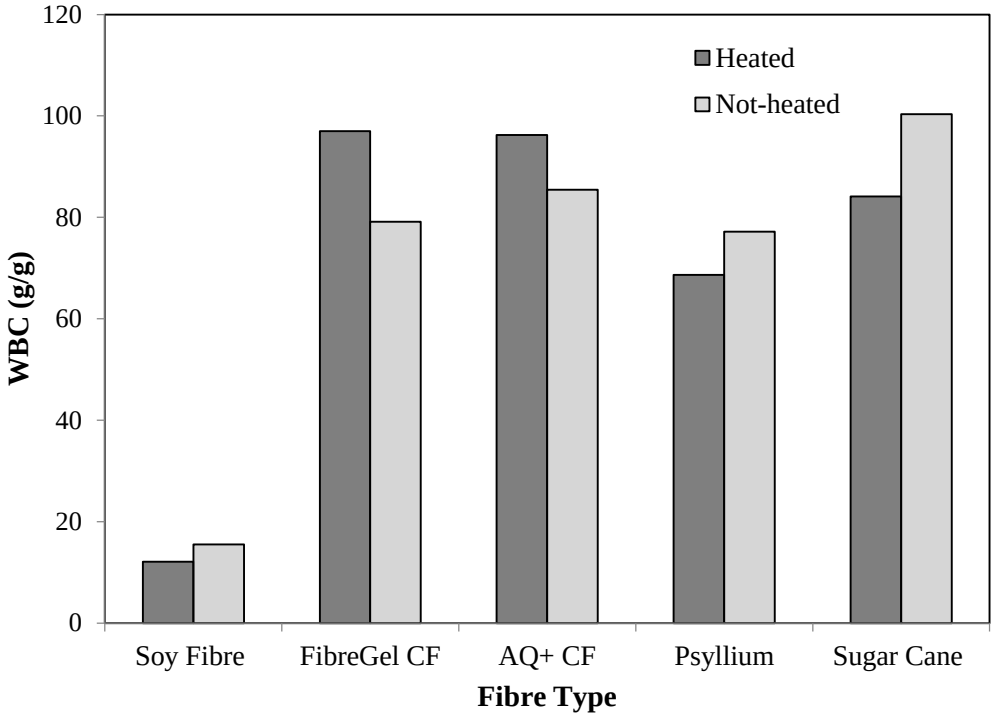

Figure 3.16 Effect of heating on the water binding capacity (WBC) of plant fibres (1% w/w fibre 20°C).

3.3.3.3 Effect of heating on rotational viscosity

The effect of heating fibres on bulk and serum viscosities are shown in Figures 3.17 and 3.18, respectively. Heating of FibreGel CF resulted in a large increase in both bulk and serum viscosities due to the solubilisation of pectin. This enhancement of both macro and micro viscosity should result in an improved control over ice recrystallisation (Goff & Hartel, 2013). With the substantial increase in WBC and serum viscosity seen with heating, it would be expected that a decrease in T_2 relaxation would correlate. However, no studies could be identified from the literature that correlate the control of pectin over ice recrystallisation as a

function of viscosity enhancement and therefore it is not known if the viscosity of 16.25 mPa.s seen here for FibreGel CF is sufficient to provide control.

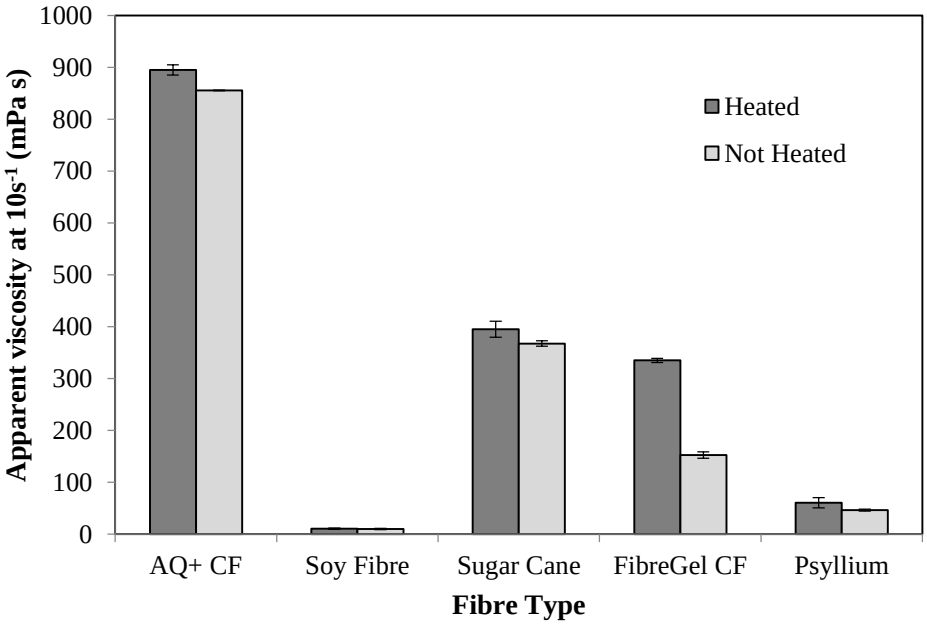

Figure 3.17 Effect of heating fibre suspensions on bulk apparent viscosity. Error bars shown represent the standard deviation (1% w/w fibre 20°C).

The apparent bulk and serum viscosity of AQ+ CF, which also contains a proportion of pectin, was also found to increase on heating, although not to the extent of FibreGel CF. Slight increases in both bulk and serum viscosities of Soy, Sugar Cane and Psyllium Fibre were also observed. This may be as a result of the solubilisation of insoluble material as a result of degradation by heating (Larrauri, 1999).

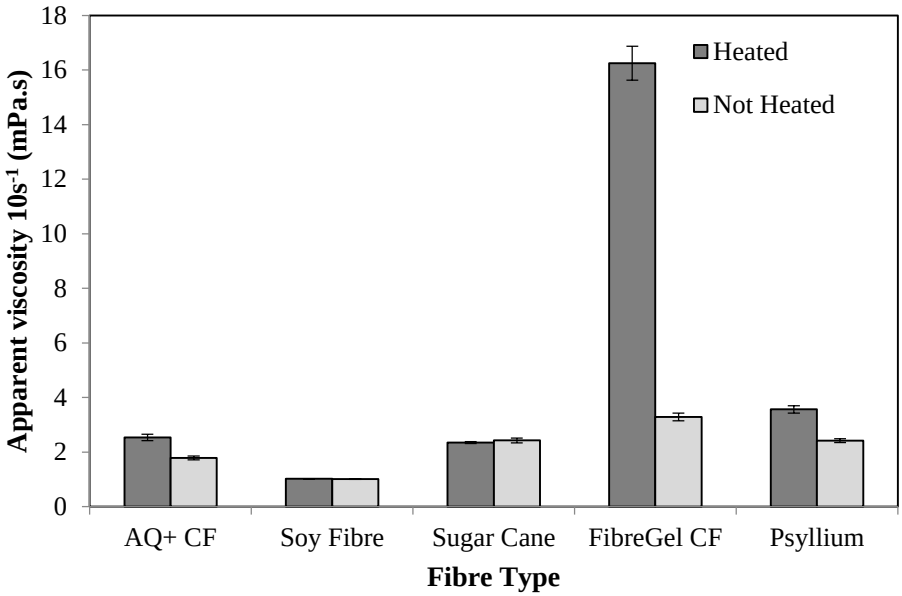

Figure 3.18 Effect of heating fibre suspensions on serum apparent viscosity. Error bars shown represent the standard deviation (1% w/w fibre 20°C).

3.3.3.4 Effect of heating on spin-spin relaxation times

Figure 3.19 shows the effect of heating on T_2 relaxation times of the bulk water peaks. As expected the increase in WBC and serum viscosity of FibreGel CF with heating as a result of solubilisation of pectin caused a reduction in peak T_2 values and a narrowing of the distribution. This indicates a more homogeneous distribution of relaxation times. This is due to a decrease in the amount of free water, suggesting that in an ice cream formulation, water is less mobile and is less likely to partake in mobility related quality deteriorations. Peak T_2 values for AQ+ CF were also found to be reduced due to the same effect. Heating caused an increase in peak relaxation times of Psyllium, Sugar Cane and Soy Fibre. This is expected due to a decrease in WBC with heating observed. However, the differences are minimal and therefore pasteurisation of an ice cream pre-mix may have little impact on the functional properties of these fibres.

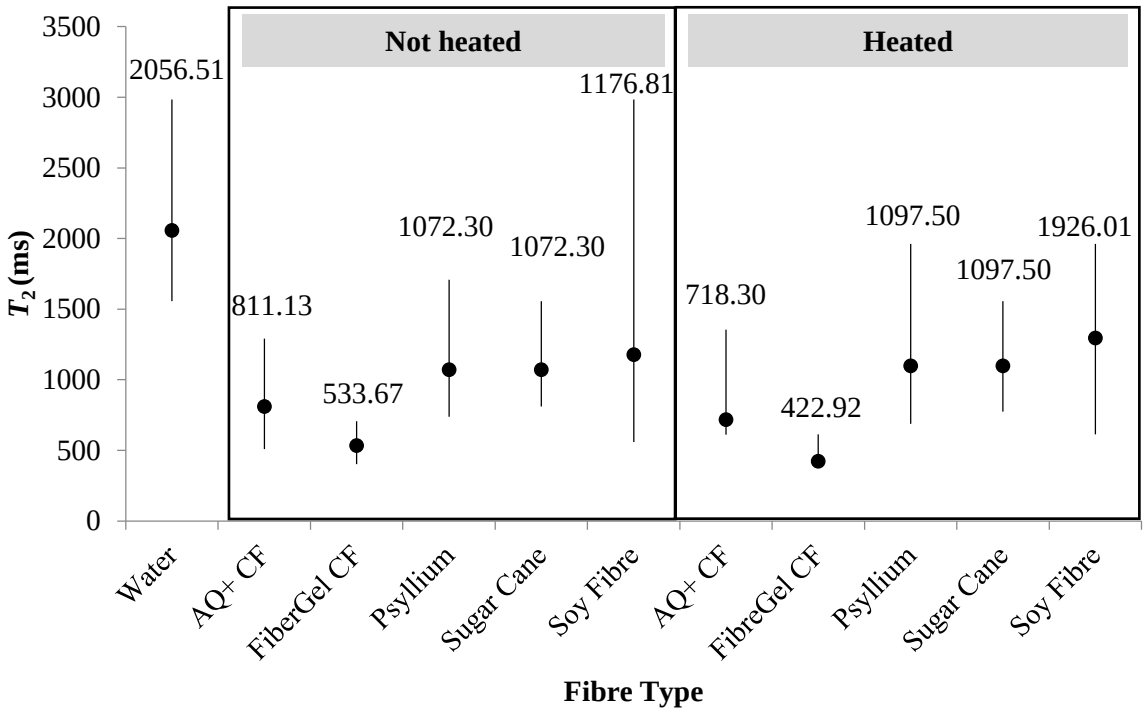

Figure 3.19 Effect of heating fibre suspensions on spin-spin relaxation times of the distributions of bulk water peaks. The data labels shown indicate peak T_2 values (1% w/w fibre 20°C).

3.3.3.5 Freeze-thaw confocal laser scanning microscopy

The effect of heating fibres on their structure and ability to recover in freeze-thaw conditions is shown in Figures 3.20, 3.21 and 3.22. For all fibres, little differences in structure can be seen with homogenising fibres at 600 bar. Heating also has little impact on the structure observed. In addition, little improvement in freeze-thaw stability of the fibre structures was identified with the exception of AQ+ CF (Figure 3.20 a) as an improvement in the ability to rehydrate upon thawing was observed. Slight differences in the structure of FibreGel CF (Figure 3.20 b) can be seen with heating, with more numerous small fibre particles forming the bulk of the

fibre mass. In addition, a slight improvement in its ability to recover on thawing can be observed with heating suggesting an improvement in functionality.

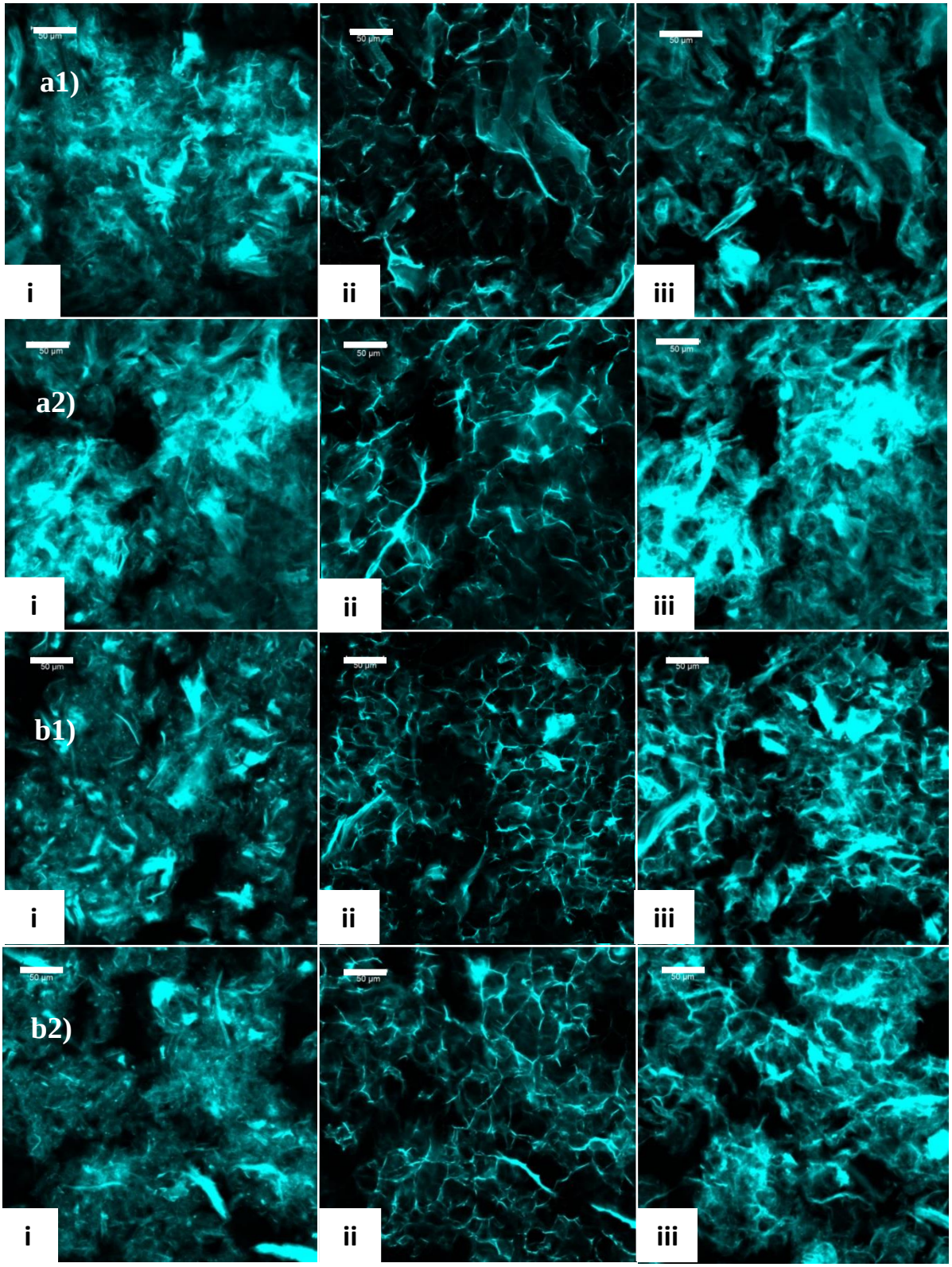

Figure 3.20 Confocal microscopy images of fibres homogenised at 600 bar and the effect of heating fibres on their ability to recover from freeze-thaw temperature cycling (a1) AQ+ CF not heated (a2) AQ+ CF heated (b1) FibreGel CF not heated (b2) FibreGel CF heated (i) initial (ii) frozen at -20°C (iii) defrosted. Scale bars shown represent 50 µm. Staining performed using Calcofluor-white.

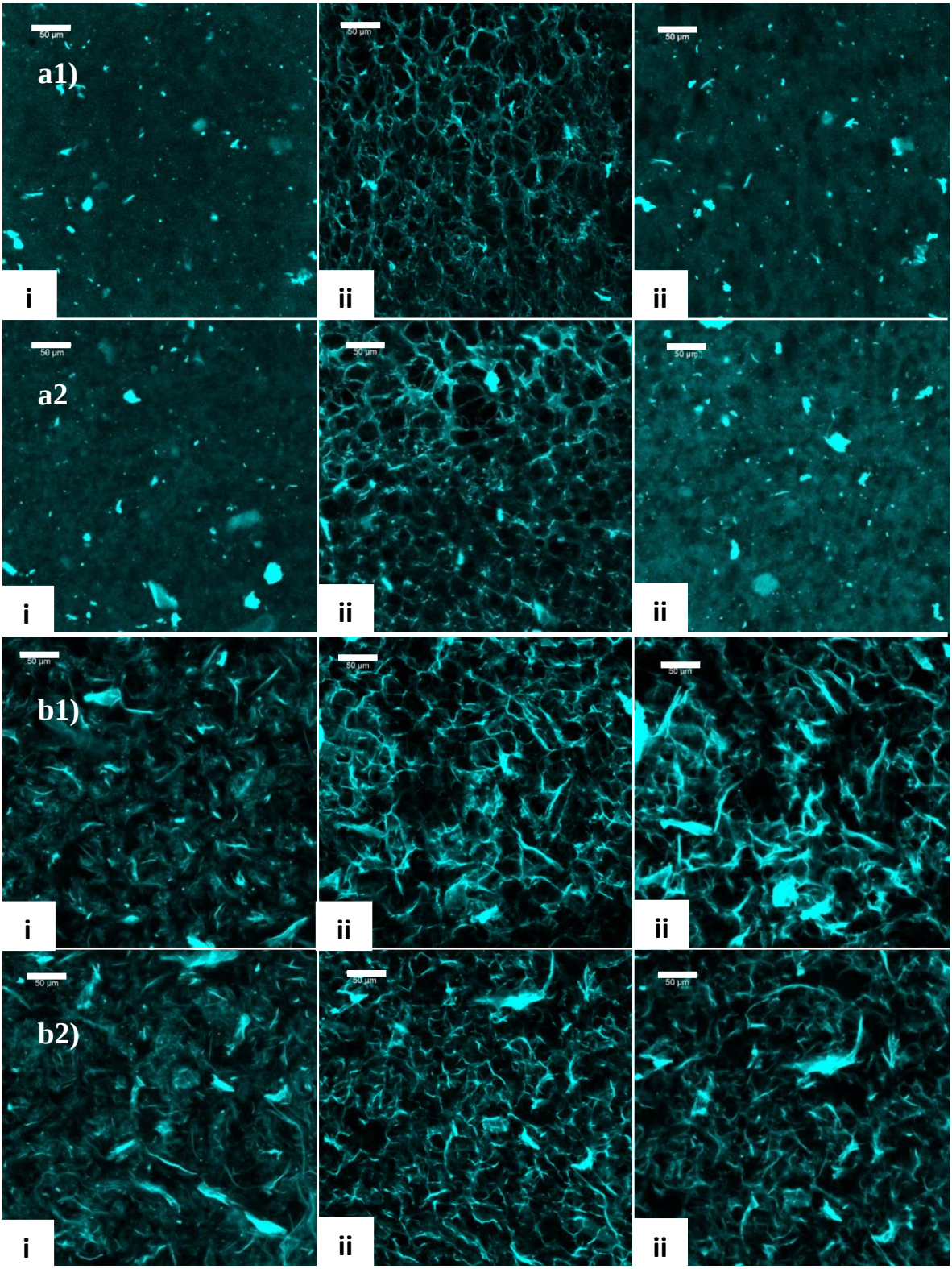

Figure 3.21 Confocal microscopy images of fibres homogenised at 600 bar and the effect of heating fibres on their ability to recover from freeze-thaw temperature cycling (a1) Psyllium not heated (a2) Psyllium heated (b1) Sugar Cane not heated (b2) Sugar Cane heated (i) initial (ii) frozen at -20°C (iii) defrosted. Scale bars shown represent 50 µm. Staining performed using Calcofluor-white.

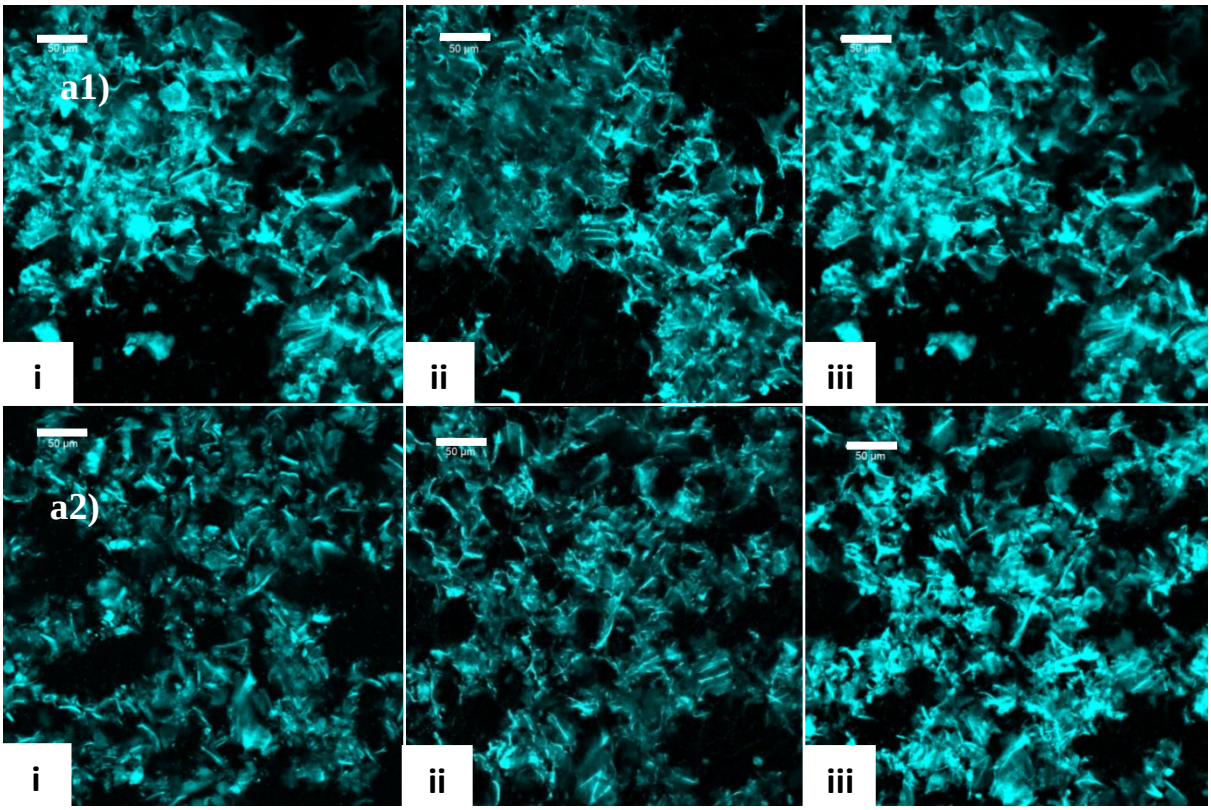

Figure 3.22 Confocal microscopy images of fibres homogenised at 600 bar and the effect of heating fibres on their ability to recover from freeze-thaw temperature cycling (a1) Soy Fibre not heated (a2) Soy Fibre heated (i) initial (ii) frozen at -20°C (iii) defrosted. Scale bars shown represent 50 µm. Staining performed using Calcofluor-white.

Of all the fibres being examined, Psyllium (Figure 3.21 a) seems to be the most space occupying with very homogeneous structures as well as an excellent freeze-thaw stability. It might be expected that such an excellent freeze-thaw stability would mean that the structure of Psyllium Fibre is not changed with repeated exposures to heat shock when in ice cream formulations. For Sugar Cane Fibre (Figure 3.21 b), heating seems to increase the presence of small fibres. However, little change in the freeze-thaw stability is observed as a poor recovery on hydration can still be seen. Sugar Cane Fibre structures also seem to be unconnected and fibre clumps like those seen in AQ+ CF and FibreGel CF are not observed. One of the functional properties of the use of plant fibres as ice cream stabilisers is that they are able to form a fibrous network within the matrix phase preventing the rate of meltdown (Dervisoglu & Yazici, 2006). Because of the lack of interconnectivity between fibre structures, it might be suggested that Sugar Cane Fibre may have weak control over the rate of ice cream meltdown. In addition, as its structure is susceptible to freeze-compression, Sugar Cane may offer little control during heat shock. Figure 3.22 shows the effect of heating Soy Fibre on its structure and freeze-thaw recovery. Little differences can be seen with heating as robust fibre structures remain with strong freeze-thaw stability. However, structures still show poor space occupancy.

3.3.3.6 Freeze-thaw recovery of water binding capacity and water holding capacity

The freeze-thaw stability of the fibres and their ability to retain their WBC and WHC functional properties are reported in Figure 3.23. As previously outlined, the aim of the research as part of this chapter was to create 'swellable hairy particles' whereby a fibre can re-swell and absorb melting water on thawing with freeze-thaw cycling having little impact on both structure and the functional properties of the plant fibres.

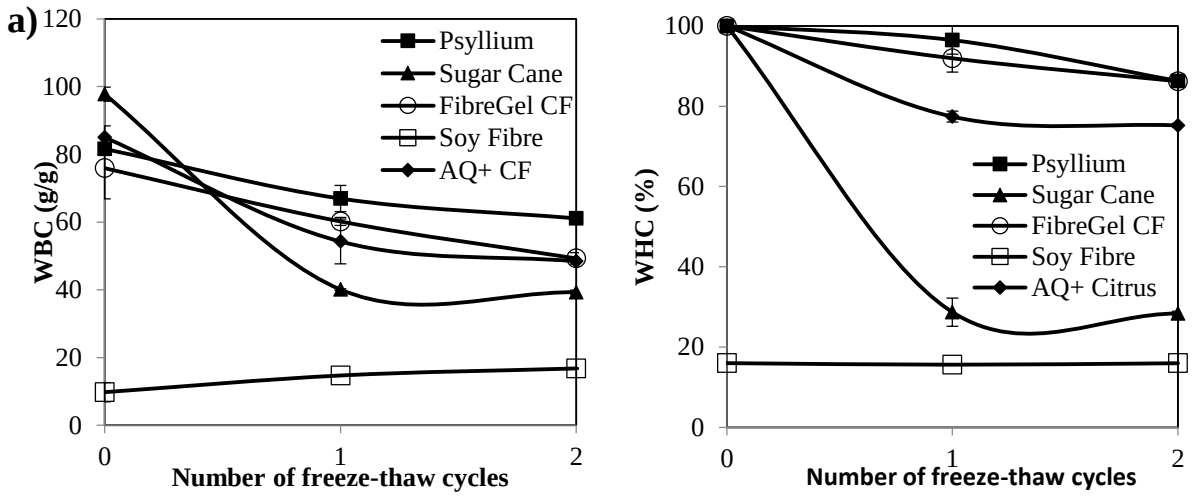

Figure 3.23 Effect of freeze-thaw cycling on (a) water binding capacity (WBC) (b) water holding capacity (WHC). Error bars shown represent the standard deviation (1% w/w fibre 20°C).

Freeze-thaw confocal microscopy images suggest that Psyllium Fibre is the most freeze stable fibre. This is also reflected in Figure 3.23 as Psyllium has the best ability to retain its properties in freeze-thaw conditions and after freezing is able to occupy the most space in a dispersion giving it the highest WHC and WBC. In contrast, Sugar Cane Fibre was observed to be the

most susceptible to freeze damage in rheological and confocal microscopy assessments and thus a large drop in both WHC and WBC upon the first freeze was observed. After the first freeze little further damage can be seen in Sugar Cane Fibre. For both AQ+ CF and FibreGel CF, the loss of WBC and WHC appears to be more progressive with freeze-cycling, reducing almost linearly with the number of freeze-thaw cycles, which is a concern with the repeated freeze-thaw cycling that can occur with commercial ice cream products. As expected, due to the robust nature of Soy Fibre, little difference in properties can be seen with freeze-thaw cycling.

3.3.3.7 Effect of freeze thaw-cycling on apparent viscosity and fibre structure

It is a concern that some fibres are showing structural susceptibility to freezing. As previously outlined, the ability of a plant fibre to retrieve its specific volume and viscosity upon repeated freeze-thaw cycling will provide a good indication of a particular plant fibres freeze-thaw stability. The effect of freeze-thaw cycling on apparent viscosity and fibre structure are shown in Figure 3.24. It could be hypothesised that if individual defibrillated fibres become compressed with freezing and are unable to rehydrate on thawing, their hydrodynamic volume will be reduced and thus a decrease in viscosity would be observed. However, as shown in Figure 3.24 a, freeze-thaw cycling, in most cases, resulted in an increase in viscosity due to clumping of the fibres as indicated in the images shown in Figure 3.24 b.

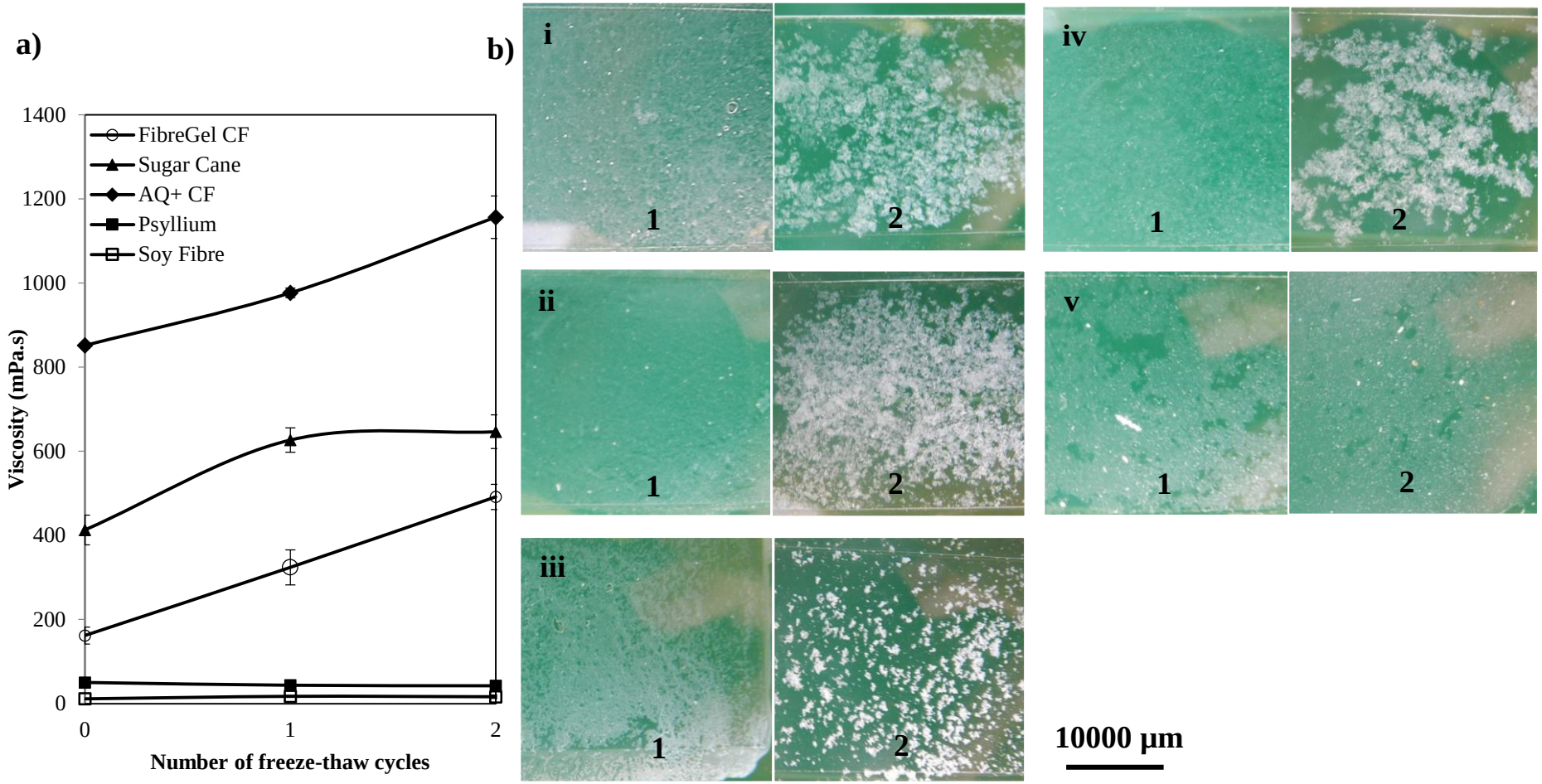

Figure 3.24 Effect of freeze-thaw cycling of homogenised fibre (600 bar) suspensions on (a) bulk apparent viscosity (b) structure of plant fibres (i) FibreGel CF (ii) Sugar Cane (iii) Soy Fibre (iv) AQ+ CF (v) Psyllium (1) Fresh (2) after two freeze-thaw cycles. Error bars represent the standard deviation (1% w/w fibre 20°C).

Psyllium Fibre, throughout the freeze-thaw cycling screening techniques used has shown excellent freeze-thaw stability. Little differences in apparent viscosity (Figure 3.24 a) and fibre structure (Figure 3.24 b, v) can be observed with freeze cycling. However, for all the other fibres, changes in structure and clumping of the fibres can be seen with freeze-thaw cycling.

Freeze-thaw WHC and WBC assessment (Figure 3.23) identified a near linear relationship between the increasing number of freeze-thaw cycles and reducing WHC and WBC of FibreGel CF and AQ+ CF. This is reflected in the apparent viscosity measurements (Figure 3.24 a) as apparent viscosity steadily increased for both fibres with the number of freeze-thaw cycles. In addition, WHC and WBC assessment identified that Sugar Cane Fibre has a large susceptibility to the first freeze-thaw cycle, but little change was observed with the second cycle. This trend in damage is also reflected in the apparent viscosity measurements.

As it is important that a suitable ice cream stabiliser increases viscosity but imparts little other textural properties, a concern of clumping of fibres forming lots of large particles is that when consuming a heat shocked ice cream, gritty, coarse or powdery sensations in the mouth may be perceived (de Lavergne et al., 2017). However, sensory assessment will shed more light on the full impact of the effect of plant fibres on ice cream sensory properties (Chapter 5).

3.4 Conclusions

Swellable hairy cellulose particles have been created by homogenising commercial plant fibres. Assessment of the properties of these fibres in a dispersion outlined large difference between fibres in their water holding and water binding properties, their viscosity in solution, and their ability to reduce the molecular mobility of water. These differences in functional properties are undoubtedly as a result of plant origin and their structure and compositional make up. That given, for the two fibres of citrus origin, heating of solutions which resulted in the solubilisation of pectin resulted in improved functionality. However, it is still unknown until fibres are assessed in ice cream formulations how fibre compositional make up including the insoluble to soluble fibre ratio determines the suitability of a plant fibre as an ice cream stabiliser.

Freeze-thaw cycling of these measurements singled out the fibres that show susceptibility to both structural and functional change. However, the reducing functionality of citrus fibres seems to be more progressive with the number of freeze-thaw cycles, whilst Sugar Cane Fibre was only affected by the first freeze-thaw cycle. In contrast, Psyllium Fibre was identified to have little susceptibility to freeze-thaw cycling. However, for Psyllium Fibre, it has one of the lowest viscosities in solution and a lower water binding capacity but its ability to refrain from freeze-thaw damage may result in it being the most suitable plant fibre being assessed. Nevertheless, the conditions of freeze-thaw cycling undertaken as part of this work are extreme as a large proportion of the water is removed to form ice. Therefore, the reductions in functionality observed after 1-2 freeze cycles may not be the same in ice cream formulations due to the presence of an unfrozen phase.

The knowledge gained about the behaviour of these fibres has allowed the identification of suitable plant fibres to be taken forward into ice cream formulations.

3.4.1 Selection of suitable fibres

The functionality of Soy Fibre could be improved by bringing the total fibre content up to that comparable with the other fibres. It was also identified to be very freeze-thaw stable. However, the overriding limitation of Soy Fibre is that it has a tendency to settle in dispersions and does not occupy 100% of the volume and therefore is unlikely to contribute to the formation of a fibrous framework within the ice cream matrix phase. In addition, Soy Fibre was found to have the lowest WBC, which makes it unsuitable as a swellable particle as a large proportion of the water remains unbound. For these reasons, Soy Fibre will be removed from the investigations from this point forward.

However, AQ+ CF, FibreGel CF, Sugar Cane Fibre and Psyllium will all be taken forward for further assessment in ice cream formulations.

Given the knowledge gained so far about the fibres, it is predicted that the higher apparent viscosity of AQ+ CF dispersions will have a higher contribution to increasing the ice cream pre-mix viscosity and therefore enhancing the meltdown properties of ice cream by reducing the rate of drainage. In addition, the higher WBC of Sugar Cane Fibre will allow it to provide the best control over the rate of ice recrystallisation. However, the lack of interconnectivity between the fibres suggests Sugar Cane Fibre will have little contribution to the formation of a fibrous framework within the ice cream matrix phase. In addition, its freeze-thaw stability may result in a reduction of the control provided over ice recrystallisation in ice creams subjected to temperature cycling or heat shock conditions.

As freeze-thaw stability is an important factor, the ability of Psyllium to retain its functional properties of a large space occupancy, WHC, WBC and its impact on viscosity suggest it is likely to behave as the most suitable ice cream stabiliser.

CHAPTER 4: IMPACT ON ICE CREAM MICROSTRUCTURE

4.1 Introduction

The main aim of ice cream manufacturers is to generate the correct microstructure to achieve the desired organoleptic properties, as it is the size, structure and morphology of the microstructural elements of ice cream that manifest themselves as sensory properties during consumption. In addition, the structure needs to be sufficiently robust to withstand transportation and storage (Crilly et al., 2008).

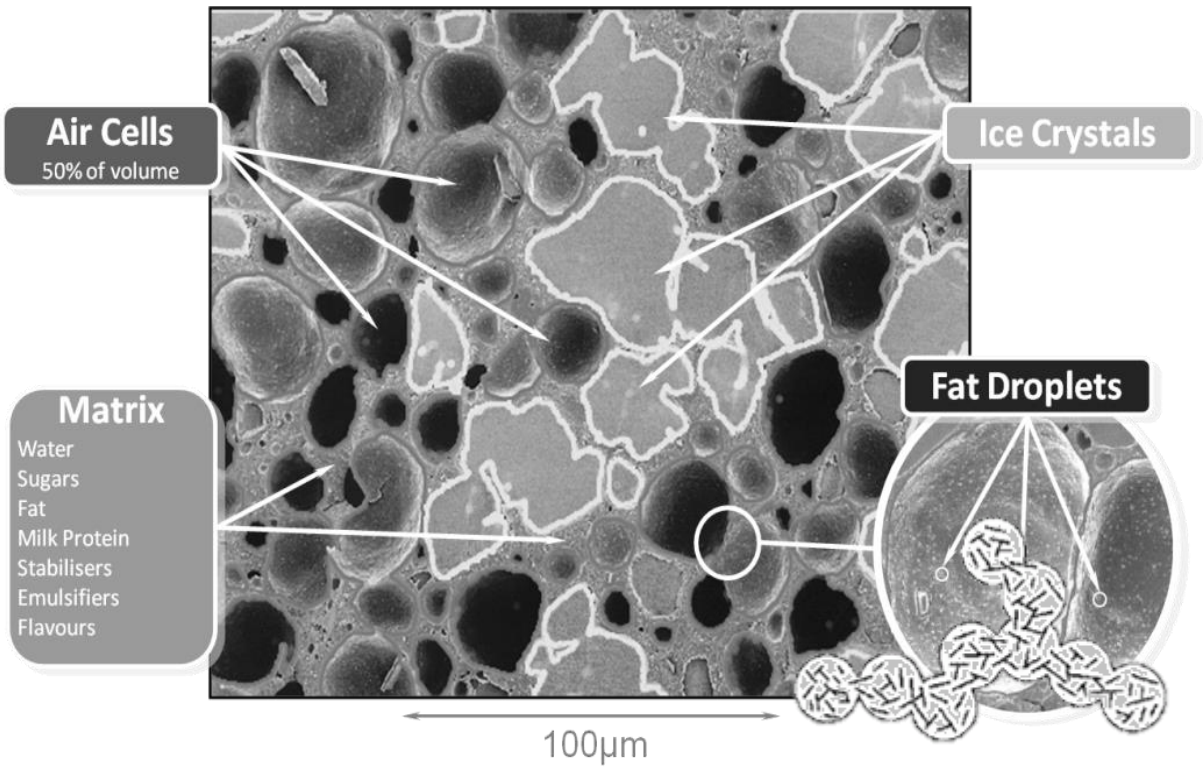

Figure 4.1 Artificially coloured Cryo-scanning electron microscopy (cryo-SEM) image of ice cream showing the four microstructural phases (provided by Unilever).

The structure of ice cream can be described as a partially frozen foam, whereby it is both an emulsion and a foam with an unfrozen continuous phase (BahramParvar & Mazaheri Tehrani, 2011). As shown in Figure 4.1, ice cream has four microstructural phases. Air bubbles and ice

crystals occupy the majority of the space. Discrete fat globules and aggregates of destabilised fat are found both at the air interface and within the serum phase. The unfrozen matrix, or serum phase, is a concentrated mixture of dissolved sugars, stabilisers and flavours. Emulsifiers are found surrounding the fat globules and proteins can be found surrounding both the air and fat as well as dispersed within the serum phase (Goff & Hartel, 2013).

The structure of each of the four microstructural phases has a large impact on the physical and sensory properties of ice cream (Goff & Hartel, 2013). Ice cream is thermodynamically unstable and even under ideal storage conditions the air and ice will coarsen overtime, resulting in a loss of quality. A process that is accelerated with heat shock.

Heat shock can result in irreversible microstructural changes that can impact on the shelf life quality and sensory experience of ice cream. Such changes include ice recrystallisation, serum drainage and shrinkage. Preventing such microstructural change is key to retaining product quality and stabilisers are included in ice cream formulation to provide such stability. Thus, in order to test the effectiveness of a new stabiliser in ice cream formulations, assessment of the microstructure of ice cream both pre and post heat shock allows identification of the suitability of the new stabilisers at preventing microstructural deteriorations.

4.1.1. Serum phase

The serum phase is the structural element that interacts and surrounds each of the other structural phases found in ice cream and is composed of unfrozen water and dissolved solutes. The properties of the serum phase are dependent on mix composition and temperature (Goff & Hartel, 2013). During freezing, the serum phase endures a concentration change. As ice cream freezes, water is removed from the serum phase to form ice. The serum phase thus becomes increasingly concentrated as the temperature is reduced. This increase in solute concentration

results in a further depression of the freezing point of the remaining unfrozen phase (Clarke, 2012). The freezing point depression as a result of freeze concentration of the solutes can be explained using a phase diagram (Figure 4.2).

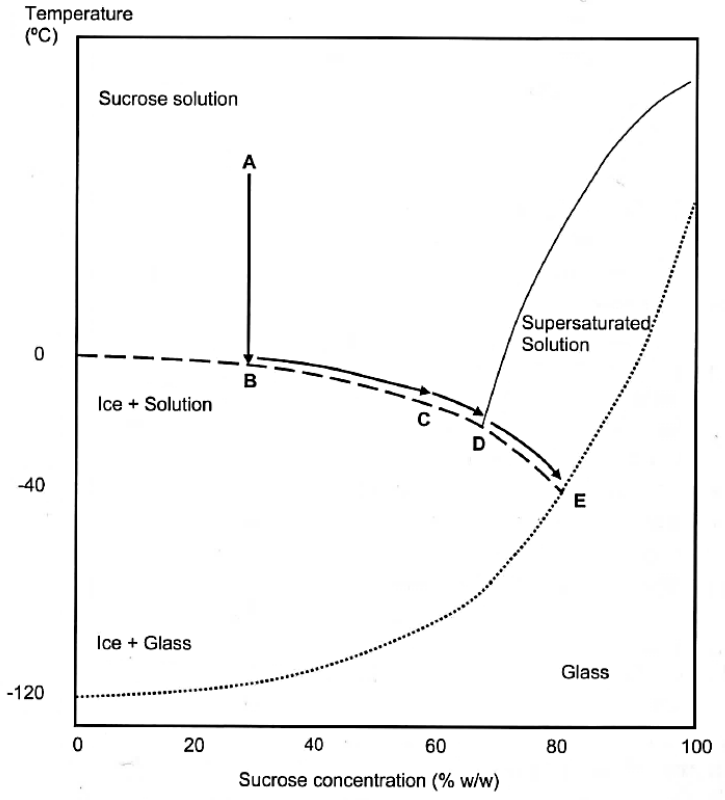

Figure 4.2 Phase diagram of an ice cream formulation (Clarke, 2012).

If, for a given formulation containing 30% sucrose (point A) is cooled, freezing point depression results in the formation of ice crystallisation at point B. As ice crystallisation proceeds, the continual increase in solute concentration due to the removal of water to form ice, further depresses the equilibrium freezing point in a manner that follows the curve along to point C. With further increasing solute concentration, ice formation ceases and supersaturation occurs without solute precipitation and the freezing point curve can continue past its eutectic point (the lowest point of solidification, point D) until its glass transition temperature (T_g) (point E) is reached. Such high solute concentrations with decreasing

temperature also results in an increase in serum phase viscosity. Eventually, high viscosity and low temperatures result in the reduction in molecular motion and diffusion kinetics resulting in an amorphous glassy state known as the glass transition temperature (T_g) (Clarke, 2012). A typical commercial ice cream formulation (although formulation dependent) has a T_g of around – 40°C whereby if stored above the T_g, little or no microstructural deterioration will occur. Upon warming of a product, ice will melt, diluting the serum phase and molecular mobility will be restored. Unfortunately, domestic freezers are only typically in the range of – 18°C to – 20°C so control over the matrix phase must be provided by stabilisers and not temperature.

A consequence of supersaturation due to freeze-concentration of the serum phase as a result of storage at low temperatures is the precipitation of sugars that may crystallise out of solution (Livney et al., 1995). This can occur for both sucrose and lactose. However, sucrose crystallisation is more common in water ices where sugar concentrations are high (Clarke, 2012). Lactose solubility is low and lactose crystallisation results in hard crystals that can result in the defect know as sandiness if the crystals reach a detectable size (above 15 μm). The crystals can be identified by their irregular or arrow head shape using optical microscopy. Increasing the milk solids-not-fat content, the source of lactose, has been identified as a promotor for lactose crystallisation. In addition, it has also been suggested that other foreign particulate materials such as nuts and fruit pieces provide a site for crystal nucleation. Plant fibre particles may have the ability to behave as crystal nucleation sites, thus this should be kept in mind. However, increasing the serum viscosity by the use of stabilisers aids in preventing the growth of lactose crystals (Hui, 2005).

The total solid content of a mix plays a critical role in governing the shape of the phase diagram. For mixes with more water and less total solids, the curve of the phase diagram will deviate due to differences in phase concentration and thus will also impact on the amount of ice in ice cream (Hartel, 1996). The freezing point of a mix is also suggested to be lowered by higher

concentrations of lactose, sugar and salts and is suggested to not be affected by fat, protein or stabilisers (Goff & Hartel, 2013).

4.1.1.1 Serum drainage

Changes in air cell structure as a result of long or abusive storage conditions and the dilution of the serum phase from melting ice during inappropriate temperature storage can result in drainage of the fluid phase (Figure 4.3). This results in an accumulation of a sugary syrup at the bottom of an ice cream container. Gravitational forces can cause the fluid to flow downwards around the air cells at the same time as buoyancy forces cause the air cells to rise. This process is governed by the interfacial tension of the air cells and the viscosity of the fluid phase. Such a process is accelerated by increases in temperature. However, significant increases in the viscosity of the fluid phase and the degree of fat destabilisation can reduce the rate of drainage (Chang & Hartel, 2002a).

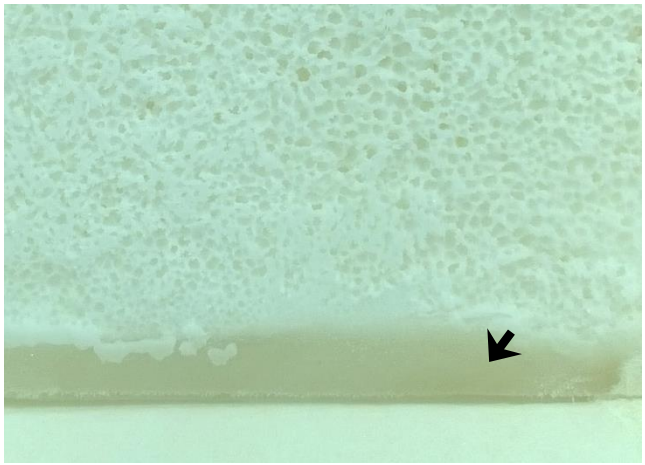

Figure 4.3 Serum phase drainage in ice cream as a result of heat shock.

4.1.2 Ice phase

The freezing of ice cream to create ice is typically accomplished in two steps: (i) dynamic freezing whereby the mix is frozen rapidly whilst being agitated to incorporate air and (ii) static freezing also known as hardening, whereby at low temperatures, to remove as much heat as quickly as possible, the remaining water available to form ice is frozen. The process of dynamic freezing using a scraped surface heat exchanger, aims to produce small ice crystals with a narrow range of mean ice crystal size distributions. Crystallisation occurs in four steps: supercooling, nucleation, growth and recrystallisation (Cook & Hartel, 2010). The freezer barrel (Figure 4.4) contains sharp, rotating blades known as dasher blades and is typically cooled by an evaporating refrigerant such as ammonia. As a cold ice cream mix enters the freezer barrel, dendrites of ice crystals nucleate on the barrel wall which is typically – 30°C as a result of super cooling promoting ice nucleation (Clarke, 2012). As the dasher blades rotate they scrape off the ice crystals that have formed on the barrel wall distributing them into the bulk of the mix where they may either melt during the early stages of freezing, cooling the mix or ripen as the temperature is sufficiently reduced. The rate of ice nucleation is of significant importance as it determines the number of ice crystals in the finished product and thus the ice crystal size distribution. Constant removal of the dendrites formed on the freezer barrel is key to a small ice crystal size distribution. However, with rapid rotation of the scraper blades, frictional energy from the blades can be dissipated to the mix causing it to warm and ice crystals to melt (Russel et al., 1999b).

There are several ways in which nucleation can occur; primary and secondary ice nucleation. Primary nucleation describes the spontaneous formation of a nucleus from solution and is underpinned by the amount of undercooling at the barrel wall (Russel et al., 1999b). Secondary nucleation is the formation of a nucleus from a pre-existing crystal or crystal fragment. However, within industrial applications secondary nucleation is the most common type

(McCabe et al., 1993). The rate of nucleation can be increased by increasing the temperature difference between the barrel wall and the bulk solution. However, if the temperature becomes too low within the bulk phase, the reduction of molecular mobility prevents the rate of ice nucleation (Schwartzberg, 1990). At temperatures below the glass transition temperature (T_g) ice nucleation can no longer occur. The rate of cooling also has a large impact on nucleation as rapid freezing to below the glass transition temperature prevents the onset of crystallisation (Hartel, 1996).

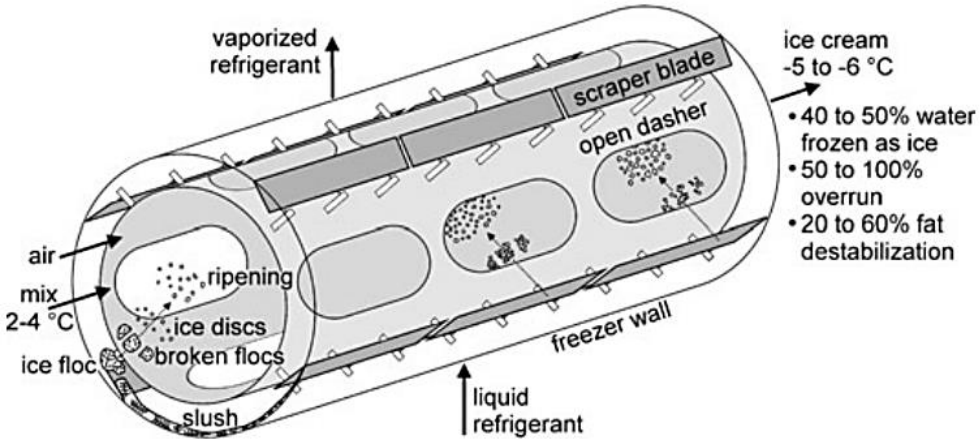

Figure 4.4 Schematic diagram of the process of ice crystallisation within a freezer barrel in a scraped surface freezer (Cook & Hartel, 2010).

An ice cream mix will typically exit the freezer between – 5°C and – 8°C (Eisner et al., 2005). The achieved exit temperature is a play-off between the increasing viscosity of the mix with freezing and the energy input required to rotate the dasher blades. Thus achieving lower exit temperatures can be difficult. However, low temperature extrusion can be utilised to overcome this self-limiting nature of scraped surface freezers (Clarke, 2012).

The maximum number of ice crystals is determined by dynamic freezing as ice nucleation can no longer occur during static cooling due to reduced rates of heat transfer. Static cooling of the

product is achieved using air with heat transfer being limited to conduction through the product (Hartel, 1996). During the hardening step the remaining ice forms by growth onto existing ice crystals (propagation) or recrystallisation (coarsening) (Goff & Hartel, 2013). Industrial processes use a hardening tunnel with air temperatures typically between – 30°C and – 45°C to remove heat as quickly as possible, preventing the degree of coarsening. Russel et al. (1999b) identified a reduction in the total number of ice crystals during static cooling as recrystallisation occurs simultaneously with ice growth.

During hardening an additional 23 – 57% of ice may be formed (Goff & Hartel, 2013). The amount of ice present in a formulation (at a given temperature) is dependent on the ice curve (Figure 4.5) which can be determined via the phase diagram. This is primarily influenced by the sugar and total solid content of the formulations. A 20% sucrose mix formulation has a freezing point of – 1.5°C as a result of freezing point depression. At - 5°C the ice cream contains around 35% ice by weight as it exits the freezer. After hardening, at a typical storage temperature of – 18 °C, it contains around 54% ice (Clarke, 2012).

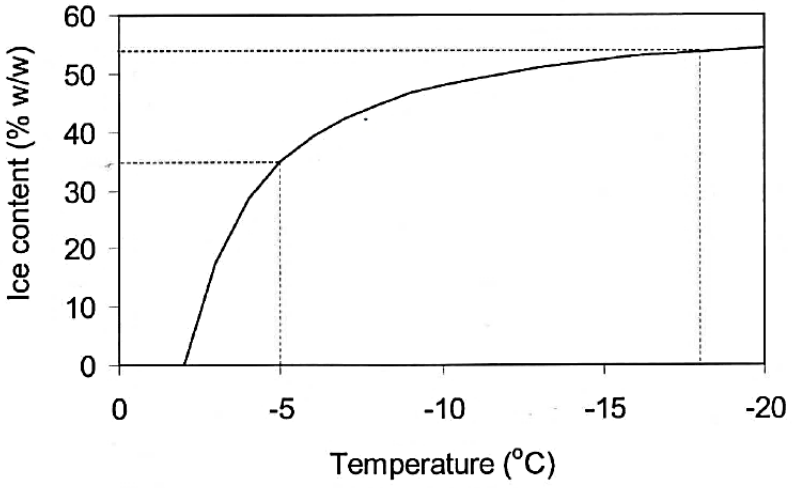

Figure 4.5 Ice content in an ice cream formulation containing 20% (w/w) sucrose as a function of temperature (Clarke, 2012).

4.1.2.1 Ice recrystallisation

Ice recrystallisation or ice coarsening is one of the most important quality deteriorations that can occur within ice cream. Ice recrystallisation is the change of shape and size of ice crystals and a decrease in the total number of ice crystals. This leads to an increase in the mean diameter of ice crystal in addition to a broadening in the distribution of sizes (Goff & Hartel, 2013). However, the amount of ice will remain constant at constant temperature as depicted by the ice curve (Clarke, 2012). There are three mechanisms by which recrystallisation can occur:

1. **Accretion**

   This is the tendency of ice crystals in close proximity to fuse together (Figure 4.6). Such a mechanism first occurs in the formation of a neck between adjacent crystals. Subsequent isomass rounding will occur to achieve the most compact, rounded structure (Donehowe & Hartel, 1996).

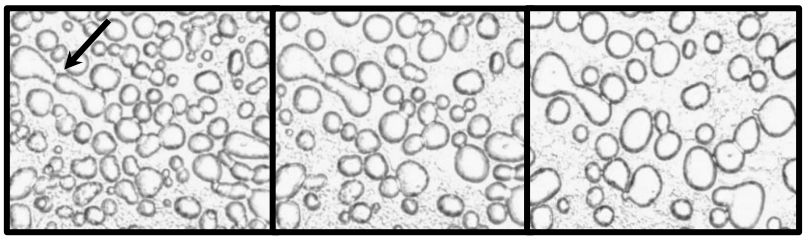

Figure 4.6 Accretion recrystallisation.

2. **Migratory and Ostwald ripening**

   Migratory and Oswald ripening is the tendency of larger crystals growing at the expense of small crystals (Figure 4.7). Migratory recrystallisation is enhanced by fluctuating freezer temperatures (heat shock) inducing a melt-refreeze behaviour due to fluctuations in ice content (Flores & Goff, 1999a). Oswald ripening refers to the

migratory recrystallisation that occurs at constant freezer temperature due to differences in surface energy between crystals involving a process of melt-diffuse-regrow (Adapa et al., 2000b). As demonstrated in Figure 4.7, the first image frame shows mixed distributions of ice crystal sizes. As time passes with constant temperature, smaller crystals disappear and larger crystals grow.

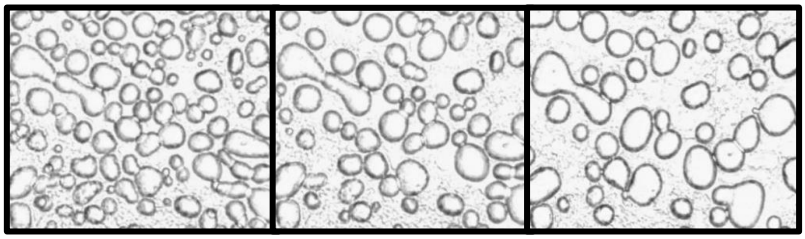

Figure 4.7 Migratory or Ostwald recrystallisation.

3. Isomass rounding

The 'rounding off' effect (Figure 4.8) is the change in surface or internal structure so that ice crystals of irregular shape with a large surface to volume ratio become more compact in structure (Donhowe & Hartel, 1996).

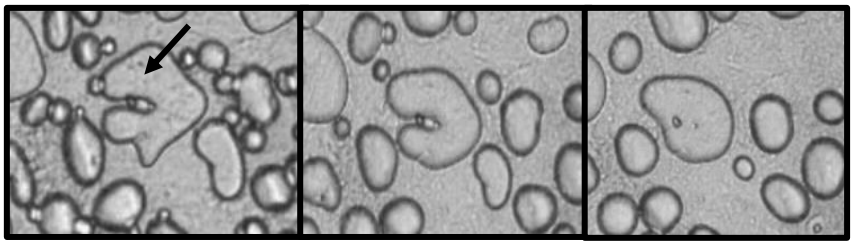

Figure 4.8 Isomass rounding recrystallisation.

Although the ice crystals formed during dynamic freezing are unstable and will undergo recrystallisation, the extent of which depends on how effectively the system has been stabilised (Hui, 2005). However, the rate of change has been identified as temperature dependent.

Donhowe & Hartel (1996) found that the rate of recrystallisation is extremely rapid at -5°C whilst Goff & Hartel (2013) reported that ice cream stored below -25°C exhibits only slight textural deteriorations. Fluctuating temperatures also contribute to increasing the rate of recrystallisation (Adapa et al., 2000b).

Changes in ice structure can also take place via water vapour. If a proportion of ice cream has been consumed from a tub and the remaining product is stored frozen for long periods of time, a layer of frost may develop on the inside of the container. This is as a result of ice sublimation to the air space within the container, which freezes forming frost (Clarke, 2012).

4.1.3 Fat phase

During dynamic freezing the combined actions of air incorporation and shearing from the scraper blades results in the partial coalescence of fat droplets resulting in the formation of a destabilised fat network (Goff & Hartel, 2013). Although, dependent on the process conditions and the emulsifier type, the structure of the fat has a large impact on the stability of ice cream (Eisner et al., 2005). Fat destabilisation also contributes to a dry and stiff product as it exits the freezer (Sung & Goff, 2010).

Homogenisation reduces the fat droplet size to around <1 µm. In the subsequent aging step, the mix cools, the fat crystallises and the newly formed interfaces become populated. Emulsifiers such a mono- or di- glycerides are added to the formulation to promote droplet coalescence as although they are called emulsifiers, their function in ice cream is to de-stabilise the fat (Clarke, 2012). After homogenisation, proteins (caseins, whey proteins) absorb to the newly formed interfaces but are partially displaced during aging by the small molecular weight surfactants which absorb to the surface. This exchange at the interface is due to the emulsifier's ability to lower the interfacial tension but produces a thinner membrane that has a reduced stability

against shearing (Goff & Hartel, 2013). Bolliger et al. (2000c) have identified a direct relationship between the desorbed protein content from displacement by emulsifiers and the extent of fat destabilisation. If the interfaces remained protein stabilised the fat droplets would be less susceptible to coalescence. The choice of fat type also has a large impact on the ability to obtain a destabilised fat network. If all the fat is liquid, droplets fully coalesce forming large droplets. In addition, if all the fat is solid, little coalescence can occur (Sung & Goff, 2010).

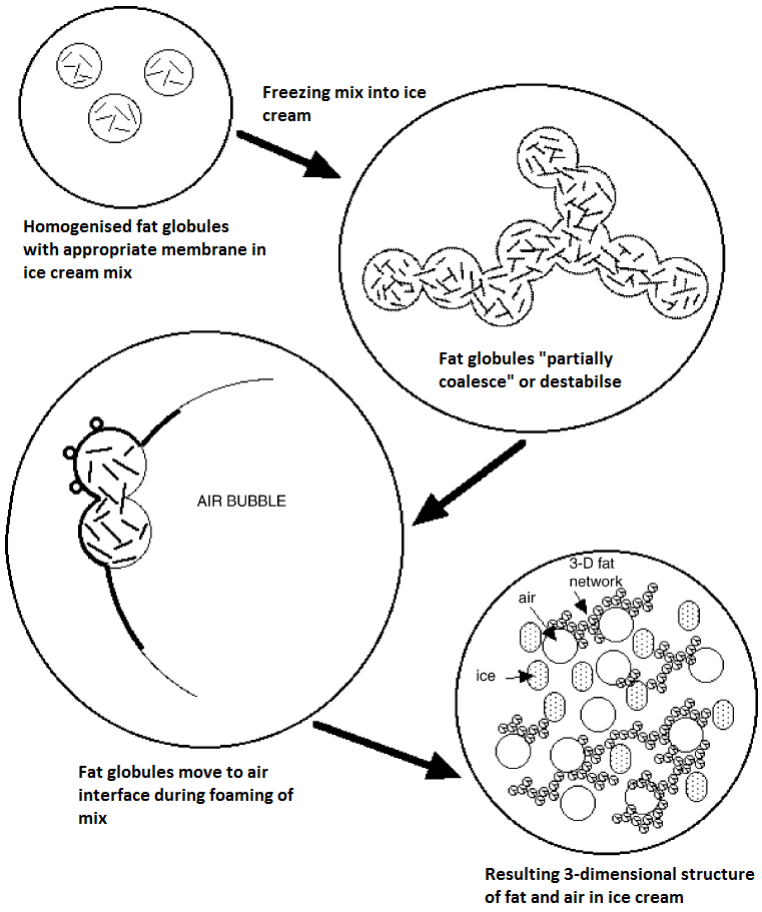

Figure 4.9 The development of fat structure in ice cream (University of Guelph, 2017).

The development of the fat structure during ice cream manufacture is shown in Figure 4.9. During the freezing of ice cream, the emulsion undergoes partial coalescence due to the partial crystallinity of the fat, preventing full coalescence (Sung & Goff, 2010). This results in the

formation of clusters of fat globules (5-10 μm) as well as discrete droplets which move to the air-matrix interface during foaming, forming an internal network that entraps air cells as well as providing stability to the continuous phase (Goff & Jordan, 1989; BahramParvar & Goff 2013a). The resulting air bubble surface is stabilised by both a protein layer and absorbed discrete fat globules and partially coalesced fat aggregates which help to provide stability against bubble coalescence (Goff et al., 1999b). However, the degree of protein interaction with the fat and air can be influenced by the protein selected and the degree of denaturation, as partly denatured proteins are more hydrophobic with greater interfacial contact resulting in better foaming properties (Eisner et al., 2005). Excessive fat destabilisation can result in churning where fat aggregates exceed 30 μm (Wildmoser & Windhab, 2001) and results in buttery or greasy sensory properties.

As previously outlined, the type of fat used has a large impact on fat structure. As consumers become increasingly more health conscious, challenges arise for the ice cream manufacturer to create the correct fat structure in reduced fat and non-dairy fat ice creams. In low fat formulations, the amount of coalesced fat network that can be formed is reduced, resulting in a compromise in both stability and sensory quality (Adapa et al., 2000a). Granger et al. (2005) investigated the use of non-dairy fats (coconut oil and palm oil) on their ability to form a destabilised fat network during dynamic freezing. They identified that palm oil was able to achieve larger destabilised fat aggregates than coconut oil which contributed to a reduction in the rate of meltdown.

4.1.3.1 Rate of ice cream meltdown

Although a variety of factors can influence the meltdown properties of ice cream, the structure of the fat has a large impact on the rate of melting as well as shape retention upon melting as a

destabilised fat network contributes to a slower rate of fluid drainage between the foam (Bolliger et al., 2000b). When an ice cream is exposed to warm temperatures, heat is transferred from the warm air surrounding the products into the ice cream to melt the ice crystals. Air cells help to act as an insulator reducing the rate of heat transfer. Initially, ice melts at the exterior of the product. The water dilutes the serum phase which drains downwards through the remaining structural elements to form drip. The development of a destabilised fat network within the matrix phase helps to reduce the rate of downward flow (Koxholt et al., 2001). In addition, viscosity enhancement of the serum phase via the use of stabilisers reduces the rate of downward flow (Goff & Hartel, 2013).

4.1.4 Air phase

Small air cells in ice cream are desirable and are an indication of a good quality ice cream as a more stable foam is produced (Halim et al., 2014). The amount of air incorporated into ice cream is termed as the percentage overrun. Commercial ice creams typically have an overrun of between 100 – 120% (Goff & Hartel, 2013). Air is incorporated into ice cream at the point of dynamic freezing by either air injection or whipping effect from the dasher blades (Eisner et al., 2005). Initially, large bubbles are introduced into the mix but the shearing effect from the blades breaks them down into numerous smaller bubbles. It is generally understood that as the applied shear stress increases, bubble size is reduced. The residence time within the freezer barrel also has a large contribution to the achieved overrun (Clarke, 2012).

The viscosity of a mix and the quantity of fat and protein has a large impact on the ability to incorporate air. If the mix is not viscous enough, the film between the air bubbles rapidly drains and the bubbles coalesce resulting in a lower overrun. However, with increasing viscosity the rate of air incorporation decreases (Javidi et al., 2016). Generally, as viscosity increases, the

resistance to melting and smoothness perception increases, but the rate of whipping decreases (Goff & Hartel, 2013). In addition, at low protein and fat contents it can be difficult to achieve overruns above 60% (Clarke, 2012).

The structure and size distribution of air cells have been reported to be one of the main factors influencing melting rate, shape retention on meltdown and the rheological properties of the melting product, which has been correlated to the perception of creaminess (BahramParvar & Mazaheri Tehrani, 2011). Higher overruns have also been reported to result in greater fat destabilisation (Sofjan & Hartel, 2014).

4.1.4.1 Shrinkage

Shrinkage can be found in hardened ice cream and is a result of changes in air cell structure and is considered a texture defect (Dubey & White, 1997; BahramParvar & Mazaheri Tehrani, 2011). It manifests itself as a reduction in the volume of ice cream in a container as a result of bubble contraction leading to coalescence and eventually collapse (Figure 4.10). The appearance of ice cream pulling away from the top and/or sides of a container are also a result of shrinkage (Goff & Hartel, 2013). Fluctuating temperatures accentuate the defect. As the temperature is decreased the volume of the air bubble is reduced. On warming, the loss of ice crystals and the increase in air cell volume can result in bubble coalescence and air cell channelling. Eventually, excessive channelling can result in the loss of the foam structure resulting in shrinkage. However, the most common cause of shrinkage is as a result of the exposure to changes in altitude (Dubey & White, 1997).

Figure 4.10 Shrinkage in hardened ice cream (Goff & Hartel, 2013).

There are three primary mechanisms by which air cells change during storage. These are disproportionation, coalescence and drainage. Chang & Hartel (2002b) reported that these three mechanisms depend on both the temperature during storage and the formulation, such as the emulsifiers and stabilisers used.

Disproportionation develops due to differences in Laplace pressure between air cells resulting in the diffusion of gas from one air cell to another whereby larger air cells grow at the expense of smaller ones (Sofjan & Hartel, 2004). Chang & Hartel (2002a) reported that disproportionation of air cells can be inhibited by addition of emulsifiers or stabilisers. Coalescence of air cells can occur between bubbles in close proximity whereby two air cells form one larger bubble. Counteracting gravitational and buoyancy forces can cause the fluid matrix to flow downwards in the process of serum drainage as previously discussed whilst large air cells begin to rise upwards. Chang & Hartel (2002) reported that control over changes in air cells can be provided by increasing the viscosity of the serum phase and thus the creation of a thick film between the surfaces of the air cells. In addition, reducing the air cell size distribution

reduces the pressure difference between air cells, thus disproportionation is reduced. However, the growth of ice crystals during recrystallisation can cause air cells to rupture. In addition, coalescence may be promoted by the presence of solid particles which can lead to a loss of stability (Dickinson, 1992; Dickinson & McClements, 1995).

4.1.5 Aims of the chapter

The literature clearly outlines the importance of creating the correct microstructure in ice cream on both its stability and sensory experience. As outlined, the formulation of a mix has a large influence on the microstructure formed and the stability of the microstructure to heat shock. With this in mind, the main aims of the research undertaken as part of this chapter are as follows:

1. To assess the impact of plant fibre on the physical properties of an ice mix and the impact this has on ice cream microstructure.
2. To assess the effectiveness of the different plant fibres to control the microstructural changes that occur with heat shock in comparison to a Standard commercial formulation.

4.2 Materials and methods

4.2.1 Ice cream manufacture

Ice cream mixes were prepared in 30 L batches. Eight different ice cream formulations were produced at pilot scale containing the four plant fibres selected (AQ+ CF, FibreGel CF, Psyllium, Sugar Cane). Formulations were prepared with just plant fibre and as a stabiliser blend with guar gum. Ice cream formulations consisted of 5% fat, 6% milk-solids-not-fat, 21% sugars, 0.2% vanilla flavouring, 0.4% mono- and di- glycerides with 0.6% plant fibre or as a stabiliser blend with 0.3% guar gum (total stabiliser concentration 0.9%). A commercial Standard formulation for comparison was also used which consisted of 5% fat, 6% milk solids-not-fat, 21% sugars, 0.2% vanilla flavouring, 0.4% mono- and di- glycerides and a 0.32% hydrocolloid stabiliser blend (Locust bean gum, Guar gum, Xanthan gum).

Each ice cream pre-mix was pasteurised at 87°C for 25 minutes. After pasteurisation, homogenisation was performed using a 2-stage homogeniser (first stage 150 bar, second stage 30 bar), cooled to 5°C and aged for 24 hours at 5°C. Freezing of the ice cream mixes was performed in a continuous freezer (MF75, WCB Ice Cream, New Jersey, USA). The ice cream was drawn and filled into 500 mL cardboard containers before being immediately transferred to a hardening room at – 40°C for 6 hours.

An overrun of 120% ± 1% was used for all formulations. The overrun was calculated using a 200 mL cup using Equation 4.1:

$$\%\ \text{overrun} = \frac{\text{weight of unit volume of mix} - \text{weight of unit volume of foam}}{\text{weight of unit volume of foam}} \quad \textit{Equation 4.1}$$

The exit temperature of ice creams was recorded by means of a thermocouple integrated in the freezer barrel door. After hardening the samples were stored at -25°C.

4.2.2 Temperature cycling

Hardened ice cream samples were placed into controlled temperature cycling chambers for two weeks. Samples were subjected to a programmed heating and cooling cycle during which the freezer was kept at -15°C for 12 hours, warmed to -5°C in 12 hours, held for 12 hours before being cooled to -15°C in 12 hours in a 48 hour heating-cooling cycle. After removal, samples were stores at -25°C until assessment. According to the ice curves obtained from Unilever, at -20°C blocks will contain a 50% ice content. At -5°C there will be a 30% ice content.

4.2.3 Mix viscosity

The steady shear rheology of aged ice cream pre-mixes were measure using the method of BahramParvar & Goff (2013) with some modification. Measurements were performed at 5°C on a MCR 301 rotational rheometer (Anton Paar, Graz, Germany) using a cone and plate geometry (diameter 50 mm, 2° angle). Samples were loaded onto the lower plate and allowed to equilibrate (10 minutes). A pre-shear step of 0.2 s^{-1} for 2 minutes was applied to samples to remove thixotropy before being subjected to shearing of 20 to 200 s^{-1}. Apparent viscosities were determined at 50 s^{-1} (Kokini viscosity) which is understood to be the sensing shear rate during oral processing of non-Newtonian fluids (Sharma & Sherman,1973; Akhtar et al., 2006). The flow behaviour of ice cream mixes were examined using the Ostwald-de Waale model as shown in Equation 4.2:

$$\tau = k\,\gamma^{n} \qquad \textit{Equation 4.2}$$

Where τ is the shear stress (Pa), k is the consistency coefficient ($Pa.s^n$) and is the shear stress at shear rate of 1.0 s^{-1}, γ is the shear rate (s^{-1}) and n is the flow behaviour index (dimensionless). The parameters k and n were determined by plotting the log of shear rate against shear stress.

The flow behaviour index *(n*) was determined using the slope of the line and linear regression allowed the identification of the y-axis intercept revealing the consistency coefficient (*k*). All measurements were performed in triplicate.

4.2.4 Ice crystal size determination

Ice cream samples were stores at -25°C until assessment. Glass slides, utensils, mounting medium and solvents were precooled to -30°C prior to use. Rectangular samples (approximate length: 4 cm, width: 1 cm, height: 1 cm) were taken from the center proportion of ice cream blocks and placed into dry ice for 30 minutes to harden. Hardened samples were placed onto a mounting medium in a microtome chamber tempered to -40°C (Cryo-microtome OTF 5000, Bright Instruments, UK). After tempering in the chamber for 30 minutes, 100 µm slices were taken and placed onto glass slides. Drops of iso-amyl alcohol were added to the sample to disperse ice crystals from the matrix phase before being covered with a glass slide. Tweezers were used to press down and disperse the sample to reduce overlap of ice crystals. Prepared slides were placed into a cold stage set to -20°C (Linkam, UK). Images were acquired using a Leica DMLM light microscope fitted with a Leica DFC 420 C imaging camera (Leica Microsystems, Heerbrugg, Germany). A Polarised lens was used to allow for easier identification of individual ice crystals. Several different fields were imaged to obtain a minimum of 500 crystals per sample and images were analysed by manually tracing the perimeter of ice crystals with a computer mouse using image processing softwear (Unilever in-house sizing software) to obtain a mean diameter (µm). Ice crystal sizes were obtained for both fresh and temperature cycled samples. The rate of ice crystal growth was calculated using the following equation:

$$\% \text{ Rate of growth} = ((r_x - r)/r) \times 100 \qquad \textit{Equation 4.3}$$

Where r is the mean crystal diameter (μm) of fresh samples and r_x is the mean crystal diameter (μm) of temperature cycled samples.

4.2.5 Recrystallisation rate

Samples of aged ice cream mix were placed on glass slides, covered with a cover slip and placed in a cold stage (Linkam, UK). Samples were cooled to -25°C at 20°C/min, held for 10 minutes, warmed to -5°C at 1°C/min and held for 120 minutes. Samples were imaged using a Leitz Diaplan microscope (Leica, Heidelberg, Germany) and a Pixelink PL-A600 (Ottawa, Canada) recording camera at a magnification of 20x. Ice crystal sizes where determined by manually outlining ice crystals using image processing softwear (Unilever in-house sizing software) to obtain a mean diameter (μm). Imaging began when samples reached -5°C (t = 0 min), however sizing of ice crystals was performed after t = 20 minutes due to image quality. The recrystallisation rate of formulations was determined using the Ostwald ripening principal (Equation 4.4) which can be applied to isothermal conditions to obtain the recrystallisation rate (Hagiwara et al., 2006):

$$r^3 = r^3_0 + kt \qquad \textit{Equation 4.4}$$

Where r is the mean crystal size (μm), r_0 is the mean crystal size at t = 0 (μm) and k is the recrystallization rate (μm^3/hour). The recrystallisation rate (k) is evaluated as the slope of the cube of the mean diameter versus time. All analysis was performed in duplicate.

4.2.6 Cryo-Scanning electron microscopy (SEM)

Sections of ice cream taken from the centre portion of a block were cooled in dry ice for 30 minutes (- 80°C). Samples (8 mm x 8 mm x 10 mm) were prepared using a pre-cooled scalpel

and mounted onto brass stubs before being immersed in liquid nitrogen (-196°C) and immediately transferred into a cryogenic preparation chamber (Gatan Alto 2500, Joel, Massachusetts, USA) and warmed to –90°C. Samples were fractured and etched. Samples were cooled to –110°C and sputter-coated with 2 nm Platinum before being transferred to the cold stage (–126°C) of the cryo-SEM (7 600 FESEM, Joel, Massachusetts, USA) and imaged using various magnification.

4.2.8 Confocal laser scanning microscopy (CLSM)

Images were obtained using a Ziess LSM780 microscope system (Ziess, Germany). Samples of aged ice cream mix or thawed ice cream were stained using the relevant stain; Calcofluor-white and/or Nile Blue, loaded onto glass slides with 50 μm spacers and covered with a glass cover slip and imaged using the appropriate magnification.

4.2.8.1 Assessment of fibre behaviour

Aliquots of 1 mL aged ice cream mix were stained with 0.1% (v/v) Calcofluor-white solution (Sigma-Aldrich, Germany) and excited using a 405 nm diode laser (emission range 395-493 nm). To assess freeze-thaw recovery, samples were placed in a cold stage at 5°C (Linkam, UK), cooled to -25°C at 20°C/min, warmed to -5°C at 5°C/min, held for 30 minutes to obtain large ice crystals via recrystallisation before being warmed to 5°C at 20°C/min. Images were obtained using a 20x lens.

4.2.8.2. Assessment of fat and protein

Samples of ice cream (30 g) were thawed in refrigeration overnight (4°C). Thawed samples were carefully stirred and aliquots of 1 mL were stained with 0.01% (v/v) Nile blue solution (Sigma-Aldrich, Germany). Samples were excited using a 633 nm laser (emission range 638-759 nm) to allow identification of proteins and excited using a 488 nm laser (emission range 498-553 nm) to allow identification of fat. Images were obtained using 40x lens with oil immersion.

4.2.9 Spin-spin relaxation NMR

Spin-spin relaxation times (T_2) of aged ice cream mixes were obtained on a R4 Benchtop NMR System (Advanced Magnetic Resonance, UK) operating at 21 MHz using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. The decay was then fitted to a distributed exponential function using Deeper softwear (Advanced FMagnetic Resonance, UK). To assess the effect of freeze-thaw cycling on T_2 values, samples were assessed at 20°C, cooled to -20°C in a water bath containing glycol (1 : 1 glycol : water) for 20 minutes and defrosted in a 20°C water bath for 20 minutes. Samples were subjected to two freeze-thaw cycles and tested in triplicate. All measurements were performed at 20°C.

4.2.10 Fat particle size analysis

The particle size distributions of fat globules and destabilised fat in samples was determined using light scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, UK). Measurements were carried out on both the ice cream pre-mix and defrosted hardened ice cream. Air bubbles were removed from defrosted ice cream using ultra-sonication. Dispersions

of 10% (v/v) of sample were prepared by dispersing samples in SDS-Urea dissociating medium (sodium dodecyl sulfate) to avoid association of fat droplets caused by protein interactions. Samples were then diluted (approx. 1:1000) in the sample chamber using MiliQ water. The particle size (μm) was generated. All measurements were performed in triplicate and averages were generated.

4.2.11 Meltdown assessment

Samples for meltdown tests (500 mL) were removed from the container, weighed and placed on a wire mesh (hole size 0.5 x 0.5 mm) in a temperature controlled cabinet (23°C) above a scale. The drip weight was recorded every 1 minute for 4 hours and plotted against time. The melting rate (% min^{-1}) was calculated from the slope of each meltdown chart. Melting onset time was determined as the time of first drip. All measurements were performed in triplicate.

4.2.12 Assessment of the glass transition temperature (T_g)

Differential Scanning Calorimetry (DSC 823e Mettler Toledo Systems, Leicester, UK) was used to determine the T_g of the stabilised aged ice cream mixes. Ice cream samples of 40 μm were weighed into sealed aluminium pans. The experimental program consisted of cooling the samples from 20°C to -60°C at 10°C/min, holding at -60°C for 1 minute, warming to -40°C at 10°C/min and holding at this temperature for 30 minutes to allow annealing of the samples. An annealing step was performed to promote optimum ice formation and remove non-equilibrium recrystallisation which can occur when rapidly cooling a mix below its T_g. The samples were then cooled to -60°C at 10°C/min, held for 5 minutes and then warmed to 20°C at 5°C/min.

4.2.13 Statistical analysis

Analysis of variance was performed using XLStat-Pro Analysis Software (Addinsoft, France). A significance value of 5% was used for all analysis.

4.3 Results and discussion

4.3.1 Serum phase

4.3.1.1 Rheological properties of ice cream pre-mixes

The apparent viscosity and rheological properties of aged ice cream mixes were evaluated to identify the impact of different fibres and stabiliser blends with guar gum on mix properties. The viscosity and rheological properties of an aged ice cream mix is considered a key attribute as it has an impact on ice cream processing (Dogan et al., 2013), as well as the body and texture of the finished product (BahramParvar & Mazaheri Tehrani, 2011). Thus, characterisation of the rheological properties of the mix is important for correlating physical parameters with sensory evaluation. Overuse of stabilisers and poor shear thinning properties of an ice cream mix has also been suggested to induce a gummy mouthfeel and poor mouth clearing properties (BahramParvar & Mazaheri Tehrani, 2011; Goff & Hartel, 2013).

Figures 4.11 and 4.12 illustrate the apparent viscosity as a function of shear rate of aged ice cream mixes prepared with different plant fibres and as stabiliser blends with guar gum, respectively. Table 4.1 reports the rheological parameters of the ice cream mixes as generated using the Ostwald de Waele model. This model was found to be an adequate model for describing the rheological properties as the R^2 values were greater than 0.984 (Dogan et al., 2013).

Fibre type was found to have a significant impact on both apparent viscosity at 50 s^{-1} (Kokini viscosity) and the consistency coefficient (*k*) (Table 4.1). Higher values of *k* indicate a higher viscous nature at zero shear because of the increase in apparent viscosity of the mix (Dogan et al., 2013). In formulations containing only plant fibre (Figure 4.11), AQ+ CF created the highest apparent viscosity whilst Psyllium created the lowest. This is in agreement with the rheological measurements undertaken in Chapter 3 as AQ+ CF had the highest viscosity in

suspensions whilst Psyllium had the lowest, indicating that fibre type has a large influence on the overall viscosity of a pre-mix.

The viscosity of a mix can have a large influence over the rate of ice cream meltdown. It is suggested that higher zero shear viscosities (*k*) correlate with a reduced rate of meltdown (Muse & Hartel, 2004). However, in general, the differences in apparent viscosity and *k* seen for fibre only stabilised mixes (Figure 4.11) and the Standard formulation were narrow and thus differences in meltdown behaviour should be as a result of the fibre physical properties and not the viscosity differences between the mixes. AQ+ CF and the Standard were also identified to be slightly less shear thinning as larger shear forces are required to achieve sufficient molecular alignment for flow (Marcotte et al., 2001). For the Standard, this will be due to the formation of an entangled biopolymer network.

The co-stabilisation of mixes with guar gum and plant fibre resulted in an increase in apparent viscosity and consistency coefficient as well as the flow behaviour index (*n*) which is primarily as a result of an increase in total stabiliser concentration (0.6% w/w fibre only stabilised to 0.9% w/w co-stabilised). All formulations of co-stabilised mixes had greater apparent viscosities than the Standard formulation (Figure 4.12), which might be suggestive of over stabilisation (Goff & Hartel, 2013). The stabiliser blend of AQ+ CF/Guar had the highest apparent viscosity and *k*. FibreGel CF had the least change in apparent viscosity on blending with guar. This may be suggestive of immiscibility (Capron et al., 2001; McKenna, 2004) and will be investigated later on in this chapter.

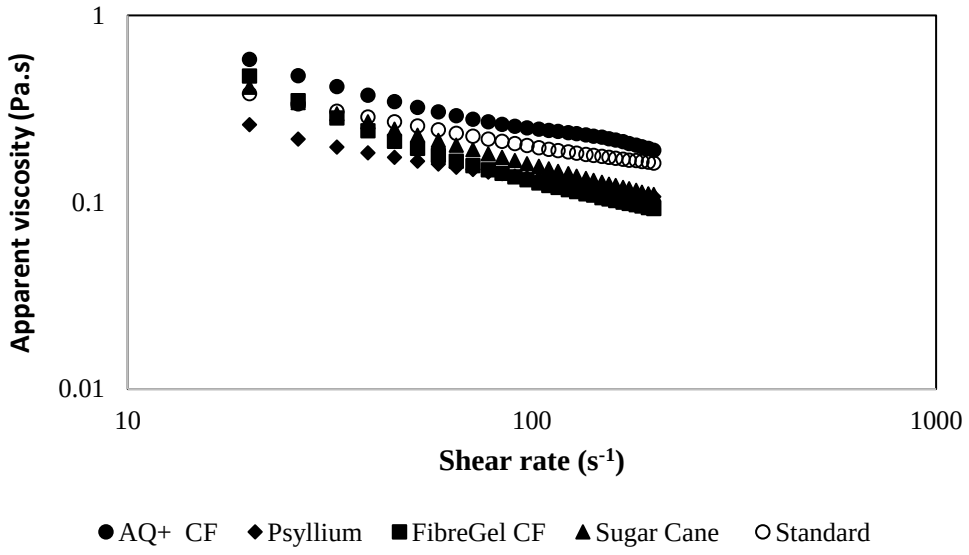

Figure 4.11 Apparent viscosity of aged ice cream mixes containing different plant fibres (0.6% w/w) at 5°C.

The flow behaviour index (*n*) represents the shear thinning behaviour of a fluid where $n < 1$ indicates shear-thinning behaviour, *n* equal to 1 indicates Newtonian behaviour and $n > 1$ indicates dilatant behaviour (Rao, 2014). Values of *n* ranging from 0.05 and 0.48 were observed, indicating all ice cream mixes demonstrate non-Newtonian shear-thinning behaviour. Accordingly, as seen in Figures 4.11 and 4.12, apparent viscosity decreases with increasing shear rate. It has been suggested that a low *n* value provides good mouthfeel characteristics and improved ease of swallowing (Szczeniak & Farkas, 1962). FibreGel CF and FibreGel CF/Guar were found to be the most shear thinning. In all formulations, the addition of guar gum weakened the shear thinning behaviour as shown by the increase in the flow behaviour index. A decrease in shear thinning behaviour of a mix may contribute to a gummy mouthfeel (Goff & Hartel, 2013).

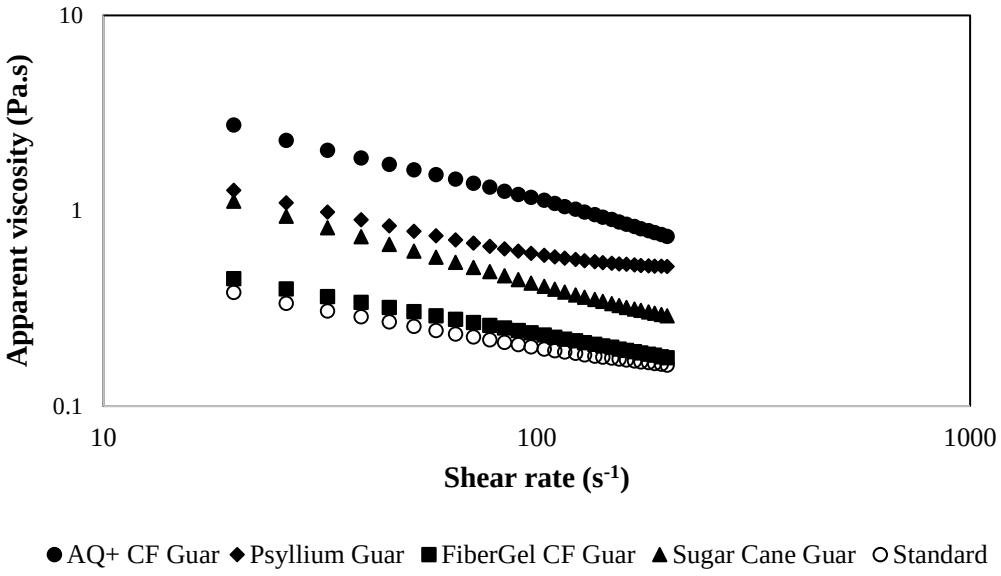

Figure 4.12 Apparent viscosity of aged ice cream mixes containing different plant fibres (0.6% w/w) with guar gum (0.3% w/w) at 5°C.

Studies by BahramParvar & Mazaheri Tehrani (2011) reported a correlation between viscosity enhancement of an ice cream mix using hydrocolloids and decreasing molecular mobility relating to control over ice recrystallisation. Thus, it may be expected that the high apparent viscosity of AQ+ CF/Guar would result in optimum molecular mobility reduction and control over ice recrystallisation.

Table 4.1 Rheological properties of aged ice cream mixes containing different types of fibre (0.6% w/w) with and without guar gum (0.3% w/w) as generated using the Ostwald de Waele model (5°C)

Stabiliser Blend	Kokini viscosity η (Pa.s)	Consistency coefficient *k* ($Pa.s^n$)	Flow behaviour index *n*	R^2 value
Standard	0.24 ± 0.011	7.35 ± 0.081	0.13 ± 0.001	0.997
AQ+ CF	0.36 ± 0.044	13.55 ± 1.65	0.15 ± 0.001	0.992
FibreGel CF	0.19 ± 0.002	7.56 ± 0.138	0.05 ± 0.001	0.994
Psyllium	0.17 ± 0.012	4.29 ± 0.402	0.09 ± 0.005	0.993
Sugar Cane	0.23 ± 0.008	8.29 ± 0.378	0.07 ± 0.009	0.986
AQ+ CF/Guar	1.67 ± 0.070	31.10 ± 3.144	0.48 ± 0.008	0.991
FibreGel CF/Guar	0.31 ± 0.004	8.37 ± 0.054	0.14 ± 0.003	0.988
Psyllium/Guar	0.79 ± 0.004	19.36 ± 1.755	0.40 ± 0.026	0.999
Sugar Cane/Guar	0.63 ± 0.017	18.07 ± 1.020	0.17 ± 0.070	0.986

± indicates the standard deviation.

4.3.1.2 Mix immiscibility

Other factors that can influence the rheological properties of a mix and the body and texture of an ice cream is immiscibility between milk proteins and stabilisers (Syrbe et al., 1998). However, such phase separation behaviour is suggested to be inhibited by the inclusion of κ-carrageenan within a mix typically used at 0.05% (Chandan et al., 2009). As an ice cream mix cools κ-carrageenan undergoes a conformational change from a coil to a helix at around 40°C. The helical form produces a weak-gel structure which can be easily broken down by shear. In

the quiescent state, the weak-gel is capable of holding dispersed particles in suspension, in particular casein micelles, preventing phase separation at a visual level (Goff & Hartel, 2013).

Assessment of the miscibility of the fat, protein and plant fibres in thawed ice cream is presented in Figure 4.13 and evidence of phase separation in all formulations can be observed. As can be seen in fibre only stabilised mixes, plant fibre is largely associated with the fat phase whilst a protein enriched phase forms the continuous phase. However, when mixes contain guar gum a change in structure can be seen. The dark areas devoid of fat, protein or fibre are assumed to be guar gum. In the presence of guar, the plant fibres become associated with the fat and protein phase with the development of a discrete guar gum enriched phase.

Within biopolymer mixtures of polysaccharide and protein incompatibility can result in phase separation. Two phase separation phenomena exist; segregative or associative phase separation. Segregative phase separation results in the formation of two different phases and is due to a thermodynamic incompatibility between biopolymers. It mainly exists where a protein is in the presence of a neutral polysaccharide or an anionic polysaccharide with a charge similar to that of the protein (Doublier et al., 2000). In associative phase separation, both biopolymers are enriched in one of the separating phases with the other phase containing mostly solvent. This behaviour usually occurs between oppositely charged biopolymers that interact. Temperature, pH, ionic strength and concentration are all important factors over phase separation (Fang et al., 2006).

Guar gum, a galactomannan, is a neutral seed polysaccharide most commonly used as a thickening agent in ice cream. Guar gum has a linear backbone of β- 1,4 linked mannose residues, substituted with galactose residues, with a mannose to galactose ratio of 2:1. Incompatibility between casein micelles and guar gum have been reported (Bourriot et al., 1999) as well as for whey proteins and guar gum (Ercelebi & Ibanoğlu, 2007) suggesting that

the phase separation seen in Figure 4.13 in the presence of guar gum is due to incompatibility between guar gum and milk proteins.

Although phase separation between pectin and whey proteins has been reported (Leng et al., 2010), not all the fibres under assessment contain pectin and the literature offers little explanation as to the driver of the phase separation observed between insoluble fibre and protein as such a phenomena has not been reported. Phase separation has been reported for sulphuric acid prepared cellulose nanocrystals in the presence of macromolecules. Sulphuric acid treatment of wood pulp produced charged rod-like polyelectrolytes that phase separated from macromolecules due to similar charges (segregative phase separation) (Beck, 2007). However, no information on the phase separation behaviour of natural plant fibres in the presence of proteins is outlined. An explanatory phenomena for the phase separation observed between fibres and protein in Figure 4.13 is segregative phase separation due to the plant fibres having a neutral charge. Although this theory cannot be confirmed because the surface charge of the plant fibres was not examined, which can be assessed by measuring the zeta-potential of the fibres, the arabinoxylan from Psyllium have been identified as a neutral polysaccharide (Fischer et al., 2004).

It is reported in the literature that in the case where spherical particles are involved such as casein micelles, phase separation has been suggested to arise form a depletion-flocculation phenomena (Doublier et al., 2000). This phenomena has only been demonstrated in the case of dispersions of solid particles (latex particles, silica) in the presence of polysaccharides and involves a depletion of the concentration of a polymer between the surfaces of the particles when they are in close proximity. This results in a volume exclusion especially when the polysaccharide is of high molecular weight and is unable to fit between the spaces between the particles and thus these spaces remain solvent enriched (Bourriot et al., 1999). Thus, it may be

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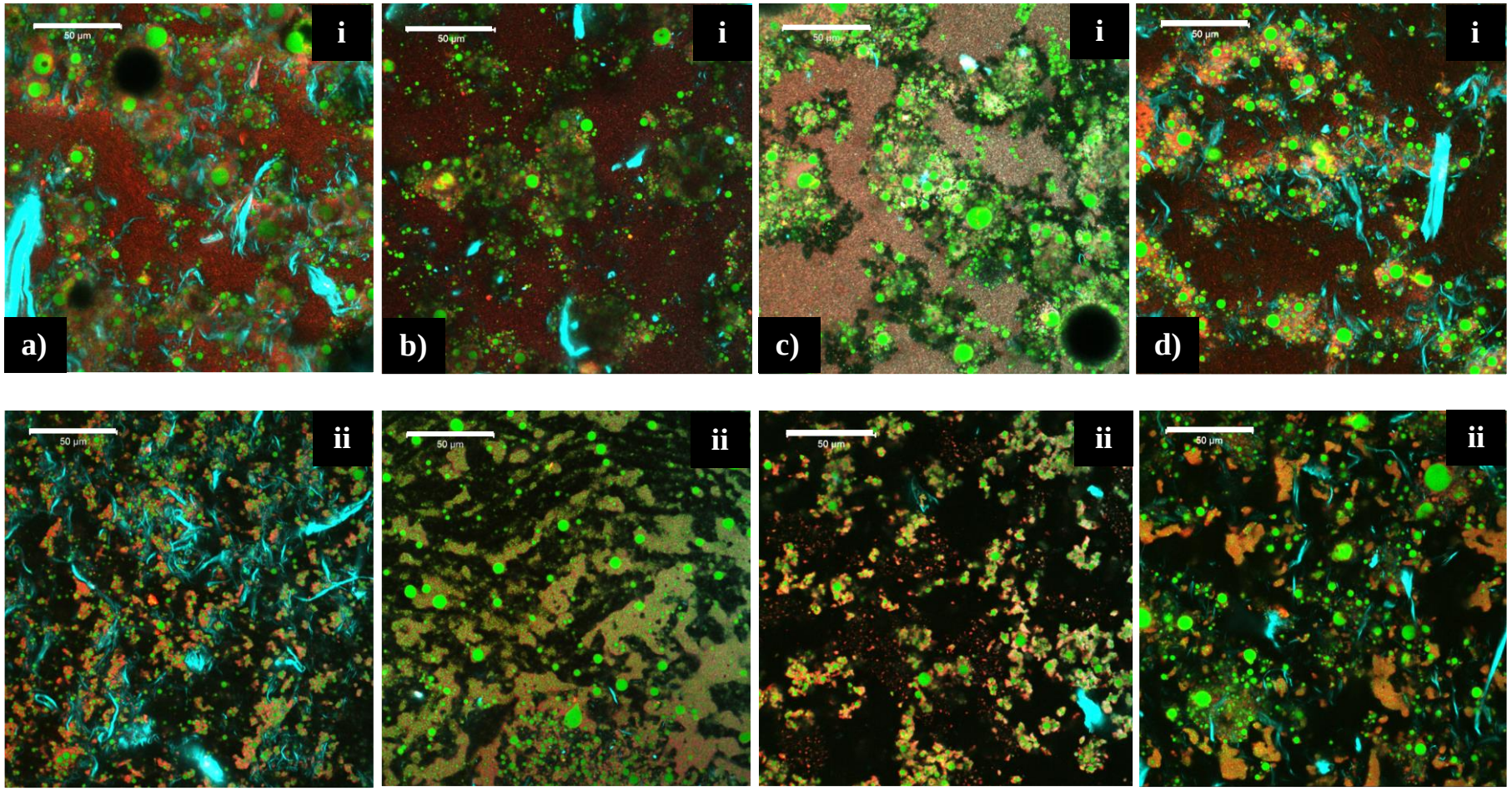

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Figure 4.13 Confocal microscopy images of the fat (green), protein (red) and fibre (blue) in defrosted ice cream imaged under ambient conditions (a) AQ+ CF (b) FibreGel CF (c) Psyllium (d) Sugar Cane (i) fibre only (ii) fibre and guar. Scale bars shown represent 50 µm. Staining of the fibres performed using Calcofluor-white and fat and protein stained using Nile Blue).

possible that depletion flocculation caused by the casein micelles, results in the large molecular weight plant fibres being pushed into a fibre enriched phase.

4.3.1.3 Meltdown behaviour

Slow meltdown, slow serum drainage, shape retention on thawing and slow foam collapse are all attractive characteristics in ice cream (BahramParvar & Mazaheri Tehrani, 2011). The rate of drainage during meltdown has been found to be reduced by increasing the degree of fat destabilisation (Koxholt et al., 20111; Segall & Goff, 2002), a small ice crystal size distribution (Muse & Hartel, 2004), viscosity enhancement of the mix (Goff & Hartel, 2013), including increasing the consistency coefficient (Alvarez et al., 2005), the presence of small dispersed air cells (Eisner et al., 2005) and the inclusion of plant fibres (Dervisoglu & Yazici, 2006; Soukoulis et al., 2009).

Table 4.2 presents the meltdown characteristics of the ice cream formulations. Differences in meltdown rates were seen between different plant fibres. It could be hypothesised that ice creams stabilised with plant fibres would exhibit reduced meltdown rates, as the presence of fibres in the serum phase would prevent rapid drainage through the creation of a fibrous network within the matrix phase. As ice begins to melt, fibres would absorb the melting water and hinder film drainage between air cells as well as provide structure within the matrix phase aiding in shape retention upon thawing. The inclusion of guar would also thicken the serum phase and further reduce the rate of drainage.

Table 4.2 Effect of fibre type (0.6% w/w) and stabiliser blends with guar gum (0.3% w/w) on the meltdown properties of ice cream. Meltdown performed at 23°C).

Stabiliser type	**Meltdown rate (% min^{-1})**	**Melting onset (min)**	**Final mass loss %**
Standard	0.053 ± 0.002	41 ± 5.567	10.089 ± 0.424
AQ+ CF	0.102 ± 0.008	98 ± 4.725	13.623 ± 0.747
FibreGel CF	0.215 ± 0.004	75 ± 5.507	32.312 ± 0.855
Psyllium	0.205 ± 0.018	113 ± 3.511	25.931 ± 0.657
Sugar Cane	0.112 ± 0.004	36 ± 9.504	21.548 ± 0.058
AQ+ CF/Guar	-	-	-
FibreGel CF/Guar	0.056 ± 0.004	42 ± 7.023	11.281 ± 0.836
Psyllium/Guar	0.003 ± 0.001	192 ± 9.899	0.220 ± 0.086
Sugar Cane/Guar	0.006 ± 0.002	70 ± 9.073	1.080 ± 0.421

± indicates the standard deviation.

It was predicted during rheological measurements of the consistency coefficient (*k*) of ice cream pre-mixes stabilised with AQ+ CF, due to its higher *k*, that it would provide the best control over the rate of ice cream meltdown in comparison to all the other fibres under assessment. This is reflected in the meltdown results as AQ+ CF had a substantially slower meltdown rate in comparison to the other fibres at 0.102 % min^{-1}. Dervisoglu & Yazici (2006) found that samples containing 0.8% AQ+ CF had slower meltdown rates in comparison to samples containing 0.8% of a commercial hydrocolloid stabiliser blend consisting of guar gum, LBG, CMC and xanthan gum. Samples containing citrus fibre also had less total mass loss during meltdown. However, citrus fibre/stabiliser blends produced the most optimum

meltdown rates. This effect of fibre on melting rate is not fully reflected in the present study. In comparison to the Standard formulation, the use of solely plant fibres did not reduce the rate of melting nor the final mass loss but did retard the time of melting onset. For the Standard formulation, melting onset occurred at 41 minutes whilst melting onset for fibre containing samples ranged between 36 – 113 mins, with the first drip from Psyllium, which had the longest time till melting onset, occurring at 1.53 hrs. This suggests that, as hypothesised, the fibres are absorbing melting water and preventing the onset of drainage. These results may also provide information on how rapidly the fibres can absorb melting water. If the rate of absorption is slower than the rate of melting with heat transfer, then drip will rapidly occur.

Blending fibres with guar gum also enhanced the meltdown properties as in general, the melting rate and the final mass loss was reduced and the time to melting onset was increased. Additionally, melting onset of Psyllium/Guar formulations occurred at the remarkably late time of 3.12 hrs. AQ+ CF/Guar had no drip during the measuring period, which could be attributed to its highest apparent viscosity and consistency coefficient (Alvarez et al., 2005). Although a fast melting product in unwanted, too slow a rate of melting can also be indicative of a defective ice cream and can result in the perception of gumminess (Goff & Hartel, 2013).

FibreGel CF and FibreGel CF/Guar had the fastest meltdown rates in comparison to all the other formulations at 0.215 and 0.056 % min^{-1}, respectively. Both these formulations also had the most mass loss at the end of testing with FibreGel CF losing 32.312% of its total mass and FibreGel CF/Guar 11.281%. This might suggest that FibreGel CF is providing little network within the matrix phase to slow down the rate of drainage as well as a lower ability to soak up melting water. The melting behaviour can also be attributed to its low apparent viscosity and consistency coefficient.

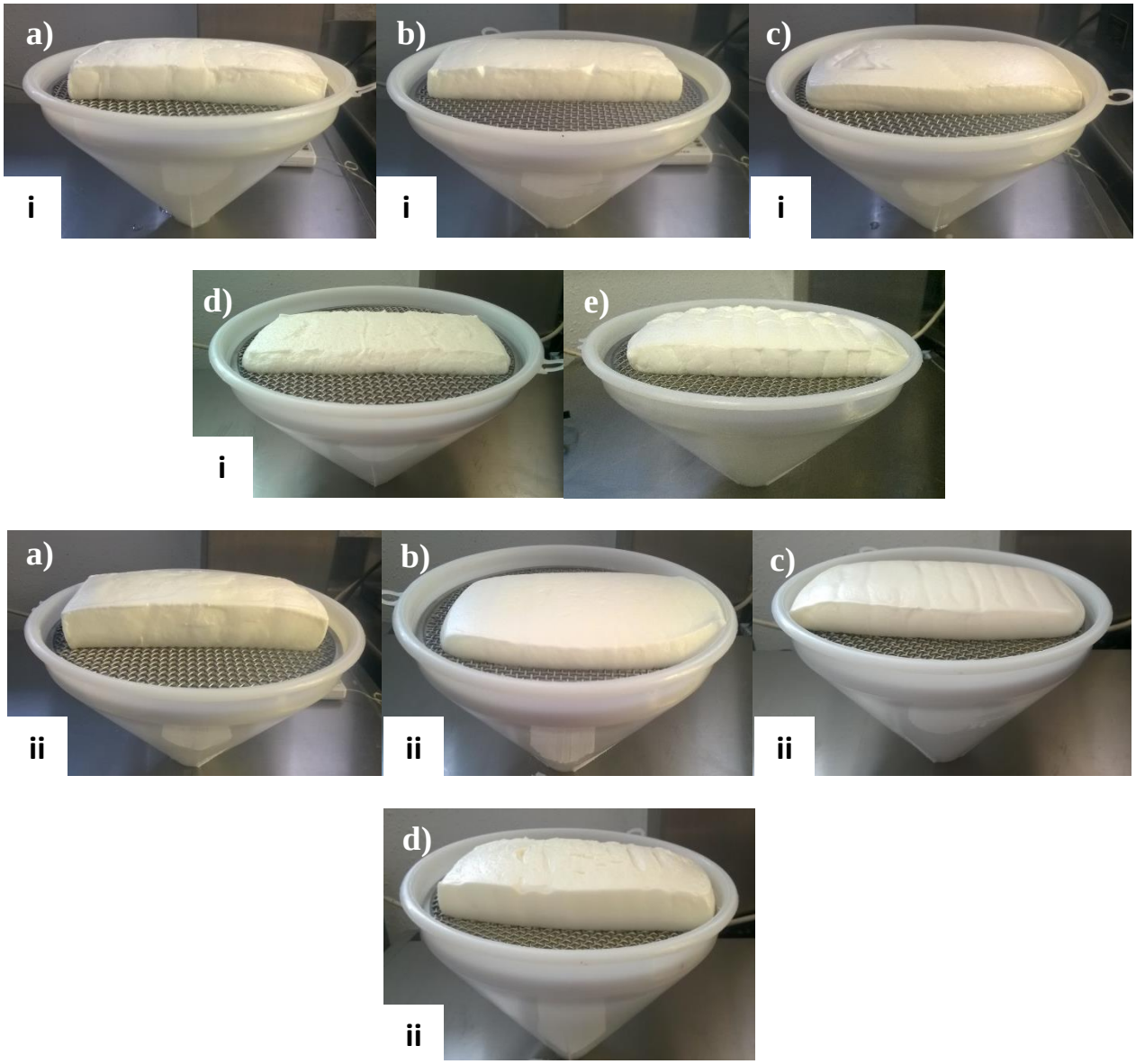

Figure 4.14 Shape of ice cream samples after four hours of meltdown assessment (a) AQ+ CF (b) FibreGel CF Guar (c) Psyllium (d) Sugar Cane (i) no guar (ii) with guar. (e) Standard formulation. Meltdown performed at 23°C.

Further information into the structure of ice creams can be gained by assessment of the shape retention on thawing (Figure 4.14). After ice crystals melt, the ice cream does not collapse until the fat stabilised foam structure collapses (Goff & Hartel, 2013). Plant fibres may aid in reinforcing the existing fat network by propping up the foam structure. Differences in shape

retention between fibre only stabilised ice creams were observed. In comparison to the Standard formulation, AQ+ CF and FibreGel CF were able to retain most of their original shape after four hours of meltdown assessment indicating that these two fibres may be aiding in reinforcing the fat network, whilst Sugar Cane fibre exhibited a collapse of structure. Psyllium Fibre also exhibited some shape retention properties. Overall, shape retention was found to be improved by the inclusion of guar gum. However, for FibreGel CF/Guar blends a large reduction in shape retention was observed.

4.3.1.4 Fibre recovery in ice cream

Assessment of fibre freeze-thaw stability using CLSM in Chapter 3 indicated that the ability of fibres to rehydrate after freezing varied between fibre type, with Psyllium exhibiting the best ability to recover from freeze-compression and freeze-dehydration. However, these tests were performed in extreme circumstances where little unfrozen water remained upon freezing and thus fibre dehydration and compression was at maximum. However, the ice cream formulations used in this study have a 50% ice content at -20°C. Thus, it is hypothesised that in the case of ice cream formulations, high concentrations of freeze concentrated sugars within the serum phase prevent the full compression of fibres and thus the effect of freezing is less destructive to fibre structure. However, successive freeze-thaw cycling over time may still results in a loss of fibre functionality similar to that seen in Chapter 3.

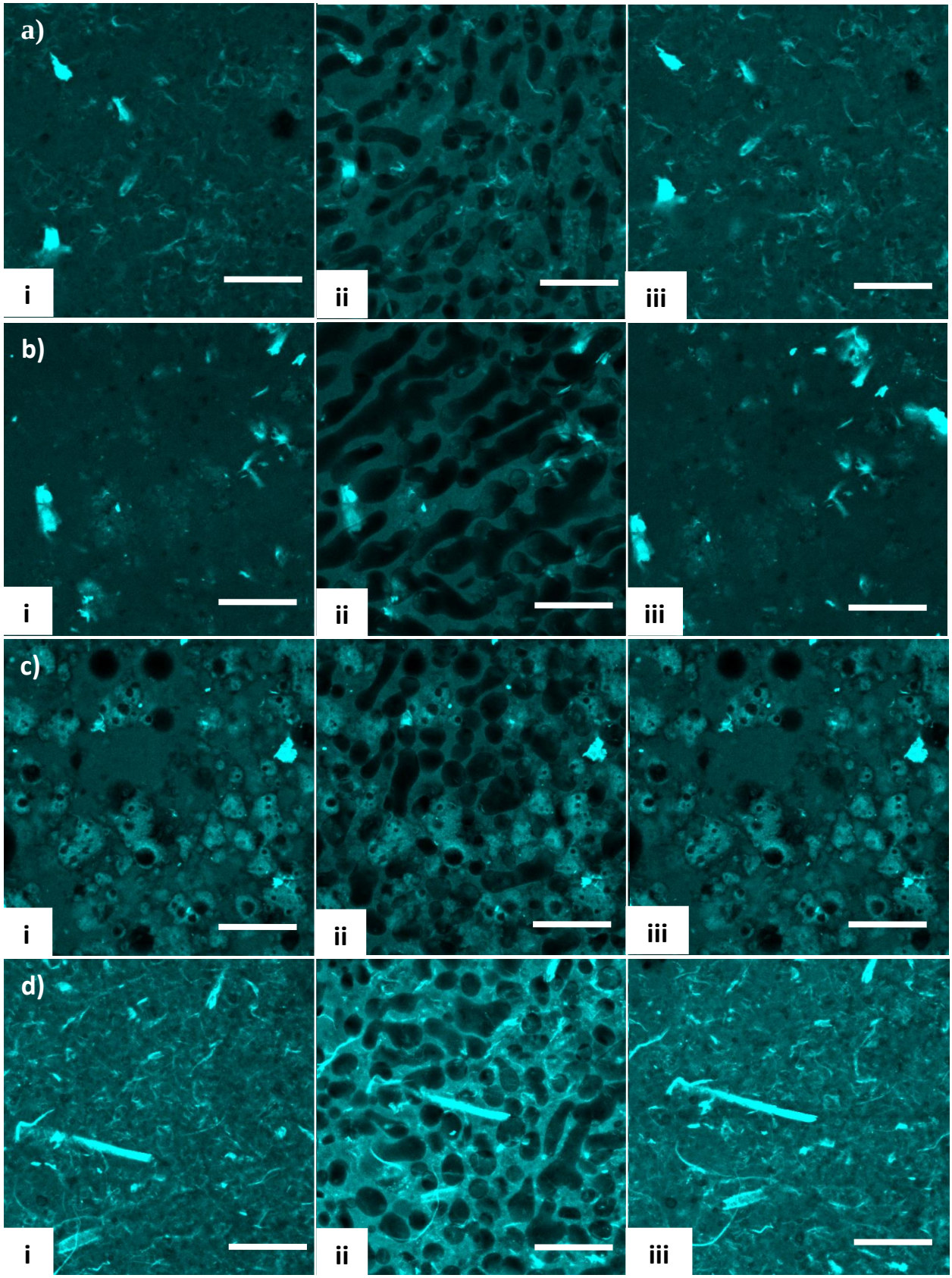

Figure 4.15 Confocal microscopy images of freeze-thaw cycled aged ice cream mixes containing different fibre types (a) AQ+ CF (b) FibreGel CF (c) Psyllium (d) Sugar Cane (i) Initial (ii) Held frozen at -5°C for 30 mins (iii) Defrosted. Scale bars shown represent 50 µm. Staining performed using Calcofluor-white.

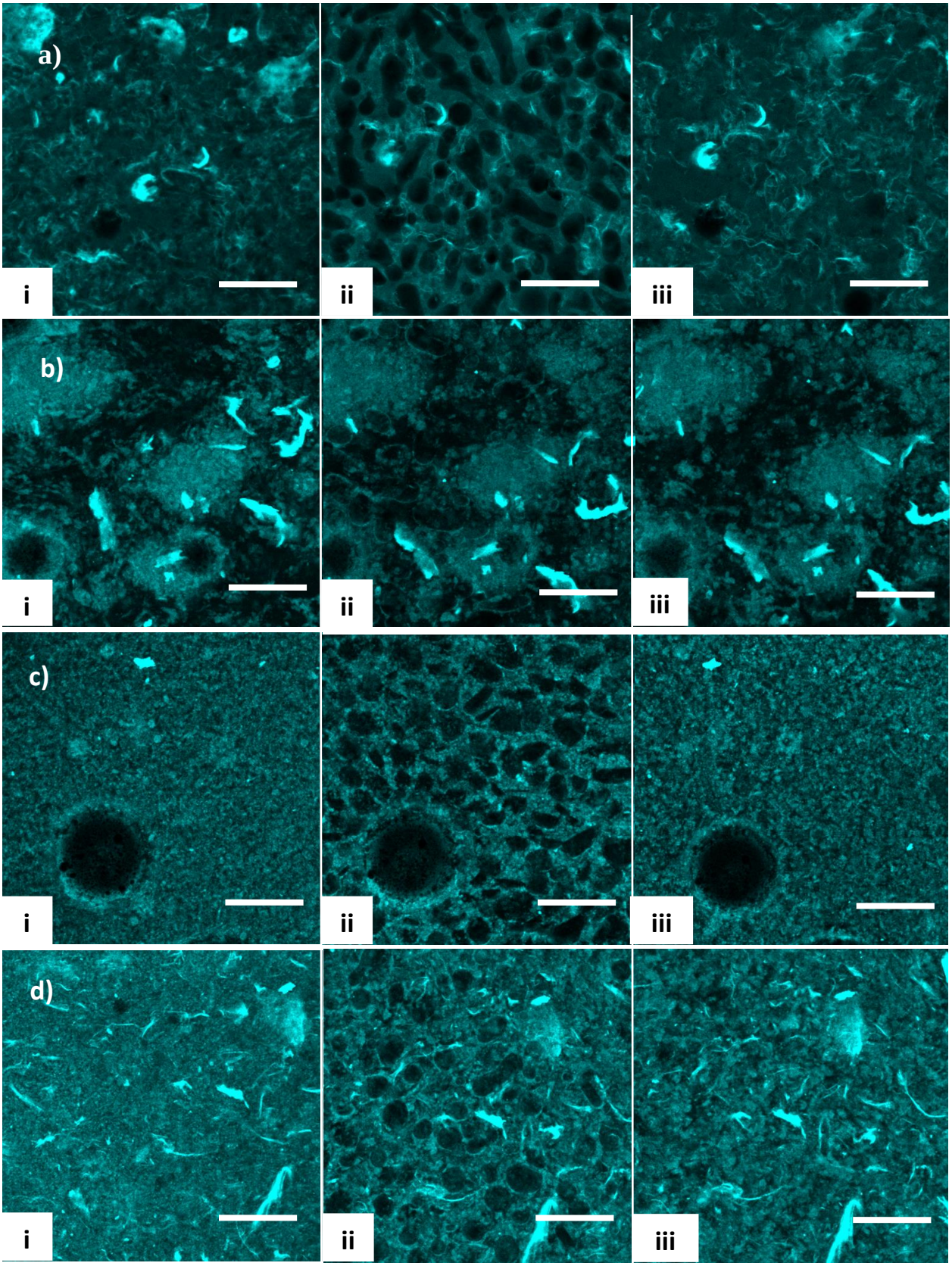

Figure 4.16 Confocal microscopy images of freeze-thaw cycled aged ice cream mixes containing different stabiliser blends (a) AQ+ CF/Guar (b) FibreGel CF/Guar (c) Psyllium/Guar (d) Sugar Cane/Guar (i) Initial (ii) Held frozen at -5°C for 30 mins (iii) Defrosted. Scale bars shown represent 50 µm. Staining performed using Calcofluor-white.

The effect of freeze thaw-cycling aged ice cream mixes containing the plant fibres and fibre blends with guar gum are presented in Figures 4.15 and 4.16, respectively. This assessment was performed using aged mixes instead of defrosted, hardened samples to remove any protection effects that may be provided by the development of a fat network during dynamic freezing and thus allowing the full ability of the fibres to retain structure upon freeze-thaw cycling to be identified. As hypothesised, in all formulations, the effect of a single freeze-thaw cycle on fibre is less destructive and little or no inability of fibres to rehydrate on defrost was observed as around 50% of the matrix phase remained unfrozen at -20°C. All formulations containing solely fibres presented a good ability to rehydrate on defrosting. However, when co-stabilised with guar gum, Sugar Cane Fibre (Figure 4.16 d iii) exhibited a slight amount of inability to rehydrate into the voids caused by ice crystal growth. This may be an effect of competitive rehydration between the two biopolymers. Alternatively, the voids seen in fibre structure may be as a result of the phase separation between fibre and guar gum originally identified, as again difference in structures between fibre only stabilised and co-stabilised mixes can be observed.

It is previously identified that the structure and compositional make up of Psyllium is very different to the other plant fibres being assessed. AQ+ CF, FibreGel CF and Sugar Cane Fibre are all cellulose based structures. Psyllium is a mucilage gum and is hemicellulose based and thus its structure in comparison are very different. Psyllium seems to form small gel-like particulate structures in an ice cream mix. Additionally, in the presence of guar gum a finer particulate gel system is produced. During homogenisation, the increase in viscosity with guar gum may cause the gel particles to be reduced in size owing to the finer particle size. The many health benefits of Psyllium have been reported to be related to its characteristic gel-like properties (Marlett & Fisher, 2003). Guo et al. (2009) have also found that the presence of Ca^{2+} enhanced the weak-gel properties of Psyllium mucilage gum to behaviours typical of a true gel.

Cryo-SEM was used to provide details on these gels. Without Ca^{2+} the gel strands were filamentous in network. In the presence of Ca^{2+} the filamentous gel strands were replaced by aggregates that were linked together to form a particulate gel network structure. TEM assessment reiterated these findings as the fine long strands of gel network around 0.1 – 1 µm in length without Ca^{2+} were replaced by strings of dense aggregates, with aggregates having a size of around 0.1 - 0.5 µm. It was postulated that calcium ions promote the aggregating of polysaccharide chains or change the conformation of the polysaccharide strands favouring aggregation. Thus, the presence of calcium within an ice cream mix may be promoting the particulate gel structure of Psyllium and it is this structure that is creating an effective stabilising network within the matrix phase of ice cream.

4.3.1.5 Spin-spin relaxation times

The effect of freeze-thaw cycling on the relaxation times of fibre stabilised and co-stabilised aged ice cream mixes are presented in Figure 4.17. The bulk peak distributions indicating the peak relaxation time in each formulation are presented in Figure 4.18. In comparison to the relaxation times of the assessment of fibres in water suspensions outlined in Chapter 3, a near ten-fold reduction in T_2 times were obtained for fibre stabilised ice cream mixes due to the water binding capacity of the other mix components.

Freeze-thaw confocal assessment of the aged mixes reported little change in structure with a single freeze-thaw cycle as a result of the existence of an unfrozen phase, reducing the amount of dehydration experienced by the fibres. As shown in Figure 4.17, this unfrozen phase has allowed the fibres to retain their ability to reduce the molecular mobility of water with freeze-thaw cycling.

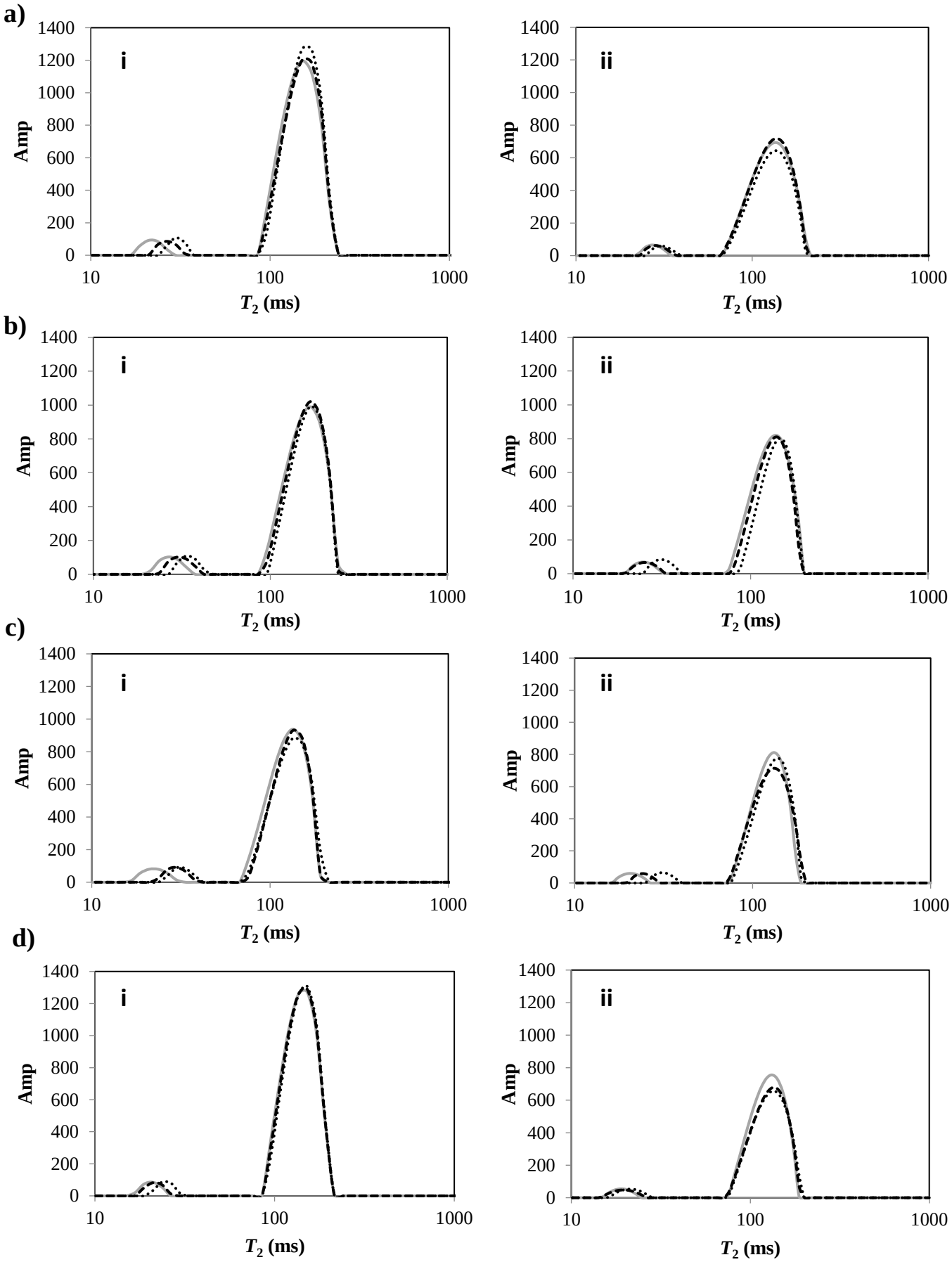

Figure 4.17 Freeze- thaw spin-spin relaxation NMR spectra of aged ice cream mixes with different fibres and stabiliser blends (a) AQ+CF (b) FibreGel CF (c) Psyllium (d) Sugar Cane (i) without guar gum (ii) with guar gum ——— Initial - - - First freeze **....** Second freeze. Performed at 20°C.

In Chapter 3, AQ+ CF, FibreGel CF and Sugar Cane Fibre all exhibited a broadening of the relaxation peaks with repeated freeze-thaw cycling. However, in ice cream formulations all fibres exhibit good freeze-thaw stability as little broadening of bulk peaks with two freeze-thaw cycles can be observed, suggesting these two cycles have little effect on the structure and functional properties of the fibres. However, with further successive cycling between 10 – 20 cycles we may begin to see reductions in functionality for the fibres that have already demonstrated to have a reduced freeze-thaw stability.

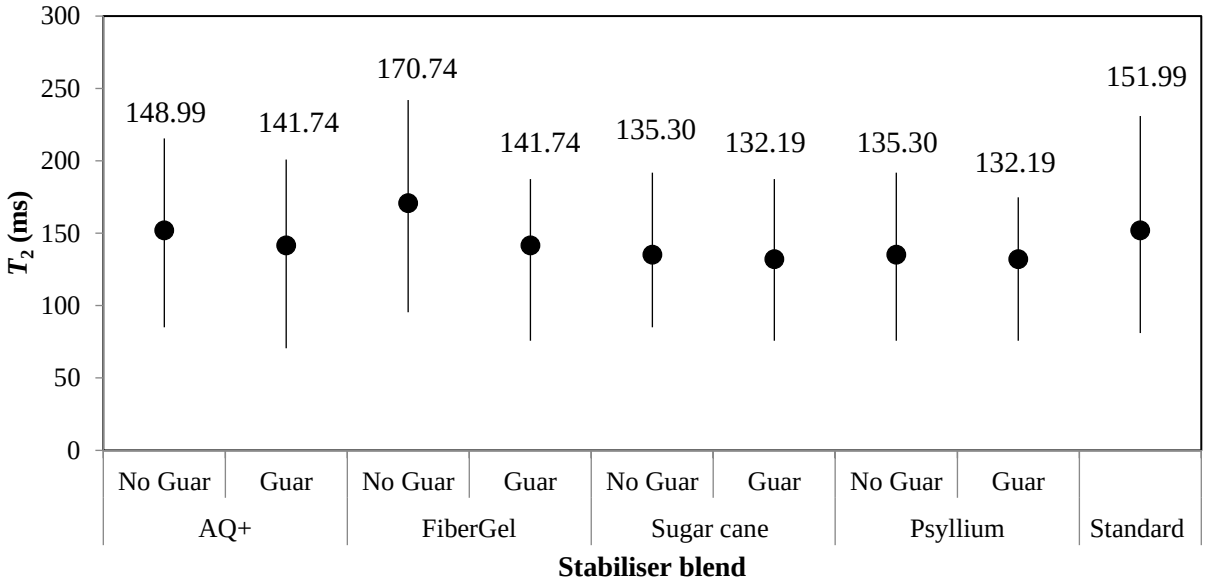

Figure 4.18 Spin-spin relaxation NMR of bulk water peak distributions of aged ice cream mixes (20°C) with different plant fibres and stabiliser blends. Numbers shown indicate peak T_2 values.

Figure 4.18 shows the distribution of relaxation times within bulk peaks of the ice cream formulations. As can be seen, a reduction in peak T_2 values and a narrowing of the distribution was observed when fibres were co-stabilised with guar gum. This is due to the water binding

contribution of guar gum. However, with the exception of FibreGel CF which has a slightly higher peak T_2, peak T_2 values of AQ+ CF, Psyllium and Sugar Cane Fibre stabilised mixes were lower than that of the Standard formulation. However, these differences are marginal and may not be reflected in their control over ice recrystallisation.

4.3.1.6 Thermal analysis of formulations

It is generally considered that hydrocolloid stabilisers have no impact on the freezing properties of a mix (Hagiwara & Hartel, 1996). However, it has been reported by Soukoulis et al. (2009) that the inclusion of plant fibres containing high levels soluble fibre (inulin and apple fibre) content elevated the glass transition temperature of model sucrose solutions and ice cream formulations. This elevation in T_g was suggested to be as a result of the restricted mobility of water molecules and thus improved thermodynamic stability of the formulations.

The effect of fibre and guar gum inclusion on the glass transition temperatures (T_g) of ice cream formulations is reported in Table 4.3. Analysis of variance revealed that no significant difference ($p > 0.05$) in T_g existed between the different formulations. FibreGel CF, a fibre high in soluble fibre content, was not identified to be significantly different to Sugar Cane Fibre, which is low in soluble fibre content. Thus, the observations reported by Soukoulis et al. (2009) are not reflected in the present results.

Soukoulis et al. (2009) used fibre concentrations in the range of 2-4 % (w/w), with higher concentrations having a more profound effect on T_g. Perhaps this might suggest that the low concentrations used in the current formulations are not sufficient enough to elevate the glass transition temperature.

Table 4.3 Glass transition temperatures (T_g) of ice cream formulations.

	Onset (°C)	Midpoint (°C)	Endpoint (°C)
AQ+ CF	-31.73 ± 1.958	-34.51 ± 0.289	-34.97 ± 1.491
AQ+ CF/Guar	-30.69 ± 1.895	-35.13 ± 2.566	-34.24 ± 0.692
FibreGel CF	-32.13 ± 0.700	-33.58 ± 0.155	-35.14 ± 0.077
FibreGel CF/Guar	-31.50 ± 0.763	-33.26 ± 0.226	-34.68 ± 0.084
Sugar Cane	-31.47 ± 1.400	-33.38 ± 0.296	-34.91 ± 0.325
Sugar Cane/Guar	-31.70 ± 0.318	-32.56 ± 0.169	-34.17 ± 0.247
Psyllium	-31.11 ± 1.294	-32.56 ± 0.502	-34.26 ± 0.304
Psyllium/Guar	-31.10 ± 0.806	-33.44 ± 0.339	-34.87 ± 0.042

N.B data generated using the DSC traces as obtained for each sample. An example trace is shown in Figure 2.13 in Chapter 2 of this thesis.

± indicates the standard deviation.

4.3.2 Ice phase

4.3.2.1 Ice crystal size determination

Assessment of the average ice crystal size in fresh and 14 day temperature abused ice cream samples are presented in Table 4.4.

Previous studies have suggested that polysaccharide stabilisers have no significant impact on ice crystal size distributions at the time of draw from dynamic freezing, nor do they limit the initial growth of crystals during quiescent freezing (Buyong & Fennema, 1988; Flores & Goff, 1999b), but do limit the rate of ice crystal growth during recrystallisation (Goff et al., 1993; Regand & Goff, 2002).

As can be seen in Table 4.4, little difference in the mean ice crystal size of fresh samples were observed between fibre only stabilised ice creams. An increase in mean diameter was observed on blending with guar. However, this difference may have been as a result of varying exit temperatures. At higher freezer exit temperatures less ice is formed during dynamic freezing and the remaining ice is formed during hardening. As nucleation can no longer occur in quiescent freezing, the remaining ice is formed by growth on existing crystals, resulting in an increase of size (Goff & Hartel, 2013).

Table 4.4 Ice crystal diameter of fresh and temperature abused ice cream samples and the rate of ice crystal growth (%). Measured at -20°C.

Stabiliser Blend	Exit Temp (°C)	Fresh mean diameter (µm)	Temperature abused mean diameter (µm)	% Rate of growth
AQ+ CF	-6.6	20.27 ± 5.27	103.15 ± 38.82	408.88
FibreGel CF	-7.2	20.21 ± 5.75	117.45 ± 36.11	481.14
Psyllium	-7.0	18.80 ± 5.32	90.71 ± 38.72	382.31
Sugar Cane	-6.7	21.15 ± 7.52	107.44 ± 42.41	406.33
AQ+ CF/Guar	-6.2	22.81 ± 7.81	102.56 ± 46.75	349.62
FibreGel CF/Guar	-5.8	21.15 ± 9.01	95.34 ± 47.69	350.78
Psyllium/Guar	-5.8	28.79 ± 8.45	91.87 ± 36.94	219.10
Sugar Cane/Guar	-5.8	23.82 ± 6.44	94.65 ± 37.32	297.35
Standard	-6.6	21.15 ± 7.39	103.77 ± 26.35	390.63

± indicate the standard deviation in µm.

A substantial increase in ice crystal size was observed with temperature abuse. It has been suggested that the rate of ice crystal growth in ice creams under fluctuating temperatures is more rapid than samples that are stored at a constant temperature (Alvarez et al., 2005). Thus, the cycling protocol used in this study will have an extreme impact on the ice crystal size.

Ice creams co-stabilised with plant fibre and guar gum had substantially reduced percentage growth rates compared to fibre only stabilised samples. Psyllium was found to have lower growth rates in comparison to all the other fibres. AQ+ CF, FibreGel CF and Sugar Cane all exhibited similar crystal growth rates. However, in samples co-stabilised with guar gum Psyllium/Guar samples also exhibited the best control over ice crystal growth whilst AQ+ CF/Guar and FibreGel CF/Guar again displayed similar properties.

It is hypothesised that plant fibres have the ability to 'soak up' unfrozen water during temperature fluctuations preventing water migration. This would enhance mechanisms of melt-regrow in a localised manner rather than melt-diffuse-regrow. However, this ability may be hindered if the plant fibre is not freeze-thaw stable. It has previously been suggested that control over recrystallisation can be attributed to the water binding capacity of the stabiliser (Alvarez et al., 2005). Sugar Cane Fibre was identified to have the highest water binding capacity in Chapter 3, however this optimum control is not reflected in the ice crystal growth rates shown here and is most likely due to Sugar Cane being freeze-thaw susceptible. In co-stabilised samples containing Sugar Cane/Guar, good control over recrystallisation was observed but the best control is provided by Psyllium/Guar. It has already been identified that the structure of Psyllium is different to the other fibres being assessed. Psyllium only stabilised mixes also have very different structures to Psyllium/Guar mixes. Perhaps this might be suggestive the best control over recrystallisation can be provided by the particulate gel structure of Psyllium whereby phase concentration in the presence of guar gum improves functionality.

4.3.2.2 Ice recrystallisation

Reducing the rate of ice recrystallisation is key to retaining ice cream shelf life quality (Goff & Hartel, 2013). The assessment of the growth of ice crystals under isothermal conditions is presented in Figures 4.19 and 4.20. The recrystallisation rates (*k*) as calculated by the Ostwald ripening principal, which can be applied to recrystallisation in isothermal conditions is shown in Figure 4.21. Although the other components of the mix (milk proteins, fat, and sugars) may aid in recrystallisation control, these are constant and therefore the differences in recrystallisation should be due to the impact of the fibres.

Differences in the distribution of ice crystal growth were observed in both FibreGel CF and FibreGel CF/Guar ice creams (Figure 4.19 c and d, respectively). Initially, large clumps of fibre could be observed in the mix. Upon freezing, ice crystals formed within the fibre clumps but as time proceeded, the ice crystals within these clumps melted, water diffused outside of the clumps and recrystallisation occurred outside of the clumps were ice crystals grew larger than in any of the other formulations. The ability of stabilisers to form elastic cryo-gels has been reported to interfere with the morphology of and provide control over ice crystal growth (Muhr & Blanshard, 1986). FibreGel CF contains the highest amount of pectin which could have the ability to form gels in an ice cream mix. Blond (1988) reported that pectin was able to form visco-elastic gels in the presence of calcium ions which inhibited the propagation rate of the ice crystal front (the building of water molecules onto the ice crystal surface). The creation of a continuous gelled network around ice crystals is reported to limit the diffusion characteristics of water within the network.

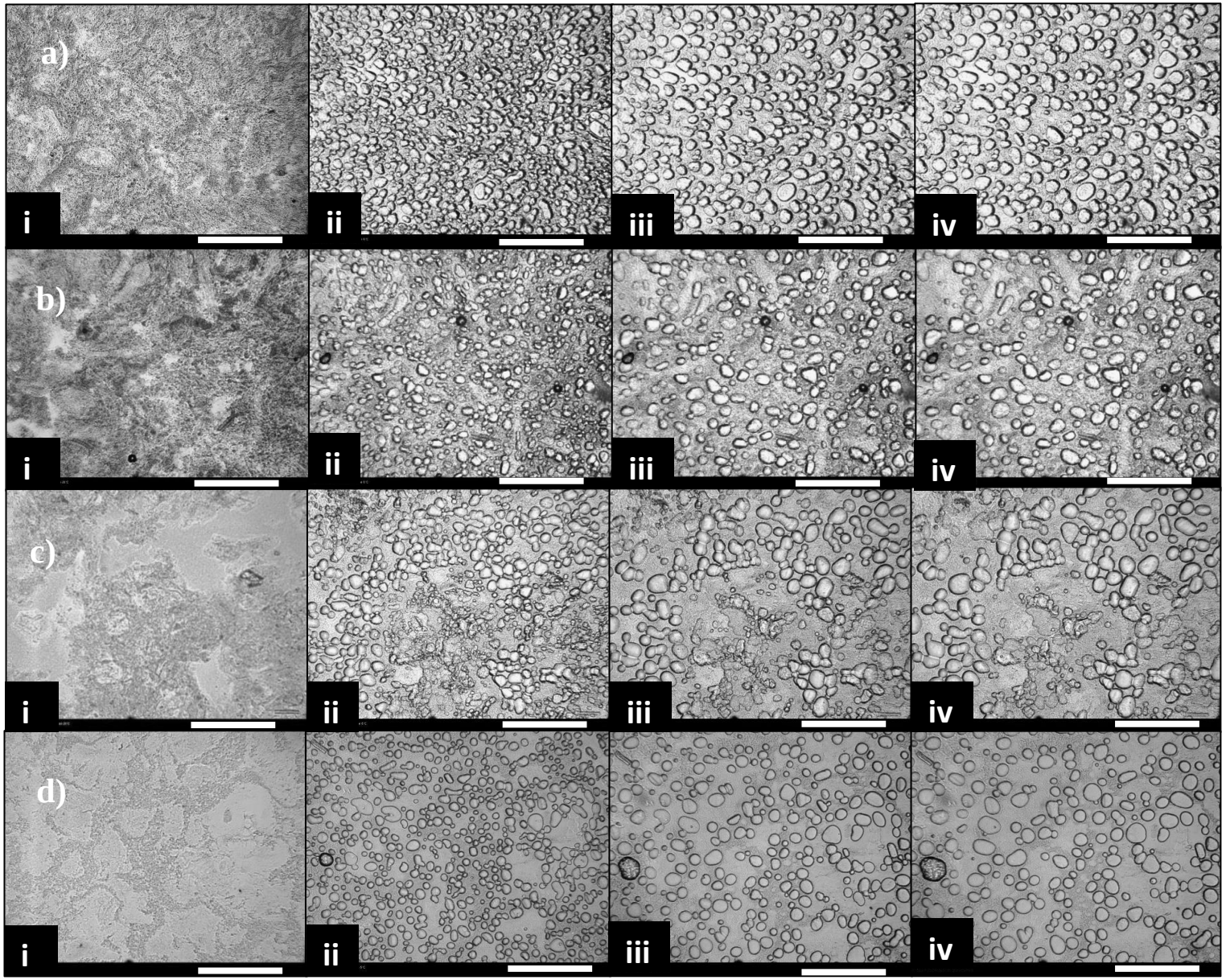

Figure 4.19 Light microscope images of aged ice cream mixes held isothermally at -5°C (a) AQ+ CF (b) AQ+ CF/Guar (c) FibreGel CF (d) FibreGel CF/Guar (i) Initial (ii) after 20 minutes at -5°C (iii) 80 mins (iv) 120 mins. Scale bar shown represents 300 µm.

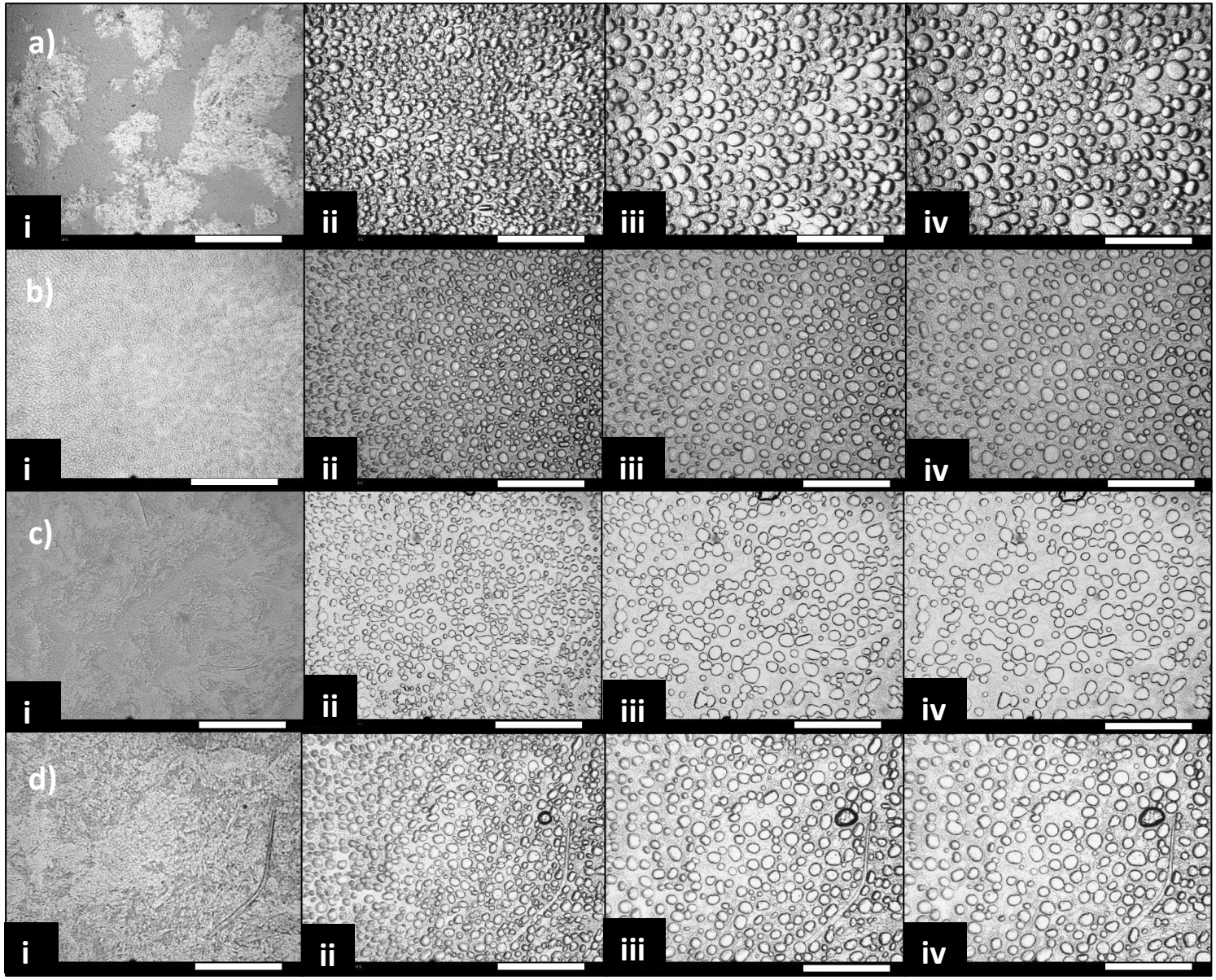

Figure 4.20 Light microscope images of aged ice cream mixes held isothermally at -5°C (a) Psyllium (b) Psyllium/Guar (c) Sugar Cane (d) Sugar Cane/Guar (i) Initial (ii) after 20 minutes at -5°C (iii) 80 mins (iv) 120 mins. Scale bar shown represents 300 µm.

Guar gum behaves as a random coil in solution. Unlike, other galactomannans such as locust bean gum (LBG) it is not able to from cryo-gels. Goff et al. (1999a) identified that phase separation of proteins and stabiliser creates a phase concentration of stabiliser that is effective at preventing ice recrystallisation. Phase separated guar gum and milk proteins offered some protection over recrystallisation but by combining phase separation with the cryo-gelling galactomannan LBG, enhanced protection was identified.

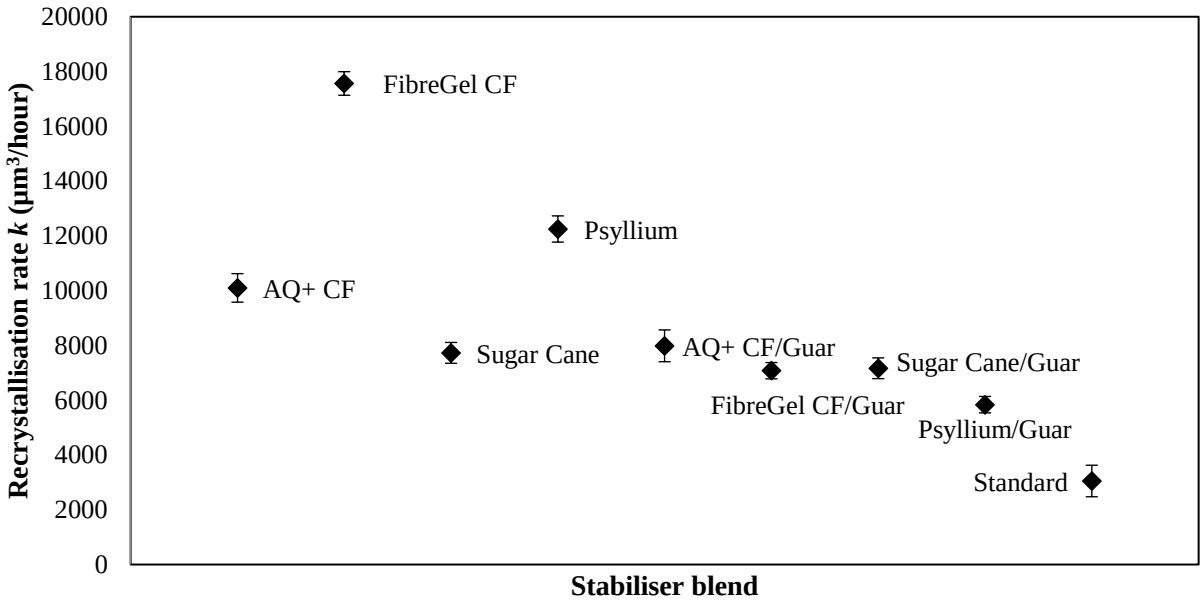

Figure 4.21 Recrystallisation rate (*k*) at -5°C of formulations containing varying fibre type (0.6% w/w) and stabiliser blends with guar gum (0.3% w/w) using the Ostwald ripening principal. Error bars represent the standard deviation.

Confocal microscopy identified a distinct protein phase and polysaccharide phase thus an impact on the morphology of ice crystal growth may occur as a result. Perhaps these fibre clumps seen are as a result of phase-separation with the enriched-FibreGel CF phase also having some gelling properties due to the content of pectin that can disturb the morphology of

ice crystal growth. However, recrystallisation assessment was performed using ice cream pre-mixes and does not necessarily suggest that these fibre clumps will survive dynamic freezing. In addition, phase separation has been identified in all mixes, but only FibreGel CF containing mixes exhibited this behaviour.

Large differences in the rate of ice recrystallisation can be seen between the different formulations (Figure 4.21). FibreGel CF was found to have the highest recrystallisation rate as although the distribution of ice crystal growth was different in these mixes, ice crystals still became quite large. A large link between the water binding capacity (WBC, assessed in Chapter 3) of the fibre and the control over the rate of recrystallisation was identified. Sugar Cane Fibre, with the highest water binding of 100 g water/g dry fibre, had the best control over recrystallisation rates. AQ+ CF had a WBC of 86 g/g, Psyllium of 81 g/g and FibreGel CF 75 g/g. and so the WBC can be used as a key measurement in providing information on the control plant fibres may provide over ice recrystallisation. However, these measurements were performed in isothermal conditions which does not take into consideration the freeze-thaw instability of the fibres.

The addition of guar as a stabiliser blend with plant fibre resulted in a reduction in the recrystallisation rate. Its addition, it also resulted in narrowing the differences in *k* between fibres. In agreement with the growth rates of crystals in fluctuating temperatures (Table 4.4) the rate of ice recrystallisation was lowest in mixes containing Psyllium/Guar. This further reiterates that the structure formed by Psyllium in the presence of guar is providing control. It has previously been outlined that the formation of cryo-gels offer some protection over the rate of ice recrystallisation (Goff et al., 1999a). Some clumps of fibre in Psyllium samples were observed (Figure 4.20 a and b) but were not seen to impact the morphology of ice crystal growth. In addition, Psyllium in the presence of guar gum seems to form small gel-like particles. Little evidence of a continuous gelled system like that of LBG with Psyllium can be

seen, however under frozen conditions, freeze-concentration of the gel-like particulates may offer a similar cryo-gel protection. It has been suggested that a firm gel may not provide optimum protection against ice crystal growth as a firm gel may be more fragile and more easily ruptured by a growing ice crystal front. A flexible gel would thus apply stronger apposing force on the ice front during growth (Muhr & Blandshard, 1986; Blond 1988). However, despite the differences seen, none of the fibre stabilised samples were as effective at controlling the rate of ice recrystallisation as much as the Standard formulation which contains a blend of guar gum, LBG and xanthan.

4.3.3 Fat phase

4.3.3.1 Fat destabilisation

During air incorporation in dynamic freezing by either whipping or air injection, air bubbles become stabilised by both proteins from the concentrated serum phase and by adsorption of fat droplets and aggregates to the serum-air bubble interface (Goff & Jordan, 1986). Increasing fat destabilisation results in the development of a more stable foam that resists meltdown and structural collapse (Koxholt et al., 2001).

The effect of ice cream formulation on the existence of fat globules and aggregates at the air bubble interface are shown in Figure 4.22. As can be seen, little difference in the amount of fat at the interface was identified between the fibre only stabilised samples. However, when fibres are co-stabilised with guar gum, a reduction in the amount of fat present at the interface was observed. Fat at the interface of fibre only stabilised ice creams seem to be mostly discrete fat droplets whereas fat at the interface in co-stabilised samples are generally larger in size.

Goff et al. (1999b) has suggested that increasing fat destabilisation results in a higher concentration of fat globules at the air interface. However, where an air interface is not

stabilised by fat droplets or aggregates, stabilisation can still be provided by proteins and thus may not hinder overall air bubble stability. Bolliger et al. (2000b) identified that the emulsifier type and concentration is one of the main factors determining the amount of fat destabilisation. With increasing emulsifier concentration, displacement of proteins at the fat droplet surface due to their higher ability to lower the interfacial tension increases droplet susceptibility to destabilisation as a result of the development of a thinner interfacial layer. It was also suggested that a critical value of protein displacement was needed to achieve sufficient destabilisation. However, in the present work the emulsifier type and concentration remains constant between the formulations and thus the addition of guar gum is proving to have an impact on fat structure.

It has been proposed that some hydrocolloids have the ability to act as emulsifying agents and protect newly formed emulsion droplets against flocculation and coalescence. Their surface active properties are reported to be as a result of their molecular structure. The non-polar nature of the chemical groups on side chains and the presence of protein moieties along the backbone of the hydrocolloid have been proposed to arise in their ability to act as emulsifiers. Understanding over the development of protein-polysaccharide complexes as emulsifying agents have also been reported (Dickinson, 2009). Fat destabilisation relies upon protein desorption and replacement by small molecular weight surfactants, rendering the interface sufficiently susceptible to destabilisation. However, it might be suggested that if proteins become more stable against desorption through complexation with hydrocolloids or if hydrocolloids themselves have emulsifying properties, are they still available to be effectively replaced by surfactants or can they increase the stability of the interface to coalescence. If this is the case, small discrete fat droplets will remain and the amount of destabilisation will be reduced. Gelin et al. (1996) have identified that the specific interactions (covalent and electrostatic) between caseins and carrageenan, strengthened protein absorption to the interface, reducing their ability to be displaced by small molecular weight emulsifiers.

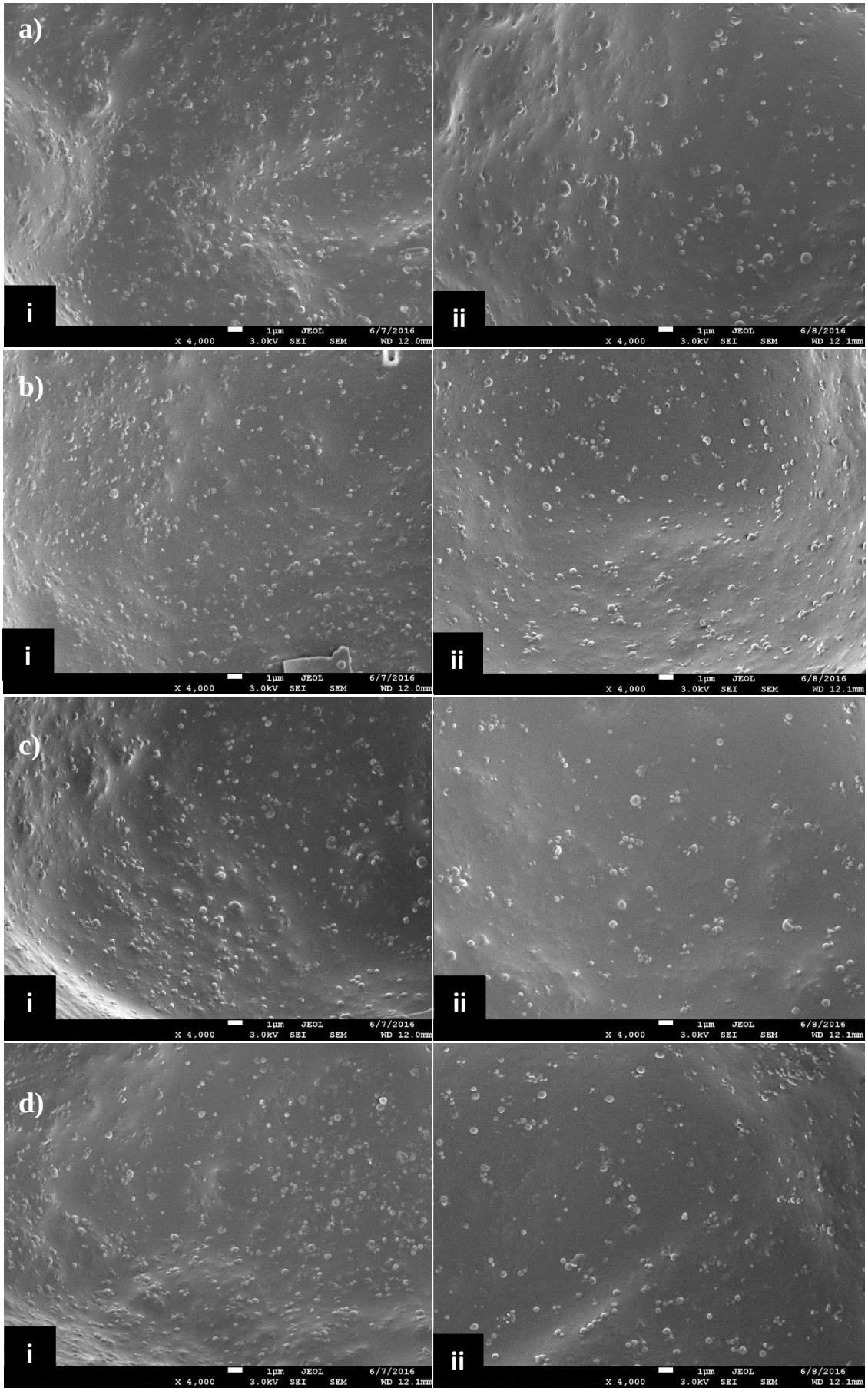

Figure 4.22 Cryo-SEM of the microstructure of air cells showing fat globules at the interface as effect of varying fibre type and stabiliser blend (a) AQ+ CF (b) FibreGel CF (c) Psyllium (d) Sugar Cane (i) without guar gum (ii) with guar gum. Scale bars shown represent 1 µm.

Garti & Reichman (1993) has reported that gum arabic has a strong ability to absorb to the oil-water interface via its protein moieties. However, galactomannans LBG and guar gum are reported to absorb weakly to an oil interface with absorbability being largely reversible and governed by the level of protein contamination bound to the hydrocolloid. As this protein material is strongly hydrophobic, guar gum can behave as an amphiphilic molecule if the level of protein contamination is sufficient (Dickenson, 2003).

Complexation may not only be limited to proteins and hydrocolloids. Electrostatic interactions between an absorbed ionic surfactant in the primary layer and an oppositely charged hydrocolloid on the secondary layer have been proposed to provide control over emulsion stability (Dickinson, 2009). However, guar gum is non-ionic so this mechanism of stabilisation is unlikely.

4.3.3.2 Fat particle size analysis

To further assess the impact of guar gum on the structure of fat observed, particle size analysis was performed on both the ice cream pre-mix and defrosted ice cream to identify the differences in the degree of fat destabilisation. As destabilisation proceeds, the particle size should become bimodal representing either coalescence and/or partial coalescence and discrete fat droplets. Fat droplets greater than 10 μm can be considered as the destabilised proportion (Goff et al., 1999b). Figure 4.23 presents the results of the effect of formulation on the particle size analysis. Due to the presence of plant fibres, their respective particles sizes are also reported.

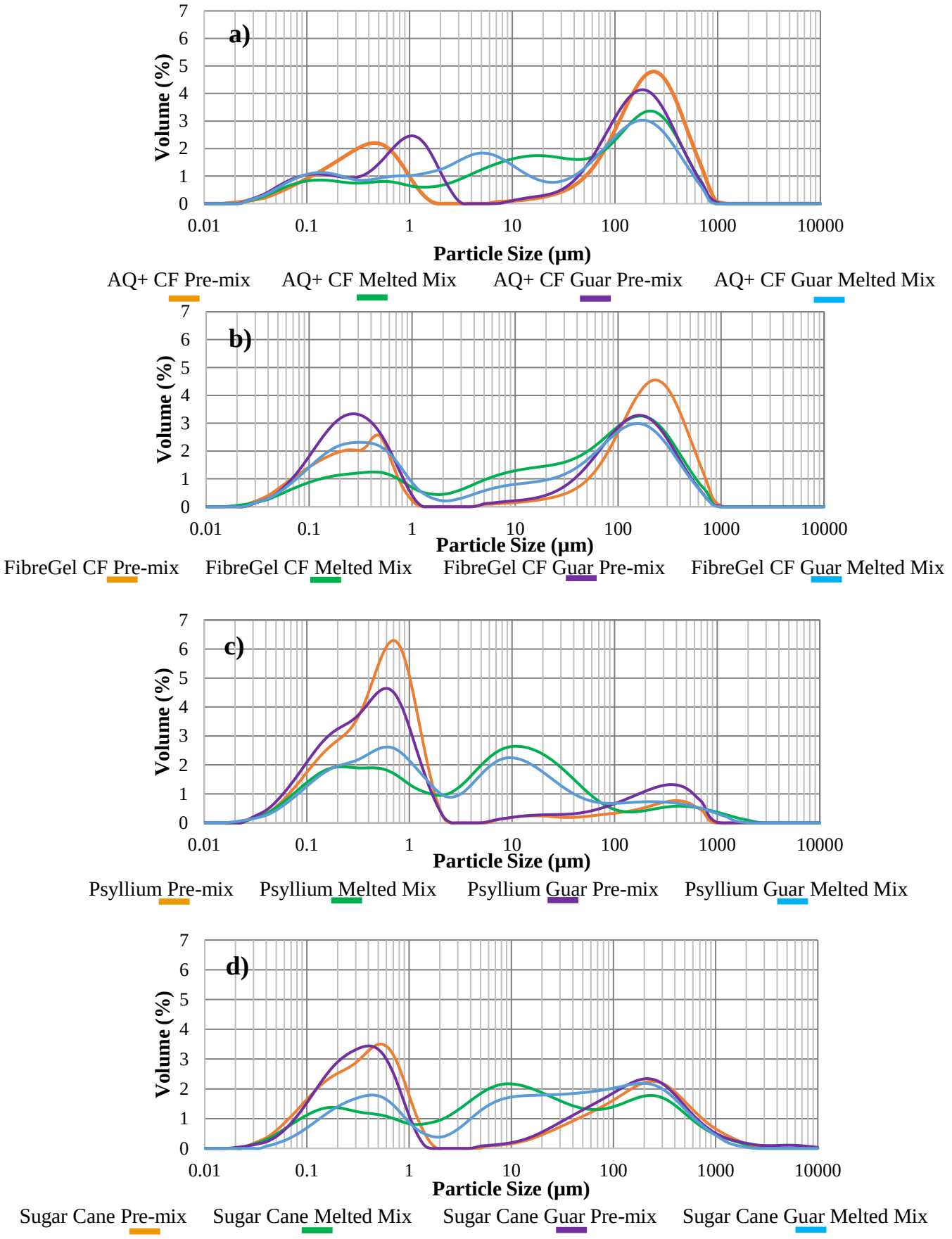

Figure 4.23 Particle size analysis of pre-mix and melted hardened ice cream of the ice cream formulations (a) AQ+ CF (b) FibreGel CF (c) Psyllium (d) Sugar Cane.

All droplets in the pre-mixes were 1-2 μm or below, suggesting that homogenisation was successful and the presence of fibre had no or little effect on emulsion droplet formation. Particle size analysis shows that fat destabilisation is greater in the absence of guar gum. The presence of a peak for fibres makes interpretation of the particle size difficult to determine the degree of destabilisation. However, the higher percentage volume of discrete fat droplets <1 um are generally higher in melted mixes containing guar than in mixes containing only fibre. This is suggestive of a decrease in the amount of fat destabilisation in the presence of guar gum.

It has been suggested that increasing fat agglomerate size aids in reducing the meltdown rate (Bolliger et al., 2000c). However, this effect on the level of fat destabilisation does not seem to have a detrimental effect on the meltdown properties and shape retention as meltdown assessment identified that the rate of meltdown was reduced by the addition of guar gum. This effect is most likely to be a result of increasing serum viscosity, reducing the rate that the serum will drain under gravity. In addition, Koxholt et al. (2001) have suggested that a critical size of approximately 1.15 μm is sufficient to provide protection over the rate of melting, which in the case of the samples containing guar gum was achieved. Fat globules and agglomerates smaller than the critical diameter will flow out of the ice cream with the serum on meltdown. This critical size however, will be dependent on the size of the width of the lamella between the air cells, which in the case of the present study, cryo-SEM suggests, is between 1 – 10 μm in fresh/unabused samples. Thus, fat agglomerate diameters of around 10 μm will be sufficiently large enough to block the space and impede the drainage of the serum. This blocking effect will also be enhanced by the presence of fibres.

The mechanisms by which fat agglomeration has increased in the absence of guar gum is still not yet fully understood and further investigations would be required.

4.3.4 Air phase

The structure of air cells has been identified as one of the main factors influencing the rheological properties during ice cream consumption, which has been correlated to the perception of creaminess (BahramParvar & Mazaheri Tehrani, 2011). Small dispersed air cells also aid in the ease of scoopability (Goff & Hartel, 2013).

The effects of different fibre formulations with and without guar gum on the air cell microstructure in addition to the impact of temperature cycling on microstructure are reported in Figures 4.24 and 4.25, respectively. The effect of temperature cycling on the microstructure of the Standard formulation is shown in Figure 4.26. Overall, initially all formulations have small discrete air cells generally smaller than 100 μm. Temperature cycling caused a dramatic increase in air cell size. All samples stabilised using plant fibres only (Figure 4.24), observed a loss of discrete air cells by coalescence and the formation of air channels which can lead to product shrinkage (Hui, 2005). These micrographs suggest that the presence of fibres in formulations are acting as antifoams during temperature cycling, by destabilising the foam structure. Ice aids in preventing air cell coalescence. However, the loss of structure from ice as a result of temperature fluctuations and changes in air pressure with temperature increase can lead to coalescence of air cells and eventually sufficient coalescence can result in air channelling (Chang & Hartel, 2002a). Large voids within the microstructure as a result of coalescence were observed for FibreGel CF (Figure 4.24 b) whereas Psyllium (Figure 4.24 c) exhibited both large discrete air cells as well as channelling. AQ+ CF also exhibited a mixture of channelling and large discrete air cells. The ability to retain discrete air cells is dependent on the structure provided by the continuous phase that aids in preventing air cell coalescence (Goff, 1997). Additionally, it could be hypothesised that plant fibres may provide an additional network structure within the continuous phase, propping up the air structure and preventing

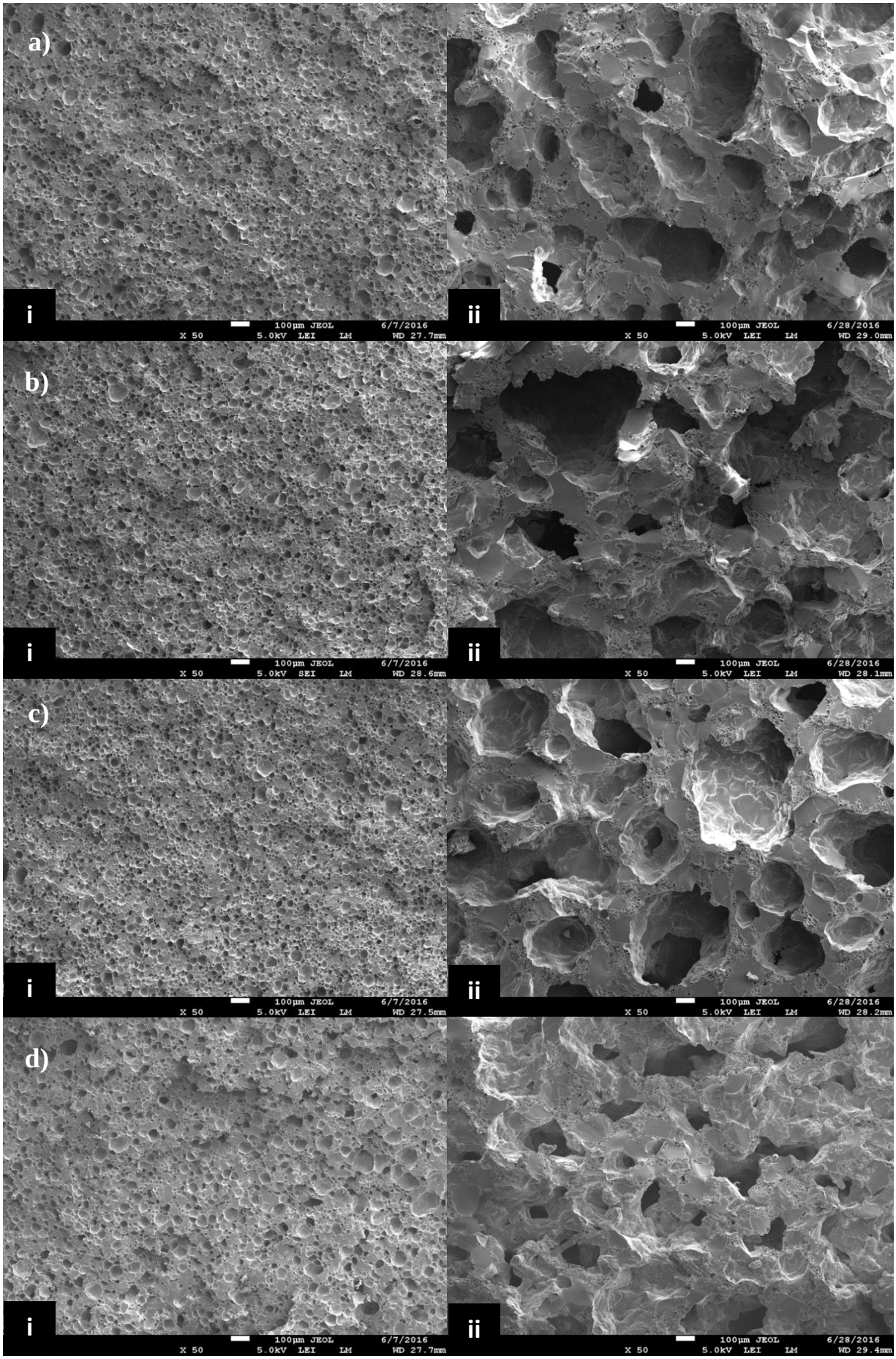

Figure 4.24 Cryo-SEM microstructure images of air cells in fresh and temperature abused samples containing different plant fibres (a) AQ+ CF (b) FibreGel CF (c) Psyllium (d) Sugar Cane (i) fresh (ii) temperature abused. Scale bars shown represent 100 µm.

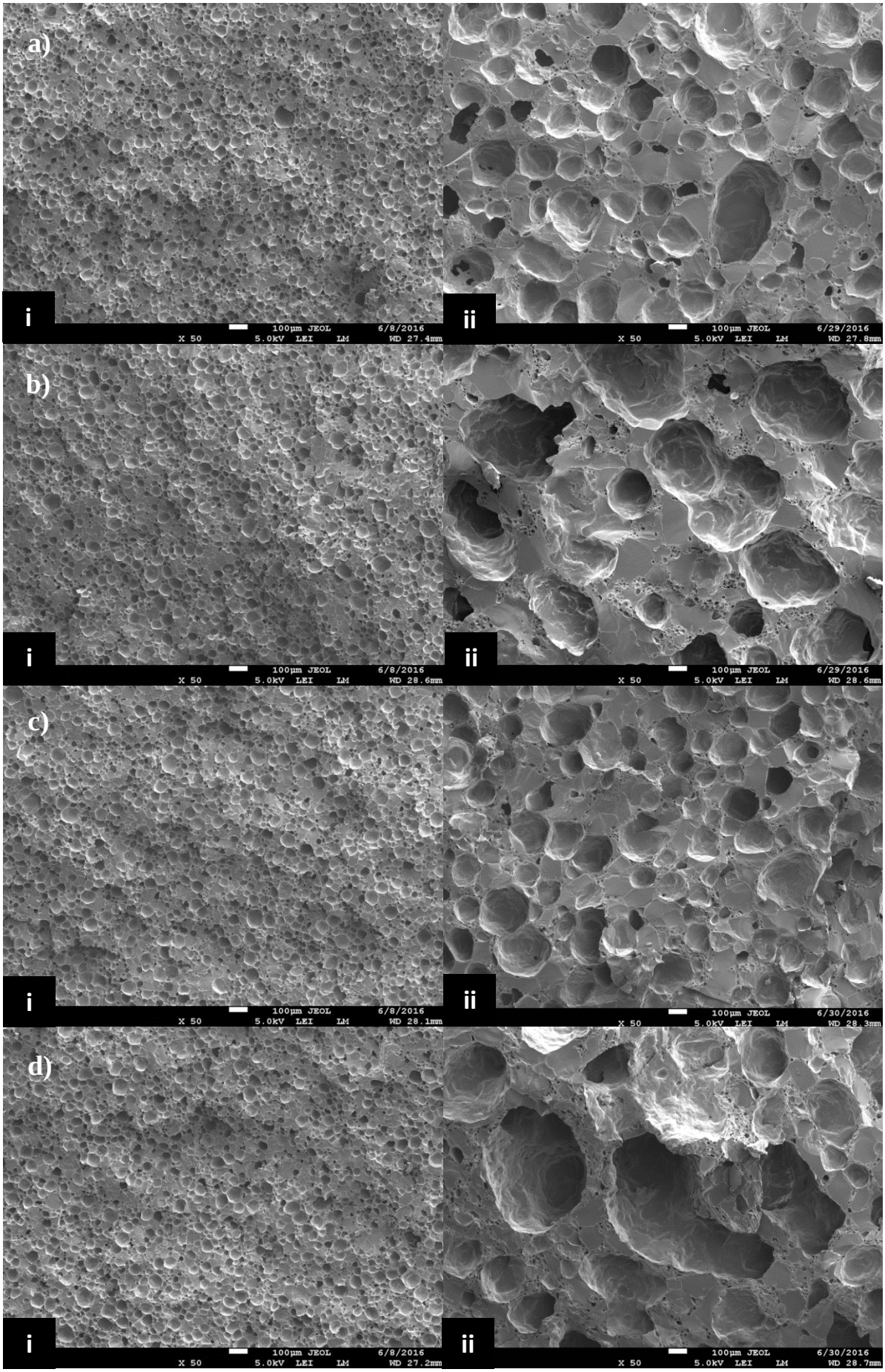

Figure 4.25 Cryo-SEM microstructure images of air cells in fresh and temperature abused samples containing different stabiliser blends (a) AQ+ CF/Guar (b) FibreGel CF/Guar (c) Psyllium/Guar (d) Sugar Cane/Guar (i) fresh (ii) temperature abused. Scale bars shown represent 100 µm.

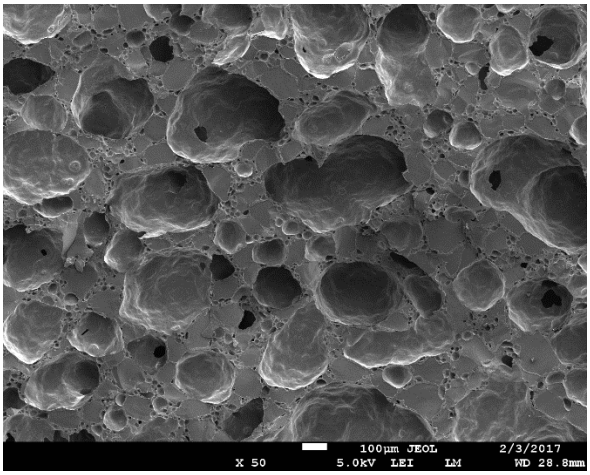

Figure 4.26 Cryo-SEM microstructure image of the temperature abused Standard sample. Scale bar shown represents 100 µm.

coalescence by a physical barrier. However, this is not fully reflected here. The microstructure of Sugar Cane Fibre (Figure 4.24 d) suggests that it has a poor ability to provide structure in the continuous phase. The microstructural images suggest structural collapse has occurred due to the presence of extremely irregular collapsed air cells. This effect was expected due to the poor shape retention of Sugar Cane Fibre stabilised samples observed during meltdown assessment (Figure 4.14).

The effect of blending fibres with guar gum is presented in Figure 4.25. An initial increase in air cell size with guar gum was observed. This is most likely due to a viscosifying effect as it becomes harder for air to be incorporated with an increase in mix viscosity (Goff & Hartel, 2013). In addition, cryo-SEM assessment of air-serum interfaces of formulations containing guar gum identified a reduction in the amount of fat adsorbed to the air interface. Sung & Goff (2010) identified a relationship between decreasing fat adsorption and increasing air bubble size. During whipping, the shearing effect of the blades can result in the rupture of air cells. A

higher degree of fat adsorption can prevent this rupture, hindering coalescence and thus preserving smaller air cells.

On the contrary, in terms of the effects of temperature cycling on the microstructure of co-stabilised samples with guar gum, the extent of microstructure change is dramatically reduced. Large coalesced air cells and air channelling were still observed in both FibreGel CF/Guar and Sugar Cane/Guar (Figures 4.25 b and 4.25 d, respectively). However, substantially less air channelling and coalescence was observed in ice creams stabilised with AQ+ CF/Guar and Psyllium/Guar (Figures 4.25 a and 4.25 c, respectively). This suggests that ice creams stabilised with AQ+ CF/Guar and Psyllium/Guar have better control over air cell microstructural changes as large discrete air cells make up the majority of the microstructure. In addition, in comparison to the Standard formulation, co-blending fibres with guar gum can achieve control over coalescence similar to and in the case of AQ+ CF/Guar and Psyllium/Guar better than the Standard formulation.

The effects of temperature cycling on ice cream macroscopic structure is reported in Figure 4.27. Generally, the addition of guar gum to formulations improved the resistance to shrinkage and drainage. Drainage involves the rise of air cells and subsequent downward flow of the serum phase. As air cells become larger with coarsening, the rate of drainage increases. The serum phase between air cells acts as the middle lamella. The drainage of the serum phase between air cells changes the film thickness between the air cells and promotes coalescence. The drained serum phase then forms as a sugary liquid phase at the bottom of the sample. Sofjan & Hartel (2004) reported that an increase in serum phase viscosity can successfully retard drainage. FibreGel CF (Figure 4.27 b1) was observed to have the most structural deterioration in comparison to all the other formulations as a large loss of volume in addition to serum drainage was observed. This is expected due to the large amount of coalescence observed in cryo-SEM images (Figure 4.24 b). Psyllium (Figure 4.27 c1) also exhibited a small

amount of drainage. This effect could be attributed to the lower mix viscosity of the two formulations. It has also been suggested that a faster melting product can be considered more prone to heat shock (Goff & Hartel, 2013). This may offer an explanation as to why the FibreGel CF formulation is not performing as effectively as the other formulations. However, the addition of guar gum, increases the viscosity of the serum phase, preventing serum drainage. It could be hypothesised that during temperature fluctuations the fibres prevent the dilution of the serum phase caused by melting ice by holding onto any melted water, thus preventing serum drainage. However, if a fibres functionality is in retarding the rate of water percolation rather than physically holding onto melted water and then releasing it again on freezing, then with enough time serum drainage will still occur.

Interestingly, in the Cryo-SEM assessment, Sugar Cane Fibre (Figure 4.24 d) had very irregular shaped air cells which might suggest extreme shrinkage. However, this effect is not seen on the macroscopic scale (Figure 4.27 d). After temperature abuse, the Standard formulation showed no evidence of shrinkage or serum drainage.

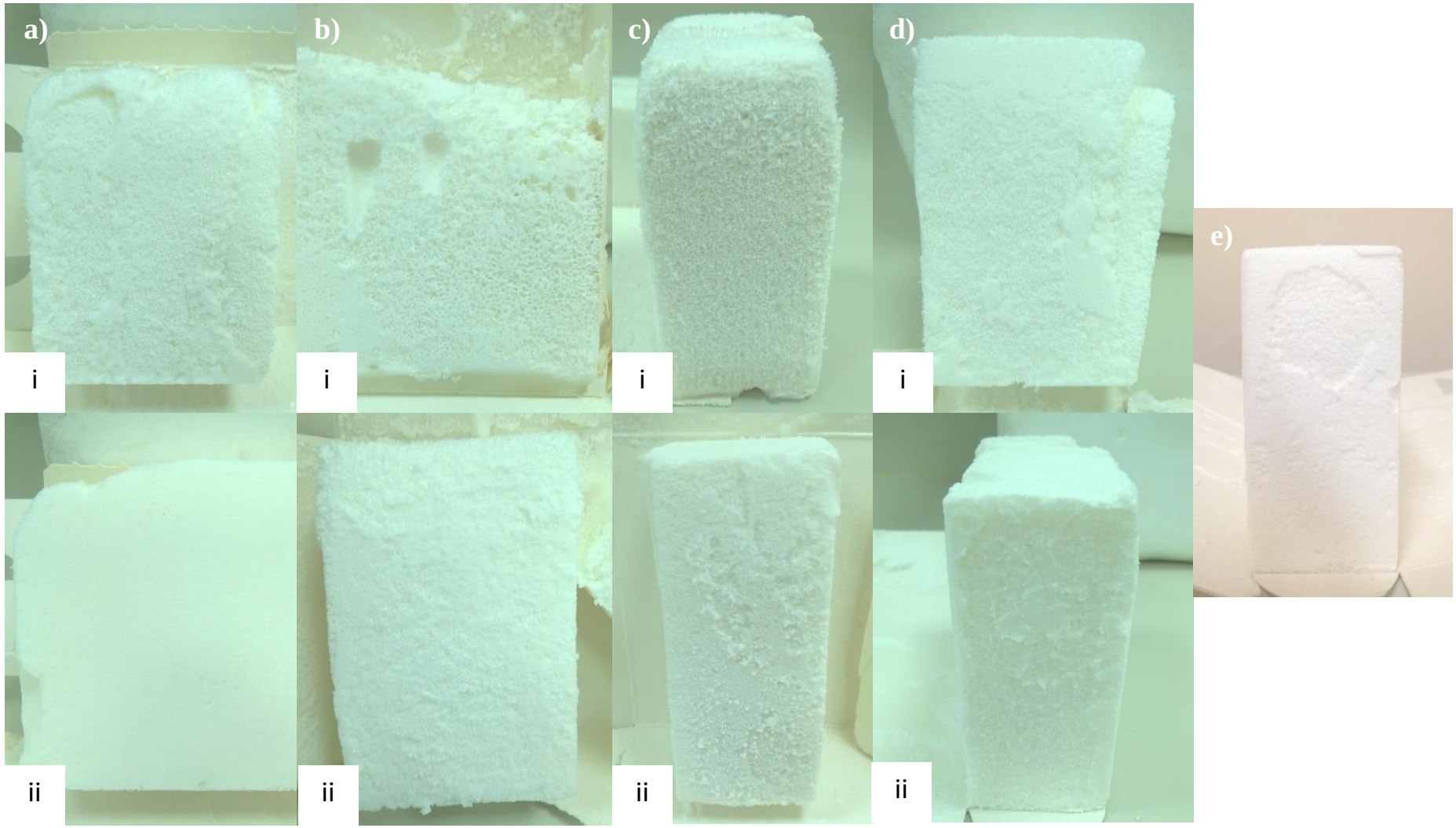

Figure 4.27 Images indicating the change in ice cream structure after temperature abuse cycling and the impact of different fibre types and stabiliser blends (a) AQ+ CF (b) FibreGel CF (c) Psyllium (d) Sugar Cane (e) Standard (i) without guar gum (ii) with guar gum.

4.4 Conclusions

The effect of plant fibres on the physical properties of an ice cream mix and the impact this has on ice cream microstructure was assessed. In addition, the efficiency of plant fibres in controlling microstructural deteriorations with temperature abuse revealed differences in the effectiveness between different plant fibres. Control over microstructural deteriorations was also provided by co-stabilising plant fibres with guar gum.

Plant fibres were not found to have an impact on the glass transition temperature of a mix but could sufficiently reduce the molecular mobility of water. Meltdown assessment of fibre only stabilised samples identified differences in the meltdown rate and the onset of melting between different plant fibres. Meltdown behaviour has been identified to vary with ice crystal size, air cell size, viscosity and fat structure. In the case of fibre only stabilised samples, little differences in the structure of ice, air and fat could be observed from the measurements undertaken in this chapter between samples. Thus, the differences in meltdown will primarily be as a result of varying apparent viscosities, in particular the consistency coefficients but also down to the ability of the plant fibres to absorb melting water and retard the rate of drip. The results show that AQ+ CF provides the best control over the rate of meltdown but Psyllium substantially retarded the onset of ice cream melting even though Psyllium mixes demonstrated the lowest apparent viscosity. Moreover, in comparison to a Standard formulation plant fibres did not reduce the rate of melting nor the final mass loss but did retard the time till first drip. Co-blending fibres with guar gum improved the meltdown properties due to an increase in viscosity. However, the large reduction in the shear thinning behaviour of co-stabilised samples may result in over-texturisation of the ice cream.

Sugar Cane Fibre exhibited poor shape retention properties during meltdown due to a poor ability to provide structure and create a fibrous network within the continuous phase. This was

reflected in cryo-SEM assessment as air cells became irregular and collapsed in shape with successive temperature cycling. FibreGel CF also exhibited a poor ability to prevent air cell coalescence and to retain discrete air cells which resulted in the macroscopic manifestation of serum drainage and shrinkage. FibreGel CF was identified to have the fastest meltdown rate. A faster melting product has been outlined as more prone to heat shock deteriorations and thus the deteriorations seen may be as a result of its rapid melting rate. On the contrary for Psyllium and AQ+ CF, a mixture of discrete air cells and substantially less air cell channelling was observed with temperature abuse and these properties were improved by co-stabilising with guar gum. However, fewer fat droplets could be identified at the air-serum interface in guar containing samples and this may have resulted in the increase in the air bubble size of fresh samples observed. Meltdown assessment was not performed on temperature abused samples. It may be expected that the increases in air bubble size, ice crystal size, and changes in the width of lamella between air bubbles as a result of temperature abuse may result in more rapid meltdown rates.

Confocal microscopy identified phase separation between plant fibres and proteins. However, in formulations containing guar gum, phase separation between hydrocolloids and proteins resulted in a change in structure with the presence of a guar enriched phase and a protein, fat and fibre enriched phase. Freeze-thaw assessment also identified that two freeze-thaw cycles had very little impact on fibre functionality. However, it is proposed that with further successive cycling fibre functionality may still be hindered. In addition, large differences in the structure of fibres were identified during confocal analysis. FibreGel CF, AQ+ CF and Sugar Cane all exhibit fibrous cellulosic structures in ice cream formulations with varying aspect ratios. FibreGel CF was found to have the smallest fibres in formulations with low specific volumes whilst Sugar Cane Fibre had the highest. FibreGel CF contains the highest amount of soluble fibre but this has not provided it with the most control over ice

recrystallisation. In fact the high water binding capacity of the highly cellulosic Sugar Cane Fibre had provided protection over ice recrystallisation in fibre only stabilised samples. However, the structure of the arabinoxylan from Psyllium is very different and it seems to behave as a particulate gel. Co-stabilising Psyllium with guar results in a phase separation and phase concentration of these gel-like particles which would freeze-concentrate at sub-zero temperatures forming a gel-like system. It is these structures that seem to be providing optimum control over ice and air coarsening with temperature abuse especially when co-stabilised with guar gum.

The results from this chapter suggest that Psyllium and AQ+ CF are behaving as the most suitable ice cream stabilisers. However, the sensory analysis to be undertaken in Chapter 5 will confirm if this is in fact the case. Therefore, given what is understood about the fibres so far and the little impact they have on the correct microstructure formation in unabused ice cream, it could be expected that the presence of fibres within formulations has little impact on the sensory properties of ice cream. However, differences may arise after samples have been subjected to heat shock as the destabilisation of the foam structure in plant fibre stabilised ice cream may affect the scoopability and hardness of the ice cream. Given that FibreGel CF has been identified to be more prone to heat shock, it can be expected that FibreGel CF samples will have a low sensory quality after heat shock.

CHAPTER 5: SENSORY EVALUATION

5.1 Introduction

Ice cream is a popular product among consumers, favoured for its sensory properties of a sweet flavour, smooth, creamy texture and cold sensation that contrasts other foods (Goff & Hartel, 2013). It is the compositional and microstructural aspects of ice cream that give rise to these desired sensory properties (Warren & Hartel, 2014). Although taste and flavour contribute largely to consumer acceptability of ice cream, mouthfeel too is an important characteristic. As ice cream is a structurally complex food product, mouthfeel is also a complex aspect involving structural and compositional properties (fat, sugar and hydrocolloids) and processing factors (homogenisation, aging, freezing and storage conditions) (Soukoulis et al., 2008). Though physical measurements can provide information on how product reformulation may impact on the properties of ice cream, due to the complex nature of ice cream, only sensory analysis can provide detailed information on the sensory properties perceived during consumption.

5.1.1 Sensory evaluation of ice cream

The sensory texture of ice cream has a large dependence on temperature. During ice cream consumption, a variety of significant temperature changes take place including phase changes from ice to water, the melting of fat, shear thinning and dilution of the matrix phase and the destabilisation of the foam structure (Moore & Shoemaker, 1981). With this, ice cream is converted from a cold semi-solid to a smooth and creamy viscous dispersion as the temperature increases (Varela et al., 2014). Previous methodologies for the sensory assessment of ice cream have revolved around descriptive analysis (Ohmes et al., 1998; Schaller-Povolny & Smith, 1999), in particular Quantitative Descriptive Analysis (QDA) (El-Nagar et al., 2002), time intensity methodology (Moore & Shoemaker, 1981; Frøst et al., 2005), discriminative tests (Balthazar et al., 2015) and assessment of consumer acceptability (Guinard et al., 1996;

Soukoulis et al., 2010a). Sensory evaluation data has often been correlated with instrumental measurements to identify if physical measurements can be used as predictors for sensory properties (Guinard et al., 1997; Aime et al., 2001) or combined with consumer hedonic data to investigate the sensory properties that drive consumer acceptability (Dooley et al., 2010; Varela et al., 2014).

Moore & Shoemaker (1981) used time intensity measurements as a means of assessment to take into account the phase changes that occur as a product melts during consumption. Ice creams stabilised with varying concentrations of carboxy methyl cellulose (CMC) were assessed by trained panellists that recorded responses in coldness, iciness, viscosity and melting time as a function of product temperature in mouth. CMC, a modified cellulose, was found to decrease the perception of coldness on increasing CMC concentration. Increasing CMC concentration resulted in a decreased intensity of iciness but an increase in the duration of perception of iciness along with a reduction in the rate of melting. It was proposed that this was due to an increase in viscosity which reduced the rate that heat could be transferred to melt the ice crystals. More recently, Varela et al. (2014) used the more sophisticated methodology of Temporal Dominance of Sensation (TDS). TDS can be used to study the appearance and evolution of different sensory attributes over time by reporting on the intensity of an attribute that is dominant at that given time. In this work, ice cream formulations were varied to obtain different textures to be assessed under TDS by varying the ice content. It was found that the addition of hydrocolloids reduced the first impact of iciness and coldness while enhancing an early perception of creaminess. The information from TDS was combined with consumer liking scores and consumer sensory data generated using check-all-that-apply (CATA) methodology to determine the sensory attributes that help to drive consumer liking. It was found that creaminess is the determining factor for consumer hedonic responses while samples that were cold and icy were not very well liked.

Many publications investigate the sensory property of creaminess in ice cream. However, creaminess is a difficult term to describe in food as it involves a number of factors including both texture and flavour attributes. In the case of ice cream, creaminess is associated with the fat content and a product free of particles that melts uniformly to a soft viscous mouth coating fluid (Antmann et al., 2011). The absence of large ice crystals and a small air cell size distribution have also been reported to boost the perception of creaminess (Wildmoser et al., 2004). Creaminess has also been related to flavour perception, particularly to vanilla (de Wijk et al., 2003). Thus it is wise during sensory testing to break creaminess down to its constituent attributes.

As consumers are becoming more health conscious, there is a large push to develop low fat and reduced sugar ice creams that poses the same organoleptic properties as full fat and sugar products. Cadena et al. (2012) used Quantitative Descriptive Analysis (QDA) to map the sensory attributes of reduced fat and sugar commercial vanilla ice creams. External preference mapping was used to combine sensory data with consumer hedonic data to determine drivers for liking. Low fat ice creams were found to be significantly paler in colour than full fat ice cream due to the yellow colour that milk fat imparts. In addition, low fat ice creams were perceived as less sweet, having a lower vanilla flavour intensity and were perceived as less creamy. The use of aspartame and sodium cyclamate in reduce sugar ice creams imparted a bitter aftertaste. Replacement of fat and sugar resulted in firmer and harder ice cream. External preference mapping separated the samples into two groups of ‘high quality products’ with creamy sensory properties which were the most accepted samples by consumers and were the full fat ice creams and ‘low quality products’ with bitter aftertastes and a hydrogenated fat aroma which were less accepted by consumers and was the reduced fat and sugar products. However, partial least squares regression analysis identified that a products appearance has a large influence over acceptability.

Further advances have been made to improve the perceived fattiness, creaminess and thickness in low fat emulsions to match that of full fat products using microstructure design. Fat perception is suggested to be a result of an interaction between emulsion droplets and the tactile receptors on the tongue. In the mouth, emulsion droplets interact with the saliva and the tongue surface. As the emulsion mixes with the saliva it changes structurally (Silletti et al., 2007). It has been found that droplet aggregation and coalescence in mouth increases the viscosity of the saliva-emulsion mixture increasing the perception of fat related sensory attributes (Dresselhuis et al., 2008). Benjamins et al. (2009) found that the in-mouth aeration to produce a foam and the partial coalescence of low fat content emulsions, by a means of the shear forces generated by the tongue during consumption, significantly increased the perception of fat related sensory attributes.

El-Nager et al. (2002) assessed the suitability of inulin as a fat replacer in low fat yog-ice cream formulations. Using QDA methodology it was found that low-fat products were harder, icy, and coarse while high fat products were smoother and softer. The addition of inulin to low-fat formulations improved the sensory properties to that similar to the full fat products suggesting that inulin has the ability to impart the correct mouthfeel properties in ice cream. It was suggested that the improved mouthfeel of the low-fat inulin products was due to a reduced meltability. González-Tomás et al. (2009) reported on the suitability of long chain (22-15, β-2,1 linked fructose units) and short chain (2-7 units) inulin as a fat replacer in low-fat custard. It was identified that short chain inulin enhanced flavour and sweetness in low-fat samples. However, the addition of long-chain inulin improved the consistency and creaminess of low-fat custards mimicking the full-fat products. It was proposed that long chain inulin is able to form crystals and crystal aggregates that impart the fat mimetic properties, though crystallisation was found to be dependent on both the chain length (degree of polymerisation) and the inulin concentration.

Following the push in trends to develop healthier and functional foods requested by consumers, ice cream is now being considered a tool for probiotic fortification. However, the manufacturing conditions involved in producing ice cream have been found to reduce microbe viability. The freezing process (Akalin & Erişir, 2008) and aeration of a mix (Ferraz et al. 2012) have both been found to cause a loss of cell viability in probiotic ice creams. To investigate a means to improve cell survival during ice cream manufacture Homayouni et al. (2008) utilised microencapsulation of Lactobacillus strains within calcium alginate microbeads to assess the survival of viable cells through the manufacturing process. Finally the impact of reformulation with probiotic microbeads on the sensory properties and overall acceptability was assessed by trained panellists using 1-9 scales. Survival rate of bacterial cells was increased by 30% when bacterial strains were microencapsulated compared to when they were freely incorporated within a pre-mix. However, culture survival was found to have no effect on yogurt/probiotic flavour as no significant differences in flavour/taste, body/texture or colour/appearance were identified between encapsulated and non-encapsulated probiotic samples. Thus, overall acceptability between samples was not found to significantly vary as no discerning sensory attributes could be identified.

Yeomans et al. (2008) investigated the role of flavour expectation in the sensory properties of ice cream based on appearance and food labels. An outlandish smoked salmon ice cream was developed and assessed along with a series of inaccurate food cues to assess how flavour cues prior to ingestion predict a flavour which in fact is in marked contrast to the actual flavour characteristics. The results found that by labelling a product as ‘ice cream’ consumers expect a sweet, fruity flavour, consistent with the visual appearance of ice cream but in fact upon consumption what they received was salty and fishy. When labelled as ‘ice cream’ acceptability of smoked salmon ice cream was very low. However, when novel labels were used – ‘a frozen savoury mouse’ consumer acceptability largely improved. This shows the importance of food

labels, expected and actual sensory qualities and the impact these can have on positive or negative responses to a new product concept.

Soukoulis & Tzia (2010c) used response surface methodology to optimise consumer acceptability of chocolate ice creams containing alternative sweeteners derived from grape juice concentrate and sugar cane molasses. The surface contours generated allowed a visual representation of the impact of formulation and varying concentration on consumer acceptability. This allowed a clear indication that grape fruit juice concentrate resulted in optimum hedonic ratings. However, acceptability of formulations containing these alternative sweeteners was largely concentration dependent which was clearly illustrated by the surface contours.

All these studies show the implications reformulation can have on consumer acceptance of ice cream and how consumer liking of a product can be significantly hindered with deviation from the expected sensory attributes. These studies also show the importance of selecting the most suitable sensory testing methodology and statically analysis method to achieve the outcomes required.

5.1.2 Correlations between ice cream microstructure and sensory perception

The impact of ice cream microstructure on the sensory properties of ice cream has been largely covered in the literature as a means to provide an understanding of how analysis of the size, structure and morphology of the microstructural components of ice cream can be used as sensory predictors.

Warren & Hartel (2014) investigated the correlations between the physical measurements and the sensory properties of commercial vanilla ice creams using Spectrum ^{TM} descriptive analysis, assessed using a trained panel. Spectrum ^{TM} methodology allows the sensory profiles

generated to be universally understandable, usable and replicated across any laboratory and panel. Panellists score the perceived intensity of attributes selected from a sensory lexicon against a reference sample with pre-defined and pre-learned intensities. It is often favourably used by R&D facilities who wish to perform sensory analysis of products across several manufacturing sites (Meilgaard et al., 2006). Pairwise comparison and principal component analysis (PCA) identified that fat content and the degree of fat destabilisation was positively correlated with the perception of creaminess. No correlation between the rate of ice cream meltdown in ambient conditions and sensory meltdown was identified. It was suggested that higher temperatures and the mechanical forces imparted by the tongue during sensory meltdown make the conditions in which ice cream melts in the mouth not comparable to ambient meltdown. In contrast, percentage fat destabilisation and mean air cell size distribution was inversely related to the sensory meltdown rate. It was found that ice crystal size did not correlate with the perception of iciness. The work identified that high fat products had the lowest intensity of iciness even though physical measurements identified that these products contained the largest mean ice crystal size distribution.

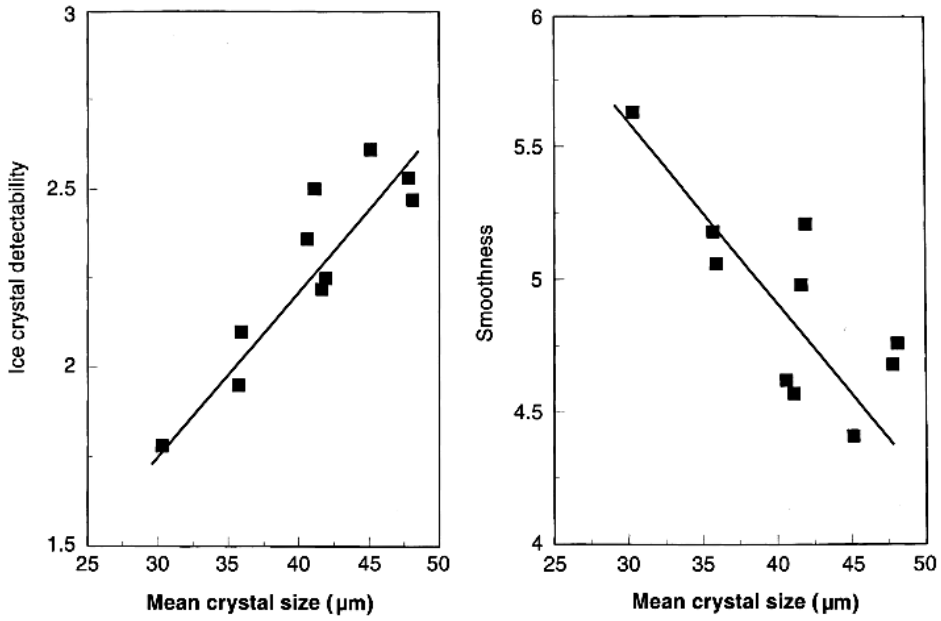

Figure 5.1 Scores from a trained panel for ice crystal detectability and smoothness versus mean ice crystal size for an 8% w/w diary fat ice cream (Russel et al., 1999b).

This suggests that formulation has a large impact on the perception of iciness in particular the thickness and firmness of ice cream. However, this work is in disagreement with Russel et al. (1999b) who identified a strong positive correlation between increasing ice crystal size and the increasing perception of iciness (Figure 5.1). In addition, it was identified that smoothness increased as ice crystal size decreased.

A small air cell size distribution aids in ice cream scoopability and creaminess during consumption (Eisner et al., 2005). Wildmoser et al. (2004) used oscillatory thermo-rheometry (OTR) at low deformation amplitudes to correlate the rheological properties of G' (storage modulus) and G" (loss modulus) with the microstructure and sensory characteristics of ice cream. The storage modulus describes the elastically stored deformation energy and the solid-body like behaviour. The loss modulus represents the viscous fluid behaviour (flowability). It was found that as the ice fraction and rigidity increased due to decreasing temperature, G' and

G" increased. Additionally, the higher the overrun and the smaller the connectivity between ice crystals the smaller the measured G' and G" at sub-zero temperatures (-15°C). In the molten state (>0°C), higher overruns resulted in higher G' and G". It was suggested that mouth viscosity is achieved by palate lubrication due to the persistence of the molten ice cream foam in the mouth between the tongue and palate. Improvements in scoopability and creaminess were correlated with a decrease in G" at -15°C and an increase of G" in the molten state. Additionally, Balthazar et al. (2015) suggested that increasing the viscosity of an ice cream pre-mix increased the sensory firmness of an ice cream. It was also found that larger air cells contributed to a firmer product.

This suggests that the differences in viscosity, in particular the consistency coefficient observed during rheological measurements of the plant fibre stabilised ice cream pre-mixes in Chapter 4, may have an influence on both the perceived iciness and firmness in the frozen state, but also on the perceived thickness and mouthcoating sensations in the molten state. Given that cryo-SEM imaging in Chapter 4 identified little difference in the foam structure and air bubble size of un-abused samples, it is suggestive that little difference in the scoopability of the plant fibre stabilised samples will be observed. However, the foam structure was found to destabilise with temperature abuse and so it may be expected that the scoopability of the temperature abused samples will decrease.

5.1.3 Impact of heat shock on the sensory properties of ice cream

It is generally accepted that a quality ice cream is one that has a smooth texture without detectable ice crystals. However, one of the largest and most noticeable quality deteriorations noticed by consumers in ice cream is the development of a coarse or icy texture due to ice recrystallisation (Goff & Hartel, 2013). Therefore there is a large amount of literature

investigating the ability of different stabilisers or new and novel ingredients in their ability to prevent a coarse and grainy ice cream texture during long storage periods or under heat shock conditions. Buyck et al. (2011) found that heat shock had a significant effect on iciness and creaminess of ice cream due to the impact of ice recrystallisation. Regand & Goff (2006) assessed the ability of ice structuring proteins (ISPs) from cold-acclimated wheat grass in their ability to prevent microstructural coarsening and sensory deteriorations with heat shock. IPS's, previously termed antifreeze proteins, are a protein that can be present in living organisms to allow them to overcome extremely low temperature environments. This special type of protein has been found in fish, insects, over-wintering plants and in bacteria, fungi and lichen (Griffith & Ewart, 1995). Using a trained sensory panel, ISP were found to significantly reduce the perception of iciness in ice cream subjected to heat shock. Their suggested mechanism of control is by an absorption mechanism to the ice crystal surface that modifies ice crystal morphology. However, light microscopy measurements assessing crystal growth rates found that ISP provided no control over the early stages of accretion but had more of a control over Ostwald ripening. A synergistic effect between stabilisers and IPS was found as ISP activity was reduced in the absence of stabilisers. It was suggested that the additional reduction in mobility and viscosity enhancement of the serum phase with the addition of stabilisers favoured ISP functionality. The use of ISP in ice cream and ice lollies is a patented technology owned by Unilever and is currently being exploited in commercial products (Linder et al., 2004).

Long storage periods of ice cream can have a similar impact on the sensory properties of ice cream. Soukoulis et al. (2008) investigated the impact of long storage periods (4 months) on the quality of hydrocolloid stabilised ice creams. It was found that storage time impacted on the temporal release and perception of vanilla flavour. The increase in numbing sensation as a result of an increase in ice crystal size resulted in a depression in the perception of vanilla flavour. It was found that the higher the hydrocolloid concentration, the better the vanilla

flavour was perceived. This would most likely be due to the cryoprotection offered by hydrocolloid stabilisers as if ice crystals remain small in size this reduces the numbing sensation and vanilla flavour can be perceived more readily. However, others have reported that hydrocolloid addition can result in flavour suppression due to increases in gumminess, although a rapid shear thinning behaviour of a hydrocolloid can aid in flavour release (Stephen, 1995). Baines & Morris (1987) investigated the taste and flavour perception in guar gum thickened systems by modifying the polymer concentration above and below the c*. Below the c*, which is the coil overlap concentration, the polymer is considered to be in the dilute regime where polymer molecules can move independently from each other. Above the c*, viscosity increases rapidly due to polymer entanglement. It was found that below the c*, guar gum had no significant impact on the perception of sweetness or flavour. However, as the concentration increased beyond the c*, where guar forms an entangled network, the perceived intensity of both attributes decreased steeply with increasing polymer concentration.

Contrary to ice recrystallisation, there are also a number of colloidal changes that occur during temperature abuse. Baer et al. (1999) assessed the stability of non-fat ice cream with and without low molecular weight surfactants to heat shock. It was found that ice creams containing surfactants received lower intensity scores for coldness than the control sample. It was proposed that surfactants improve the stability of the foam structure to heat shock by stabilising the air-serum interface. Maintenance of the finely dispersed foam structure improved heat shock stability, dampened the perception of coldness and therefore improving sensory quality.

Given that increases in the perception of iciness of heat shocked ice cream appears to be a large issue noticed by consumers, it is desirable that the plant fibres in the present study, to be considered as a suitable alternative to hydrocolloid stabilisers, should either meet or exceed the control provided by hydrocolloid stabilisers.

5.1.4 Sensory evaluation of plant fibre stabilised ice creams

Dervisoglu & Yazici (2006) used a semi-trained panel and category scale methodology to assess the impact of citrus fibre as a stabiliser (AQ+ Citrus Fibre, from Herbafoods Inc.) on the sensory properties of ice cream. Panellists gave samples scores for overall texture, appearance and flavour using 1 – 9 scales in comparison to a reference sample used during panellist training (slight 9, moderate 7, moderate-strong 3, strong 1). Sensory assessment identified that citrus fibre-only stabilised samples achieved lower scores for texture and body in comparison to hydrocolloid stabilised samples. Their results suggest that AQ+ CF was unable to impart the correct mouthfeel properties in ice cream even across a variety of concentrations from 0.4% to 1.2%. However, the citrus fibre samples were formulated without emulsifiers and assessed in comparison to stabiliser/emulsifier sample. As the research undertaken in Chapter 4 of this thesis suggests citrus fibre cannot provide emulsifying properties, the absence of emulsifiers which aid in the development of a destabilised fat network that encases and stabilises the foam structure will result in a large loss of mouthfeel properties due to a poor microstructure. However, in this work the authors did not investigate the microstructures of these samples, and so the differences in scores for mouthfeel can only be suggested to be due to large differences in microstructure due to the absence of emulsifiers. It was also found that citrus fibres at a high concentration of 1.2% imparted unwanted flavour defects.

BahramParvar et al. (2010) assessed the relationships between physical measurements and the sensory properties of ice creams stabilised with carboxy methyl cellulose (CMC), Balangu seed gum (BSG) and palmate-tuber salep (PTS). Strong correlations between instrumental viscosity and sensory viscosity were identified due to the assessment of apparent viscosity at a shear rate of 51.8 s^{-1} which is similar to the sensing shear rate in-mouth (Rao, 2014). High viscosity was also positively correlated with a reduced perception of coldness, increased firmness and reduced sensory melting rate. Trained panellists also rated overall acceptance of ice creams

using a 9-point hedonic scale and identified that CMC and BSG stabilised samples were more accepted. However, it is generally considered that trained panellists should not be used to generate consumer hedonic data in amongst sensory profiling due to their heightened sensitivity to sensory testing. Also the panellist sample size of 10 panellists is far below 100 consumers required to generate reliable consumer hedonic data (Meilgaard et al., 2006).

Yangilar (2015a) assessed the sensory impact of banana peel and pulp fibre on ice cream formulations. Their research found that fibre obtained from banana pulp had a higher water binding capacity (WBC) after heating to 80°C (6.42 g water/g fibre) than fibre obtained from the peel (5.60 g/g), suggesting that the pulp has higher contents of pectin, which solubilises on heating and would contribute to an increase in water binding. However, the differences in WBC were not found to have an impact on the sensory properties of ice cream and in fact fibre addition was found to have no impact on texture, body and flavour in comparison to a control sample. In Chapter 4 of this thesis is was found that control over ice recrystallisation improved by a fibre with a higher WBC. Unfortunately, Yangilar (2015a) did not investigate the impact of WBC on sensory iciness. Subsequently, Yangilar (2016) compared fibres obtained from peach peel and peach pulp on the sensory properties of ice cream. Fibres obtained from peach peel had a higher total fibre content of 57g/100g and higher soluble fibre content 12g/100g than peach pulp with a total fibre of 51g/100g and a soluble fibre content of 8g/100g. It was identified that fibre composition had an impact on the sensory properties of ice cream in comparison to a control sample. Peach peel fibre obtained higher sensory scores for body and texture, resistance to melting and mouth feeling suggesting a higher total fibre content and/or soluble content can improve the sensory properties of ice cream.

These studies suggest that good correlations between the physical measurements of the fibre stabilised ice creams in Chapter 4 and the sensory properties may be observed. These studies also suggest that plant fibres may impart some unwanted flavour defects but the remainder of

the sensory properties of ice cream should not be affected greatly and plant fibres should also be able to impart the correct mouthfeel properties in ice cream. However, the ability to impart the correct mouthfeel properties may be dependent on the compositional makeup of the fibres.

5.1.5 Aims of the chapter

The main aims of the research carried out in this chapter are as follows:

1. To identify which sensory attributes are affected by temperature abuse. This allows the identification of which sensory attributes plant fibres must control to behave as suitable ice cream stabilisers.
2. To determine the sensory attributes that change with varying fibre type (formulation effect). It is of great importance that plant fibres are bland/do not impart any unwanted sensory attributes.
3. To determine if plant fibres are able to impart the correct mouthfeel properties that are usually imparted by hydrocolloid stabilisers.
4. To determine the ability of the different plant fibres in preventing sensory deteriorations with temperature abuse.

Based on a pre-tasting of the fibre and fibre/guar gum stabilised samples performed in-house at Unilever, it was found that the fibre/guar samples were largely over-stabilised masking many of the other sensory properties. Based on this, the decision was made to perform sensory analysis on the fibre only stabilised ice cream samples. However, in terms of a research deliverable for Unilever, their aim was to discover a plant fibre that can be used on its own and exploited in clean label products, so no further attempts were made to reduce the guar gum/fibre concentrations to obtain a less over-stabilised product. However, given that it has been shown in Chapter 4 that the addition of a hydrocolloid stabiliser improves the functional properties of fibre-stabilised ice cream, it would be worth in future studies to lower the level of stabiliser addition to achieve the correct mouthfeel properties.

5.2 Materials and methods

Sensory evaluation was performed on formulations containing 0.6% fibre (AQ+ CF, FibreGel CF, Psyllium and Sugar Cane) and a Standard formulation stabilised using a 0.32% hydrocolloid stabiliser blend (Locust bean gum, Guar gum, Xanthan gum). Ice cream samples for sensory evaluation were produced one month prior to testing using the manufacturing method as outlined in Chapter 4. All samples exited the freezer at -6.2°C. Samples were put into 500 mL cardboard containers and stored at -30°C when not in use. Sensory evaluation was performed using fresh (un-abused), 7 day abused and 14 day abused ice cream samples. The abuse protocol consisted of temperature cycling the samples between -5°C and -15°C every 12 hours in controlled temperature cycling cabinets. All sensory evaluation was performed using trained panellists. Fifteen panellists were invited to attend a screening session and were asked to complete a series of sensory acuity tests including taste sensitivity, descriptive ability, reproducibility in assessment and texture recognition. Screening tests were designed to reflect the common sensory attributes of ice cream based on that of the literature (Cody et al., 2007; Soukoulis et al., 2010; Hettiarachchi & Illeperuma, 2015). Panellists that achieved 70% correct answers were considered suitable panellists. Based on the outcomes of panellist screening, nine panellists were recruited for training and assessment (all female assessors, with an average age of 43).

All testing was performed in a purpose built sensory facility under Northern Hemisphere lighting, under controlled air, temperature and humidity conditions. Panellists were provided with mineral water (Evian) and plain crackers (Rakusen's, Matzos) as palate cleansers.

5.2.1 Sample preparation

Prior to sample preparation, sample blocks were transferred to a -15°C freezer for 12 hours. Sample blocks were cut into 20 g samples and placed in 50 mL lidded polystyrene cups labelled with a random three-digit code. Samples were stored at -15°C and freshly prepared for each assessment. Samples were removed from the freezer and served at -15°C.

5.2.2 Attribute Generation

Sensory attributes were generated over three sessions. In the first session, to generate a list of sensory attributes, panellists were presented with pairs of samples and asked to describe differences in texture, flavour and appearance. These samples were selected from the fresh (un-abused) and 14 day abused ice cream samples and were chosen to represent all attributes. In booths, panellists received a total of five pairs of samples. The following questions were asked to aid in attribute generation:

1. How does it look?
2. How does it feel when you put the spoon in?
3. When you first put the sample in the mouth, how does it feel/taste?
4. Press some sample down between the molars, how does it feel?
5. When it starts to melt while agitating in the mouth, how does it feel/taste?
6. Once it is completely melted, before swallowing, how does it feel/taste?
7. After swallowing, how does it feel/taste?

In a second session, a total of 30 attributes were compiled and Check-all-that-apply (CATA) questioning was conducted to determine which attributes significantly varied among the sample set. In individual booths, panellists were presented with individual ice cream samples and asked

to tick all attributes from the list that most apply to that particular sample. The samples used for CATA analysis were the five fresh and five 14 day abused samples. The order of presentation of samples followed a randomised balanced design. Sensory attributes were also randomised. CATA analysis generated frequency counts on the number of times an attribute was ticked off for a particular sample and a Cochran's Q test was used to determine which attributes significantly varied between samples.

In a final session a group discussion was conducted to confirm the sensory attributes that largely differed between samples based on the attributes identified from the frequency counts of the CATA analysis. Panellists were asked to describe attributes to remove duplicates of similar terms, or opposites as well any ambiguous attributes. A total of 17 attributes were taken forward for sensory evaluation (Table 5.1 and 5.2).

5.2.3 Training

Training on the sensory attributes was conducted over five 2 hours training sessions (10 hours of training in total). Reference samples from the sample set of fresh (un-abused) and 14 day abused ice creams were provided to help aid in generating attribute definitions, assessment protocols and to position the panel on attribute intensity on a 10 cm linear scale. Reference samples for each sensory attribute were selected based on the results generated by the Cochran's Q test of CATA data. Panellists were then tested at the end of each session on the attributes covered using a series of ranking-rating tests whereby they received three samples, were asked to rank the samples and then rate the intensity. This was performed to ensure alignment of the panel and consistent rating by panellists between replicates.

5.2.4 Sensory Evaluation

Sensory evaluation was performed using Quantitative Descriptive Analysis (QDA) methodology across six 2 hour sessions following a balanced incomplete design. Panellists assessed 8 samples per session with 3 minute rest breaks between samples to prevent cold sensation carry over with a dummy sample at the beginning of each session to reduce first order effects. Panellists received three freshly prepared pots of each sample during evaluation to prevent the impact of sample melting on attribute intensities. In individual booths, panellists rated the intensity of each attribute on a 10 cm linear scale anchored at each end with '0' (no intensity) and '10' (high intensity). Each sample was evaluated in triplicate.

5.2.5 Sensory statistical analysis

Statistical analysis was performed using XLStat-Pro (Addinsoft, France). The frequency of use of each sensory attribute in the CATA questionnaire was determined by counting the number of panellists that used a term to describe each sample. Cochran's Q test was used to identify the significant differences between samples for each of the terms included in the CATA questionnaire.

Two-way analysis of variance (ANOVA) with interactions was used to identify the impact of heat shock and formulation on the significant differences in intensity of sensory attributes of plant fibre stabilised samples. Tukey's honestly significantly different (HSD) post hoc analysis of the difference categories with a confidence of 95% was used to identify significant differences in intensity of each attribute between samples. Principal component analysis was used to generate a biplot representing the relationships between samples and samples with attributes. A significance value of 5% was used for all statistical analysis.

Table 5.1 Sensory attributes and definitions relating to appearance, texture on spooning and mouth texture used during descriptive analysis.

Attribute	Definition
Appearance	
Cream Colour	The intensity of the cream colour from white to cream. Assessed by comparing to a white piece of paper.
Texture on Spooning	
Harness on Spooning in	Measuring the level of hardness by pushing the spoon in 90 degrees into the middle of the ice cream. The higher the resistance and force required to push the spoon in the harder the sample.
Pliable	Measuring the level of flexibility and elasticity perceived when pushing the spoon in 90 degrees. Bending of the sample is perceived as a high intensity, a clean cut of sample is perceived as a low intensity.
Dry	Measuring the level of dryness of the sample along with its lustre. The dryer the sample the more the lack of moisture in the sample. Assessed by putting a spoon in vertical and breaking the sample up.
Crumbly	Measuring the level of crumbliness perceived when pushing the spoon into and removing a sample. The higher the amount of breakage and shattering of the sample, the more crumbly it is.
Stickiness	Measuring the level of stickiness and tackiness of the sample adhering to the spoon upon removing the spoon from the sample. Measured by manipulating the sample for 15 seconds.
Mouth Texture	
Thickness	While the sample melts in the mouth, measuring the level of thickness/viscosity when it is moved around the mouth by the tongue.
Rate of Melting	Measuring the speed of melting by moving a sample around the mouth. Measured by counting the seconds taken to melt the sample. The higher the number the lower the intensity, the smaller the number the higher the intensity.
Watery	Measuring the level of wateriness perceived on melting in the mouth. A sample that melts unusually quickly to an uncharacteristically thin water like fluid or the sensation of a burst of water upon melting is a high intensity.
Firmness	Measuring the level of firmness when the sample is pressed between the tongue and palate. The higher the resistance to pressing, the firmer the sample.
Icy	Measuring the level of iciness of the sample after 1-2 chews between the molars before the sample has melted. The louder the breaking of ice crystals perceived, the higher the intensity.

Table 5.2 Sensory attributes and definitions relating to taste and flavour and after texture used during descriptive analysis.

Attribute	Definition
Taste and Flavour	
Caramel Flavour	Measuring the intensity of Caramel flavour detected in the ice cream, independent of sweetness.
Overall Vanilla Flavour	Measuring the overall vanilla flavour perceived.
Overall Sweetness	Measuring the intensity of overall sweetness perceived.
After Texture	
Powdery	Measuring the level of powdery feeling or particles left on the tongue perceived after the ice cream has melted, after the first swallow.
Mouth Coating	The degree of film formation on the tongue once the ice cream has melted after the first swallow.
Mouth Drying	The sensation of drying out of the tongue or the feeling of absorbance of moisture from the mouth once the ice cream has melted, after the first swallow.

5.2.6 Transmission electron microscopy

Portions of frozen ice cream samples were removed from the centre of the block and placed on an aluminium plate resting on a bed of dry ice. Several small pieces of samples were prepared (5mm x 2 mm x 2 mm) using a precooled scalpel and tweezers. Samples were then placed in a freeze substitution preparation chamber (Leica EM AFS2, Milton Keynes, UK) containing 4 mL of fixation solution. The fixation solution was prepared in three parts (A: 6 mL of glutaraldehyde in 100 mL methanol; B: 1 g osmium tetroxide in 46 mL methanol; C: 0.4 g uranyl acetate in 4 mL methanol) then combined to produce a fixation solution consisting of 0.5% osmium tetroxide (w/v), 1.49% glutaraldehyde (v/v), 0.4% uranyl acetate (w/v) and used immediately. In order to freeze substitute the ice by solvent the following program was carried out: -80°C for 96 hrs, increased to -40°C at 2°C/hr, held at -40°C for 48 hrs, increased to -20°C at 2°C/hr, held at -20°C for 24hrs, followed by 3 x 1 hr washes with ethanol and a 1 hr wash with acetone. All solvent washings were performed at -20°C. Resin infiltration was performed using the following protocol: acetone 9:1 resin for 12 hrs; acetone 7:3 resin for 12hrs; acetone 3:7 resin for 12 hrs. After the final infiltration the samples were removed from the preparation chamber and allowed to warm to room temperature before being placed in 100% resin for 24 hrs. After this the samples were placed into embedding moulds and cured with fresh resin for 48 hrs at 65°C. Ultrathin section (130 nm) of embedded material were cut using a Lecia UC6 microtome (Milton Keynes, UK), stained with a lead citrate solution, washed with distilled water and allowed to air dry. Embedded sections were examined in a JEM-2100 (JEOL, Massachusetts, USA) at 200kV and imaged using a Gatan 4K Ultrascan camera and associated digital micrograph software.

5.2.7 Ice crystal sizing

Assessment of the size of ice crystals in the samples used during sensory analysis was performed using the same method as outlined in Chapter 4.

5.2.8 Rotational and oscillatory rheology

Plant fibres (2% w/w) were dispersed in 80°C ± 2°C water and allowed to cool and hydrate overnight (4°C). Suspensions were homogenised in a single stage homogeniser at 600 bar (APV2000, SPX Flow Technology, UK). Rheological measurements were performed on an MCR 301 Rheometer using parallel plate geometry (Anton-paar GmbH, Germany). Samples were loaded and allowed to equilibrate (10 min) before being subjected to shear rates of 0.1 to 100 s^{-1}. Oscillatory measurements of the storage modulus (G') and loss modulus (G'') were conducted. Strain sweeps were first performed to identify the linear viscoelastic region. Samples were subjected to a frequency range of 0.1 to 100 rad/s at a strain of 0.5%. All measurements were performed at 20°C. The tan δ was determined at 1 Hz.

5.3 Results and discussion

5.3.1 The impact of heat shock on the sensory properties of ice cream

Analysis of the sensory attributes that change with temperature abuse of the samples was performed to identify the attributes that need to be controlled by the plant fibre stabilisers in order for them to behave as suitable ice cream stabilisers. These attributes are the sensory properties associated with deteriorations in product quality when ice creams have been subjected to heat shock or have been stored in inappropriate temperature conditions.

Principal component analysis (PCA) allows a visual representation of the relationship between samples and attributes and the attributes that positively or negatively correlate. Clustering of samples suggests similar sensory properties (Meilgaard et al., 2006). The PCA biplot of the fresh and temperature abused samples is shown in Figure 5.2. PCA analysis identified two main components (F1 and F2) which account for 82.60% of the data. The fresh samples are separated along F1 showing that formulating ice creams with different plant fibres has an impact on the sensory properties. Fresh samples of AQ+ CF, Sugar Cane and Psyllium are grouped with the Standard sample showing similar sensory properties to the Standard and being described as mouth coating. However, FibreGel CF stabilised samples are separated from the other fresh samples along F1 showing this sample to have different sensory properties and being described as watery and crumbly.

The PCA biplot allows an indication of how effective certain plant fibres are at controlling sensory change with temperature abuse. Seven day abused samples of AQ+ CF and Psyllium remain within the positive loading of F1 along with the Fresh samples indicating little change in sensory properties whilst Sugar Cane Fibre becomes separated from the fresh samples along F2 suggesting that Sugar Cane fibre is less effective at retaining sensory quality after 7 days of temperature abuse.

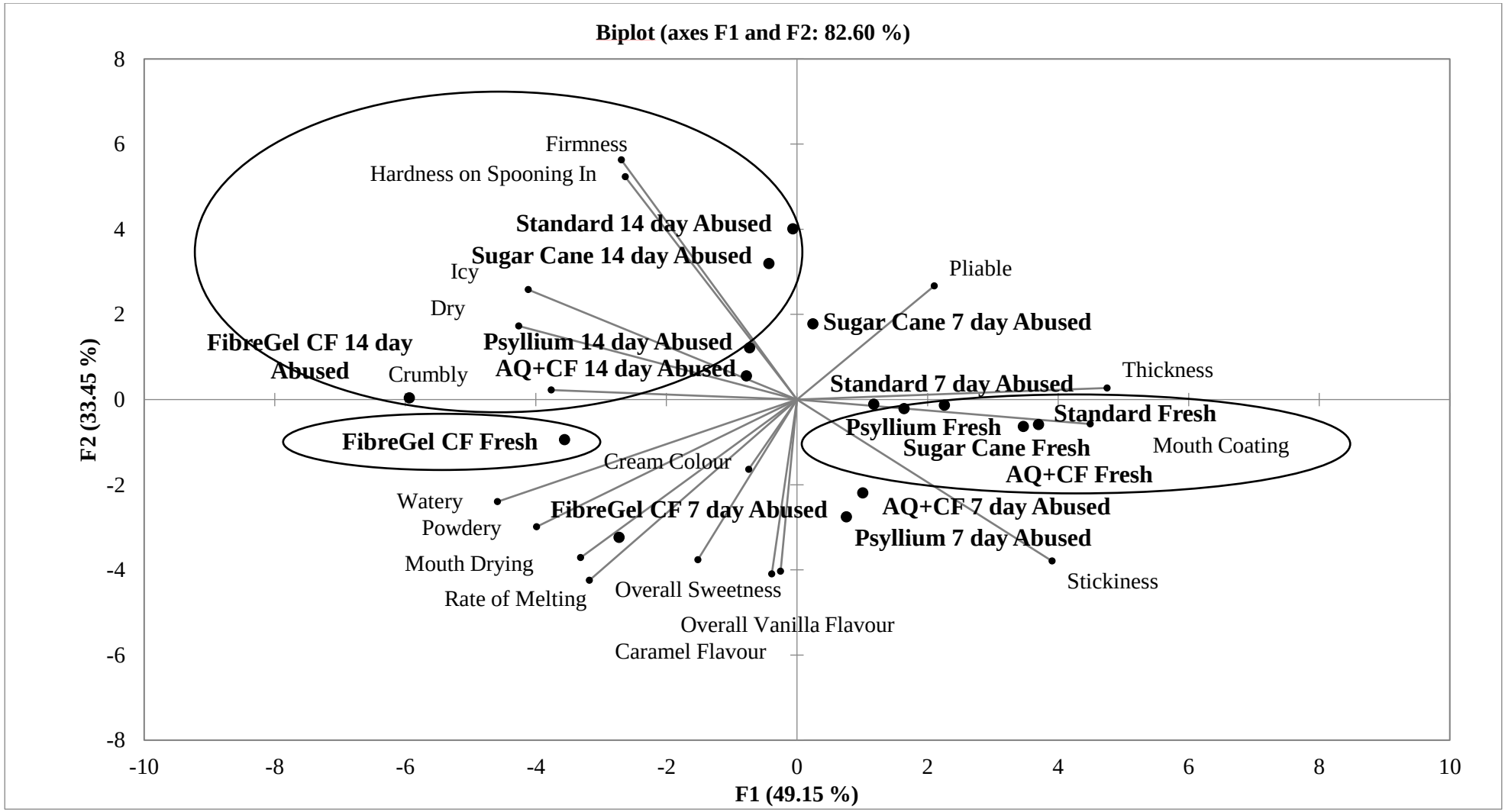

Figure 5.2 Principal component analysis (PCA) of fresh and temperature abused ice cream samples.

After 14 days of temperature abuse we can see a large change in the sensory properties as all ice creams become firmer, harder on spooning in, icy, dry and crumbly suggesting that these are the main sensory attributes that are associated with deteriorations in product quality with exposure to heat shock. These attributes are negatively correlated with the mouth coating sensory properties of the fresh samples.

The attributes that significantly change with heat shock treatment are presented in Table 5.3. It shows that no significant difference ($p > 0.05$) in caramel flavour, overall sweetness, overall vanilla flavour and mouth drying were identified between fresh and temperature abused samples and thus these attributes remain unaffected by temperature abuse. However, all the remaining attributes were identified to significantly differ ($p < 0.05$) between fresh and temperature abused samples. After 7 days of abuse, significant differences in the intensity of pliable, dryness, crumbly, stickiness, icy, watery, rate of melting, mouth coating, thickness and powdery were identified. After 14 days of abuse significant differences in hardness on spooning in and firmness were identified. This allows the food manufacturer knowledge of when consumers may begin to detect certain quality deteriorations using these particular formulations.

PCA identified that the main attributes associated with the 14 day abused samples were changes in firmness, hardness on spooning in, icy, dry and crumbly. However the other attributes that are identified to significantly change with temperature abuse (Table 5.3) may be more specific to one formulation. The details of these sensory quality deteriorations and the microstructural changes that occur to contribute to these will be discussed throughout this chapter.

Table 5.3 The effect of temperature abuse on the sensory properties of ice cream.

	p - value	Fresh	7 day abused	14 day abused	Overall impact of abuse on attribute
Cream colour	0.037	2.87 [b]	3.18 [ab]	3.27 [a]	Increases
Caramel flavour	0.052	2.46 [a]	2.25 [a]	2.07 [a]	No change
Overall Sweetness	0.681	5.84 [a]	5.99 [a]	5.88 [a]	No change
Overall vanilla flavour	0.532	3.26 [a]	3.43 [a]	3.22 [a]	No change
Hardness on spooning in	<0.001	3.47 [b]	3.10 [b]	5.86 [a]	Increases
Pliable	<0.001	3.69 [b]	5.08 [a]	5.09 [a]	Increases
Dry	<0.001	3.46 [b]	2.61 [c]	4.23 [a]	Increases
Crumbly	<0.001	2.33 [b]	3.25 [a]	3.19 [a]	Increases
Stickiness	<0.001	5.15 [a]	4.51 [b]	3.25 [c]	Decreased
Firmness	<0.001	3.21 [b]	2.79 [b]	5.43 [a]	Increases
Icy	<0.001	1.84 [c]	4.58 [b]	6.09 [a]	Increases
Watery	<0.001	3.92 [b]	4.70 [a]	4.65 [a]	Increases
Rate of melting	0.041	4.93 [b]	5.55 [a]	5.09 [ab]	Increases
Mouth coating	0.001	5.62 [a]	4.72 [b]	4.05 [c]	Decreases
Thickness	<0.001	5.06 [a]	4.51 [b]	4.07 [b]	Decreases
Powdery	0.015	2.80 [b]	3.38 [a]	3.23 [ab]	Increases
Mouth drying	0.127	3.63 [a]	4.09 [a]	3.77 [a]	No change

[a,b,c] Letter superscripts indicate significant differences between means within a row as determined using Tukey HSD with a confidence level of 95%.

5.3.2 Impact of ice cream formulation on the sensory properties

The effect of plant fibres on the sensory properties of the fresh ice creams are presented in Figure 5.3. Analysis of variance identified that varying plant fibre type within formulations had no significant effect ($p > 0.05$) on overall sweetness and overall vanilla flavour. FibreGel CF is reported to be standardised with dextrose with the amount of dextrose incorporation unknown, so it is encouraging to see that this has no significant effect on product sweetness.

As the concentration of vanilla flavouring remained the same across all formulations, differences in vanilla favour intensity were not expected unless interactions between ingredients or the matrix occurred. The viscosity of a system, the ease of physical breakdown of the food matrix and the melting rate of ice cream have all been identified to have an impact on the release of volatile flavour compounds (Chung et al., 2003). The fat content of an ice cream has also been identified to have a large impact on the intensity and release of volatile compounds due to its solvent properties (King, 1994). Therefore there is a large amount of interest in the literature into creating the flavour release profiles of full fat ice cream in low-to-no fat formulations (Chung et al., 2003). Hydrocolloid stabilisers have been found to have a positive impact on flavour release in ice cream (Soukoulis et al., 2008) whilst whey proteins have been found to modify the flavour release profile due to interactions with some flavour compounds. Increasing the whey protein concentration from 0.125 to 0.5% has been found to cause a decrease in vanilla flavour (Hansen & Heinis, 1991). Higher perceptions of vanilla flavour are also associated with increasing sweetness (Koeferli et al., 1996). Crizel et al. (2014) used orange fibre as a fat replacer in low-fat ice cream formulations. It was found that complete substitution of fat for orange fibre significantly affected ice cream flavour as orange fibre was not able to act as a solvent reservoir to flavour compounds and so the release of flavour compounds during consumption was different to a control sample.

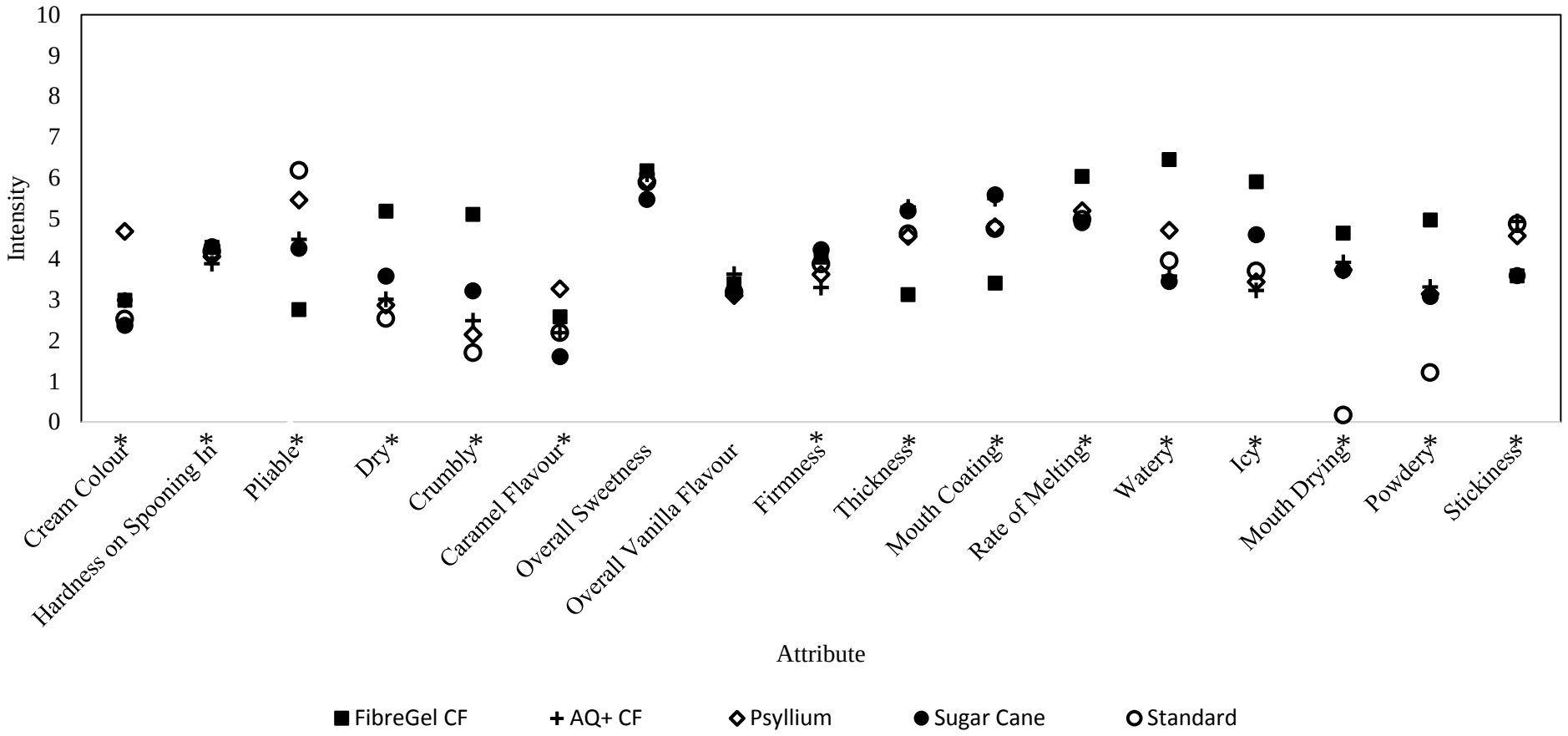

Figure 5.3 The effect of different plant fibres on the sensory properties of fresh ice cream. Asterisks mark the sensory attributes that were found to significantly vary in intensity between samples.

Hydrocolloid stabilisers have been reported to impart a selection of mouthfeel properties that are desirable to the consumer such as a mouth coating sensation and thickness in mouth which will be related to the rheology of the mix (Varela et al., 2014). Therefore it is important that for plant fibres to be suitable alternative stabilisers, they must also impart such properties. In general, AQ+ CF, Sugar Cane and Psyllium all impart similar mouthfeel properties to that of the Standard with little deviation from the trend. However, FibreGel CF fails to impart such mouthfeel properties. FibreGel CF stabilised samples can be described as thin, low mouth coating, fast melting, watery and icy which are all attributes associated with an ice cream being understabilised (Goff & Hartel, 2013). FibreGel CF during compositional analysis (Chapter 3) was identified to contain the lowest total fibre content and thus the effective stabiliser, fibre, will be in low concentration and insufficiently providing enough stabiliser per gram of ingredient to impart the correct mouthfeel properties.

Although the persistence of a molten foam structure and the extent of fat destabilisation have been found to influence the perception of mouth coating and thickness, which is primarily influenced by the addition of an emulsifier (Wildmoser et al., 2004), it is the rheological properties of a pre-mix, in particular the consistency coefficient that have a primary impact on the sensation of these attributes (Aime et al., 2001; Varela et al., 2014). Ice cream mixes stabilised with FibreGel CF were identified to have the lowest apparent viscosity and consistency coefficient (Chapter 4) which would offer a further explanation as to why FibreGel CF is unable to impart the correct mouthfeel properties. El-Nagar et al. (2002) has suggested that the improved mouthfeel in low-fat ice creams stabilised with inulin is due to the reduced meltability of low-fat inulin products. As FibreGel CF in meltdown assessment (Chapter 4) was found to have the most rapid meltdown rates, this may offer further explanation to the low mouthfeel properties it imparts. However, this suggests that large links exist between total fibre

content, pre-mix viscosity, the rate of melting and the perceived mouthfeel imparted by a plant fibre.

The Standard ice cream sample was identified to be lacking in the attributes of mouth drying and powderyness whilst the plant fibre stabilised samples exhibit low to moderate intensities of these sensory attributes. This shows that the presence of particles of plant fibre in ice cream imparts the perception of these two attributes. FibreGel CF stabilised samples were identified to have the highest intensity of sensation of powderyness of the molten ice cream on the tongue. This may suggest that FibreGel CF has the largest, most dense particles and so their perception is more intense. However, if we compare the particles sizes of the homogenised fibres in ice cream pre-mixes using the confocal microscopy images shown in Chapter 4, we can see that FibreGel CF stabilised samples do not exhibit the largest and most dense particles. Thus, it may be suggested that interactions from other sensory attributes are contributing to the higher intensity of powderyness perceived. Perhaps the low mouth coating properties and thickness of the molten mix of FibreGel CF samples allows the particles on the tongue to be more easily perceived. Considering the perception of a mouth drying sensation imparted by the plant fibres during aftertaste assessment, Crizel et al. (2014) also found that orange fibre imparted a bitter and astringent aftertaste in low fat ice creams. It was suggested that this was due to the organic compounds (polyphenols & flavonoids) that can be found in plant material. FibreGel CF was also found to be the most mouthdrying. McRae & Kennedy (2011) found a relationship between increased sensations of astringency (the drying and puckering sensation in the mouth) and increased roughness in mouth in wine. This might suggest that the increased perception of powderyness may also be related to the higher perception of a mouth drying sensation.

As previously mentioned, for plant fibres to be suitable alternative stabilisers they must also be bland and not impart any unwanted sensory properties. Psyllium fibre was identified to impart a slight caramel flavour and cream colour to samples. Derivisoglu & Yazici (2006) also found

that citrus fibre imparted unwanted flavour characteristics in ice cream resulting in a negative impact on flavour scores. Similarly, Yangilar (2015b) identified that the addition of date fibre to ice creams imparted an unwanted date flavour, whilst Crizel et al. (2014) found that orange fibre imparted an unwanted colour in low fat ice cream formulations thus suggesting the some plant fibres can impart unwanted colour and flavour defects.

5.3.3 Ability of fibres to control sensory deteriorations

Principal component analysis identified that firmness, hardness on spooning in, icy, dry and crumbly were largely related to samples that had received temperature abuse treatment (Figure 5.2). However, other attributes were also found to significantly vary with temperature abuse (Table 5.3). Although, these attributes are largely formulation dependent, as outlined in Table 5.4. Cream colour was only found to significantly vary in Psyllium stabilised samples. As ice creams become heat shocked, the matrix phase containing the fibre becomes more compressed due to the coarsening of ice crystals and air cells, resulting in a concentration of cream coloured particles. However, after 7 days of temperature abuse, no further significant increase in cream colour was observed. Soukoulis et al. (2008) also found changes in colour from white to yellowish of hydrocolloid stabilised samples during a 4-month storage period. It was suggested that the dehydration effect caused by sublimation with long storage periods resulted in the freeze concentration of the serum phase. Significant changes in pliable were also identified in Psyllium stabilised samples and the Standard sample.

Table 5.4 Mean intensity scores of different sensory attributes as an effect of different stabilisers and temperature abuse.

	AQ+ CF			FibreGel CF			Psyllium			Sugar Cane			Standard		
	Fresh	**7 day**	**14 day**	**Fresh**	**7 day**	**14 day**	**Fresh**	**7 day**	**14 day**	**Fresh**	**7 day**	**14 day**	**Fresh**	**7 day**	**14 day**
Cream colour	2.78^{c}	3.24^{bc}	2.95^{c}	2.76^{c}	3.01^{bc}	3.18^{bc}	4.20^{b}	4.97^{a}	4.86^{a}	2.43^{c}	2.34^{c}	2.33^{c}	2.17^{c}	2.35^{c}	2.30^{c}
Pliable	4.41^{bcd}	4.80^{bcd}	4.19^{bcd}	1.58^{e}	3.10^{de}	3.57^{bcd}	4.26^{bcd}	5.28^{abc}	6.79^{a}	3.47^{cde}	5.37^{ab}	3.93^{bcd}	4.72^{bcd}	6.84^{a}	6.95^{a}
Dry	2.40^{bc}	2.71^{bc}	3.91^{b}	6.17^{a}	2.98^{bc}	6.37^{a}	3.48^{bc}	1.72^{c}	3.40^{bc}	2.62^{bc}	4.25^{b}	3.86^{b}	2.63^{bc}	1.76^{c}	3.22^{bc}
Crumbly	2.27^{bcd}	2.37^{bcd}	2.80^{bcd}	6.43^{a}	2.67^{bcd}	6.16^{a}	3.29^{bc}	1.32^{d}	1.81^{cd}	2.27^{bcd}	3.79^{b}	3.58^{b}	2.34^{bcd}	1.11^{d}	1.62^{cd}
Stickiness	5.79^{a}	5.18^{ab}	3.78^{bcd}	4.13^{abcd}	3.84^{bcd}	2.52^{d}	4.72^{abc}	5.40^{ab}	3.56^{bcd}	4.91^{abc}	3.11^{cd}	2.75^{d}	5.93^{a}	5.03^{ab}	3.63^{bcd}
Watery	2.83^{f}	3.88^{def}	4.03^{def}	5.88^{abc}	6.41^{ab}	7.03^{a}	4.24^{cdef}	5.21^{bcd}	4.65^{cde}	3.18^{ef}	3.93^{def}	3.23^{ef}	3.46^{ef}	4.08^{def}	4.31^{cdef}
Rate of melting	4.26^{b}	5.26^{ab}	5.18^{ab}	5.58^{ab}	6.61^{a}	5.89^{ab}	4.95^{ab}	5.91^{ab}	4.67^{b}	4.93^{ab}	4.61^{b}	5.10^{ab}	4.92^{ab}	5.37^{ab}	4.62^{b}
Mouth coating	6.17^{ab}	5.57^{ab}	4.69^{abcde}	3.64^{de}	3.65^{de}	2.93^{e}	5.49^{abc}	4.44^{bcde}	4.45^{bcde}	6.39^{a}	5.60^{abc}	4.73^{abcd}	6.43^{a}	4.34^{cde}	3.46^{de}
Thickness	6.08^{a}	5.13^{abc}	4.61^{abcd}	3.33^{de}	3.25^{de}	2.79^{e}	4.79^{abcd}	4.65^{abcd}	4.21^{bcde}	5.31^{abc}	5.21^{abc}	5.01^{abc}	5.80^{ab}	4.31^{bcde}	3.75^{cde}

[a,b,c] Letter superscripts indicate significant differences between means within a row as determined using Tukey HSD with a confidence level of 95%

5.3.3.1 Hardness on spooning in and firmness

Relationships between hardness on spooning in and firmness were identified. The firmness in mouth of ice cream has been found to be related to the viscosity of a pre-mix, the number and size of air cells (Balthazar et al., 2015) and the distribution and connectivity of ice crystal sizes. Large clusters of ice crystals have also been found to increase product firmness (Warren & Hartel, 2014). These same factors have been found to influence the hardness and scoopability of an ice cream (Warren & Hartel, 2014).

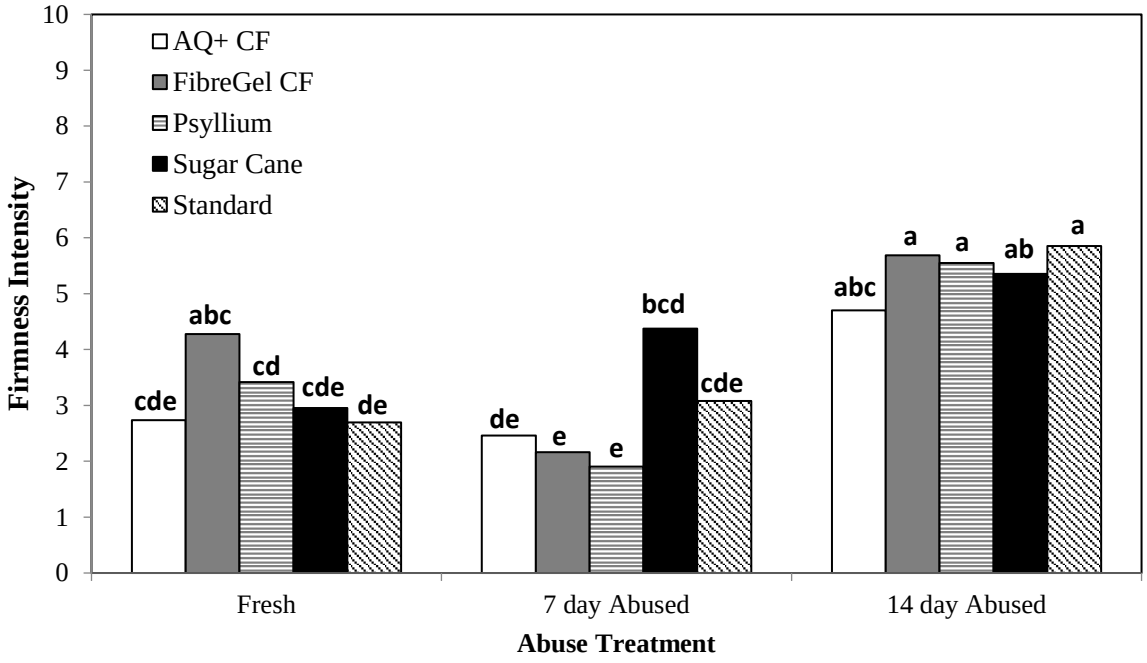

Figure 5.4 The effect of temperature abuse on mean firmness intensity scores. [a,b,c] Letter superscripts indicate significant differences between means as determined using Tukey HSD with a confidence level of 95%.

Figure 5.4 reports on the effect of heat shock on the perception of firmness in the plant fibre and Standard stabilised samples. In Chapter 4, little differences in the foam structure were identified in cryo-SEM imaging of fresh/un-abused samples. In addition, assessment of the rheological properties of the plant fibre stabilised pre-mixes identified that AQ+ CF stabilised samples had the highest apparent viscosity at 5°C whilst FibreGel CF samples had the lowest apparent viscosity. However, in disagreement with Balthazar et al. (2015) higher pre-mix viscosities did not correlate with higher firmness intensities in the fresh samples. In fact, FibreGel CF stabilised samples were identified to be the most firm. FibreGel CF stabilised samples were identified to be the most icy (Figure 5.3). In addition, assessment of the ice crystal sizes of the sensory samples identified that FibreGel CF samples had the highest mean ice crystal size. This suggests that the size of ice crystals has a larger impact on the perception of firmness rather than the pre-mix viscosity.

As the number and size of air cells influence the firmness of ice cream it might be expected that a loss of discrete air cells and air cell channelling might result in an increase in product firmness. After 7 days of temperature abuse, increases in firmness were not seen with the exception of Sugar Cane Fibre stabilised samples. Although the microstructure of 7 day abused samples was not assessed in Chapter 4, after 14 days of abuse Sugar Cane Fibre samples demonstrated very different foam microstructures compared to the other samples with a large amount of irregular shaped air cells and a complete loss of discrete, spherical air bubbles. It is possible that this complete loss of foam structure can be achieved after 7 days of temperature cycling as very little further increases in firmness can be identified after 14 days of cycling. However, after 14 days of abuse, a significant increase in firmness was seen for all samples, although the intensity of firmness was not found to be significantly different between formulations.

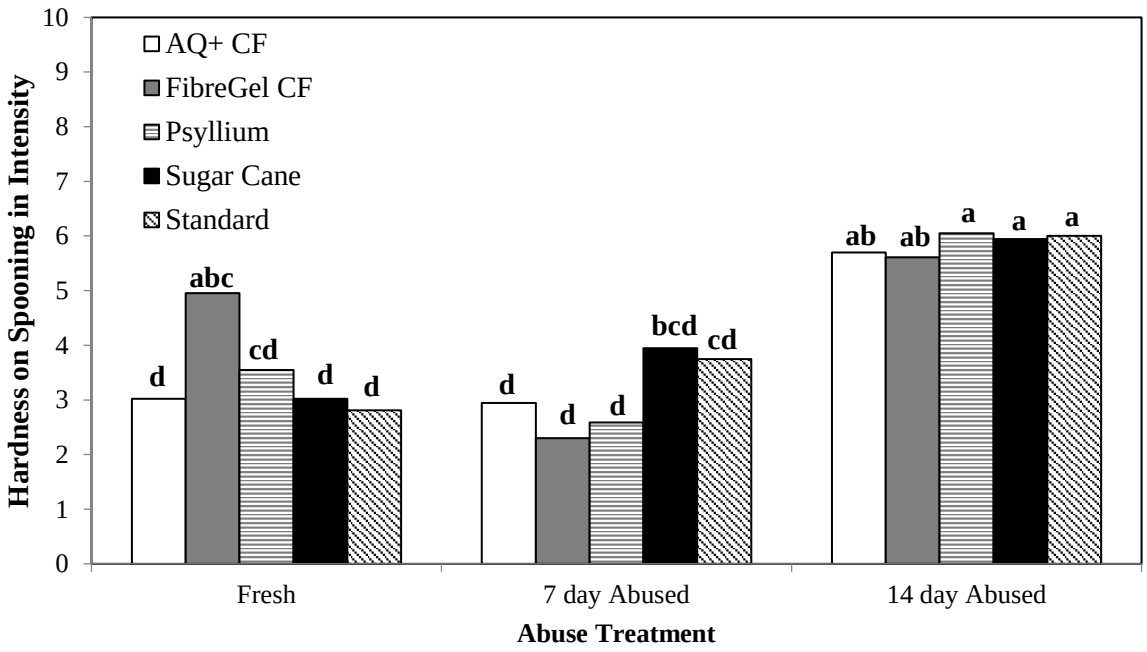

Figure 5.5 The impact of temperature abuse on the intensity of hardness on spooning. [a,b,c] Letter superscripts indicate significant differences between means as determined using Tukey HSD with a confidence level of 95%.

It was found that samples that received high firmness intensity scores also received high scores for hardness on spooning in (Figure 5.5) due to the relationships between the microstructure and sensory perception. Fresh FibreGel CF samples which had a high firmness were also hard upon spooning. Although, with the exception of FibreGel CF due to its other sensory properties (most icy), no significant difference in hardness was identified between the other fresh samples. This is in agreement with Crizel et al. (2014) who found that citrus fibre (<1%) has no significant impact on hardness, as hardness was dependent on the fat content. Similar to firmness, in 7 day abused samples Sugar Cane Fibre was also found to be the hardest. After 14 days of temperature abuse a significant increase in hardness was seen suggesting that the foam structure has been sufficiently destabilised to cause an increased in hardness. However, given that cryo-SEM imaging of 14 day abused samples in Chapter 4 identified the foam structure of

plant fibre stabilised samples to be largely more destabilised than the Standard formulation it is encouraging to see that this has no significant impact on firmness or hardness on spooning in.

These results show that after 14 days of cycling no significant benefit of the control over increases in firmness and hardness on spooning in were provided by the plant fibres in comparison to the Standard formulation. Therefore, this suggests that the control plant fibres have over controlling microstructural changes with heat shock is not sufficient to control changes in firmness and hardness due to their poor control over air cell coarsening.

5.3.3.2 Dry, crumbly and pliable

The impact of temperature abuse on the perception of dry, crumbly and pliable is shown in Figure 5.6. A fresh (un-abused) ice cream that is dry and crumbly can be indicative of the inappropriate use of or low concentration of stabiliser as well as a low total solids content (Goff & Hartel, 2013). As the total solid content remained constant across all formulations the high intensity scores of dry and crumbly of fresh FibreGel CF stabilised samples of 6.17 and 6.46, respectively, further reiterated the impact a plant fibre with a low total fibre content has on the sensory properties of an ice cream.

With increasing temperature abuse, increasing dryness can be due to a loss of moisture from the ice cream due to sublimation (Soukoulis et al., 2008). This can also end in the perception of crumbliness and results in a product that breaks apart easily on scooping. Increasing air cell size, loss of discrete air cells and air cell channelling may also result in the loss of good ‘scoopability’ (Wildmoser et al., 2004). Although in all formulations mean intensity scores of dry and crumbly increased after 14 days of temperature abuse, this was not identified to be statistically significant (Table 5.3) suggesting the large changes in air cell structure after

temperature abuse seen in Chapter 4 have no significant effect on the perception of these properties.

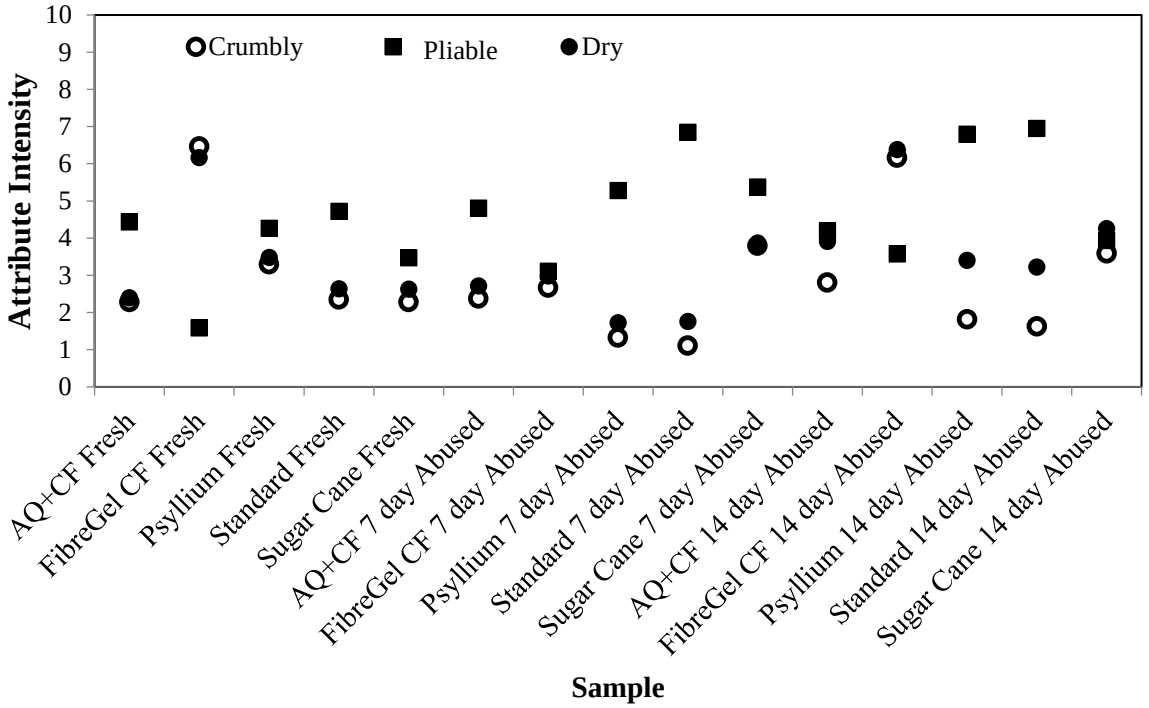

Figure 5.6 The effect of temperature abuse on the perception of dry, crumbly and pliable.

It was found that a sample described as pliable was low in dry and crumbly properties. Pliable can provide information on the elastic nature of the matrix phase. Proceeding temperature abuse can result in shrinkage of the serum phase causing a phase concentration of the stabilisers changing their viscoelastic properties. This effect is more apparent in the Psyllium and Standard formulation suggesting that their rheological properties at high concentrations are different to the other stabilisers. Schaller-Povolny & Smith (1999) also found that Inulin stabilised samples increased in chewiness, the elastic nature in mouth, as temperature abuse proceeded. It was proposed that this was due to a loss of moisture and shrinkage of the ice cream.

To further investigate the rheological properties of the plant fibre dispersions the viscoelastic properties were investigated (Figure 5.7).

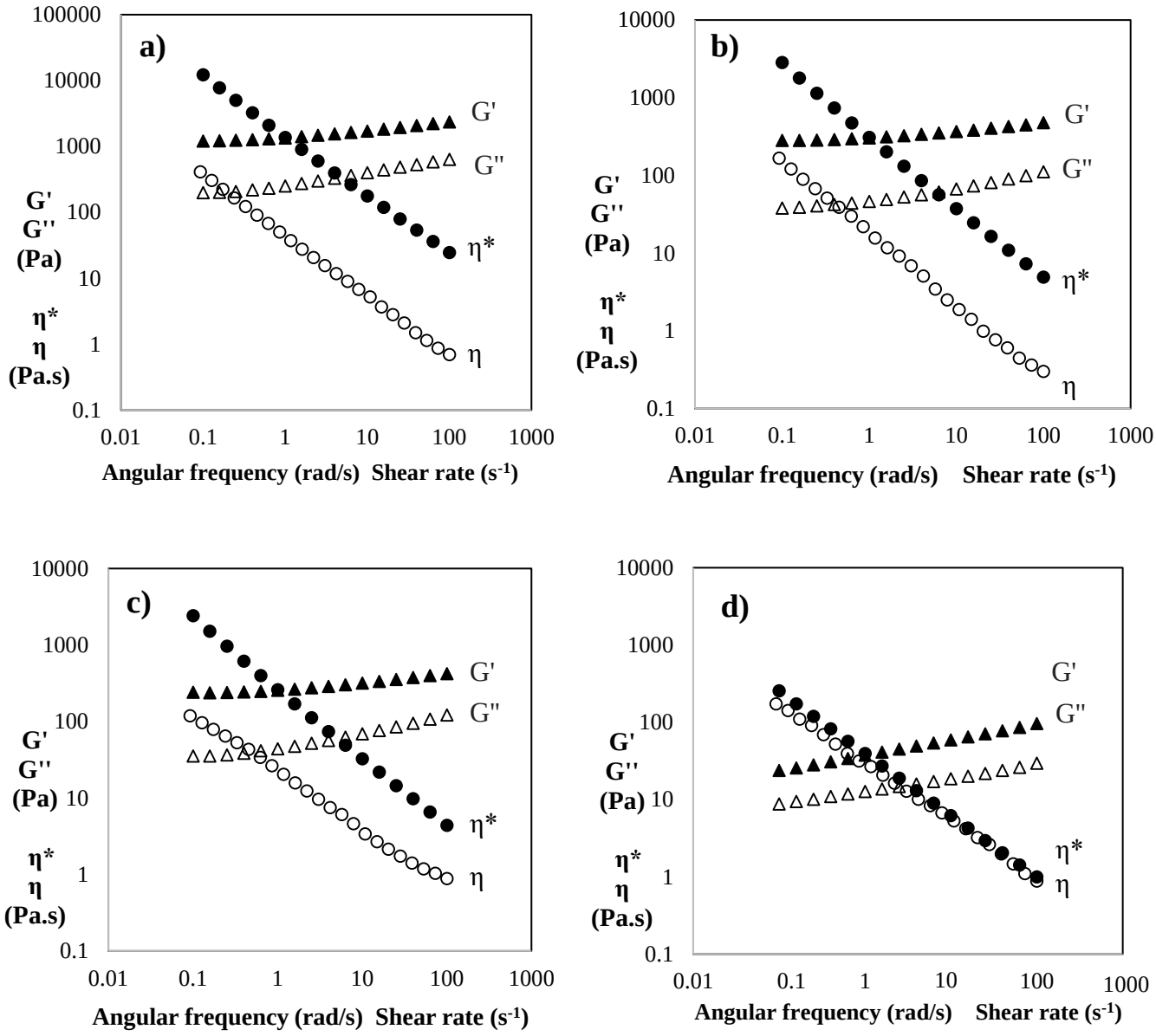

Figure 5.7 Rheological properties of 2% (w/w) plant fibre dispersions homogenised at 600 bar (a) AQ+ CF (b) FibreGel CF (c) Sugar Cane (d) Psyllium. Storage modulus (G') loss modulus (G") complex viscosity (η*) shear viscosity (η) performed at 20°C. Oscillatory measurements performed at 0.5% strain.

Homogenised fibre suspensions at a higher concentration than were incorporated into the ice cream formulations are used as a means to assess the rheological properties of the fibres similar to that of the freeze-concentrated conditions. In all four fibre suspensions the elastic nature (G') is greater than the viscous nature (G") across the whole frequency range suggesting solid-like responses to oscillation. This is in agreement with Fischer (2008) who reported that 2% homogenised dispersions of AQ+ CF behave like a solid. However, preparations that give predominantly solid-like responses to low-amplitude oscillations but are not self-supporting and can be poured or spread, such as in the case of the fibre suspensions in the present study, are known as weak-gels (Sweetnam et al., 2009). Three types of systems can be recognised by frequency sweep measurements; dilute solutions, concentrated solutions and gels. In dilute polysaccharide solutions G" is greater than G', but intercept at higher frequencies. For concentrated systems, G' is smaller than G" and cross over each other in the middle of the frequency range. For gels, G' is always greater than G" and are independent of frequency (Mezger, 2006). Assessment of the tanδ indicated that all suspensions exhibit weak-gel structures. AQ+ CF had a tanδ of 0.19 ± 0.04, FibreGel CF of 0.15 ± 0.01, Sugar Cane of 0.17 ± 0.02 and Psyllium 0.34 ± 0.01. The higher tanδ value for Psyllium suggests it has the weakest network association. Further to this, differences in the relationships between η and η^* were seen between the different fibre suspensions. According to Cox & Merz (1958), the variation in dynamic viscosity (η^*) as a function of angular frequency has a relationship with steady shear viscosity (η) as a function of shear rate. If biopolymer solutions are devoid of strong interactions like in the case of locust bean gum or guar gum, dynamic and steady shear flow curves are the same or overlap in some way. However, if strong interactions occur such as in the case of xanthan gum which deviates from the rule of Cox & Merz (1958), dynamic viscosity remains higher than the steady shear viscosity with no overlapping of the curves. Such a behaviour is shown for AQ+ CF, FibreGel CF, and Sugar Cane suggestive of interactions

between the fibre particles. However for Psyllium, the η* is equal to the η suggesting a lack of strong interactions. This suggests that Psyllium behaves like a weak gel but can easily flow with applied shear due to weak interactions. This is reflected by the flow behaviour index of the ice cream pre-mixes in Chapter 4, the low flow behaviour index of Psyllium suggests it is a rapidly shear thinning material. Farahnaky et al. (2009) found that as the concentration of Psyllium mucilage increased, the consistency coefficient increased but the flow behaviour index decreased. This suggests that higher mucilage concentrations are more shear thinning. The rheological properties of Psyllium gum have been further reported. Psyllium gum exhibits a gel-like structure similar to that of xanthan gum which generates a weak-gel network by entanglement of the rigid, ordered molecular structures. Increasing the concertation from 1% to 2% gives a significant increase in the elastic modulus. However, freshly prepared dispersions of Psyllium gum show flow properties similar to that of disordered coils. Upon aging, Psyllium gum solutions form cohesive gels and show obvious syneresis. The gels continue to contract on storage over long storage periods (to about 30% of their original volume after three months). This contraction process can be accelerated by freezing and thawing cycles (Hui, 2005). This offers an explanation to the perceived increase in elasticity of the matrix seen after temperature abuse after freeze-thaw cycling.

A limitation to the data presented in Figure 5.7 is that sucrose has not been included in the system which may have an impact on the weak-gel properties. Previous studies on the impact of sugar on the rheological properties of plant fibres in ice cream could not be identified from the literature. However, Sweetnam et al. (2009) assessed the impact of sugars on the formation of the weak-gel networks of konjac glucomannan, a hydrocolloid that possesses the same β-1,4 linked glucose backbone as cellulose. It was found that when in the presence of sucrose, the rheological properties were significantly enhanced. Konjac glucomannan (1% w/w) alone exhibited dilute solution behaviour under frequency sweep assessment, whilst in 60% w/w

sucrose, G' and G" were independent of frequency, exhibiting a more solid-like behaviour. This suggests that in the case of the present study, the weak-gel properties of the fibre suspensions may be enhanced in the presence of sucrose and G' and G" will exhibit less frequency dependence.

5.3.3.3 Iciness

The ability of plant fibres to prevent increases in the perception of iciness in comparison to the Standard sample under heat shock treatment is shown in Figure 5.8. The results show that the perception of iciness increases with temperature abuse as a result of ice recrystallisation. However, differences in the intensity of iciness was observed between different plant fibres. In fresh samples, FibreGel CF was found to be the most icy with a mean intensity of 4.07. No significant difference in iciness was identified between AQ+ CF, Psyllium, Sugar Cane and Standard stabilised samples. After 7 days of temperature abuse, FibreGel CF and Sugar Cane samples were found to be the most icy, whilst AQ+ CF, Psyllium and Standard were identified to be the least. After 14 days of temperature abuse, AQ+ CF and Psyllium were found to be the least icy with no significant difference in iciness identified between 7 day and 14 day abused samples. However, FibreGel CF 14 day abused samples were found to be the most icy with a mean intensity score of 8.10. However, this was not found to be significantly different to that of Sugar Cane and the Standard with intensity scores of 6.73 and 6.69, respectively.

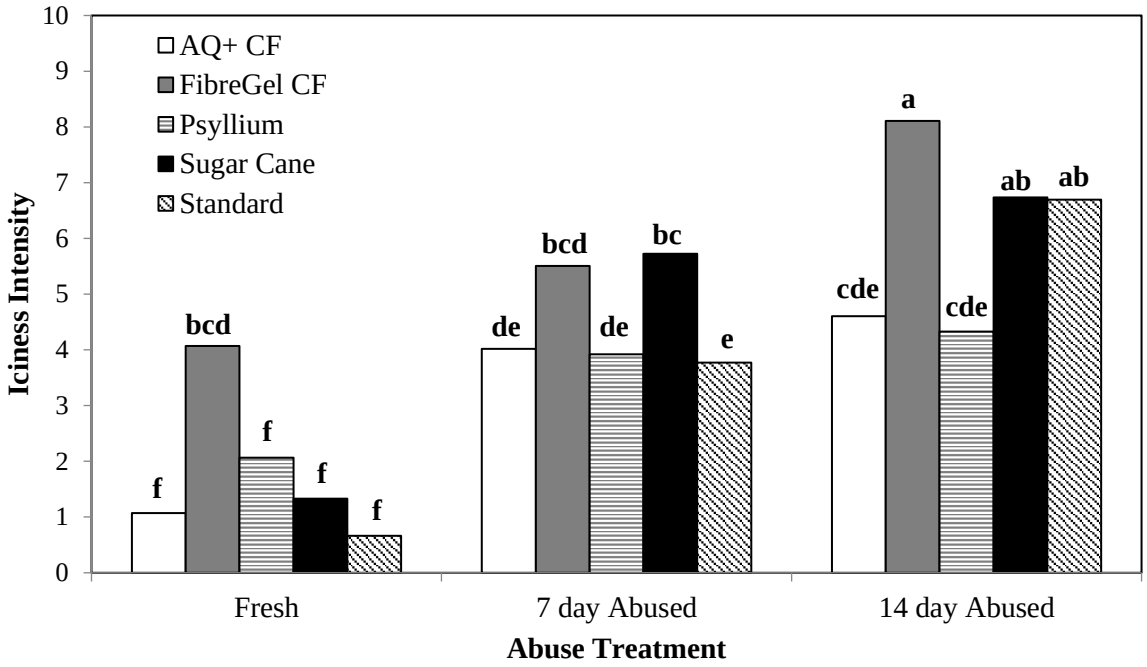

Figure 5.8 The impact of temperature abuse on the mean intensity of iciness of plant fibre stabilised samples. [a,b,c] Letter superscripts indicate significant differences between means as determined using Tukey HSD with a confidence level of 95%.

The intensity of change in the perception of iciness with temperature abuse between samples is shown in Figure 5.9. It shows by comparison of the width of change in iciness that Psyllium fibre is suggested to have the best control over increases in iciness and thus ice coarsening. In Chapter 4 it was identified that the largest control over the rate of ice recrystallisation in isothermal conditions was provided by Sugar Cane Fibre, with Sugar Cane Fibre > AQ+ CF > Psyllium > FibreGel CF and control was found to be directly proportional to the water binding capacity of the fibres. However, it was found in Chapter 3 that Sugar Cane Fibre, AQ+ CF and FibreGel CF were all freeze-thaw susceptible and so their control in freeze-thaw conditions could be hindered. Thus, although Sugar Cane Fibre was found to have the best control over ice recrystallisation in isothermal conditions its effectiveness may be lost due to a loss in functionality during temperature cycling. It was proposed that as Psyllium is the most freeze-

thaw stable, that it would provide the best control over ice coarsening in fluctuating temperature conditions. This hypothesis seems to be reflected in the present results as Psyllium has the narrowest distribution of change and therefore possibly the best control over ice recrystallisation. Additionally, AQ+ CF and Psyllium seem to provide a better cryoprotective action than the Standard formulation suggesting that a suitable plant fibre is more effective at controlling the main quality deteriorate in ice cream – increases in iciness.

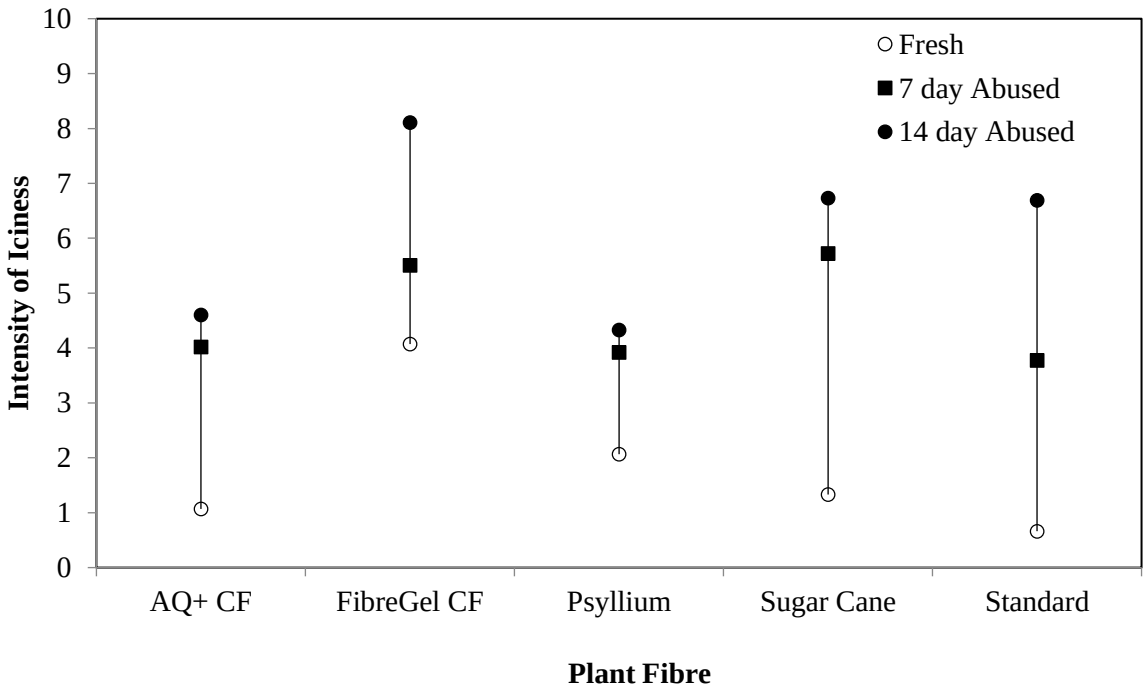

Figure 5.9 Plot showing the distribution of change in iciness perception with temperature abuse treatment.

However, a large amount of controversy exists in the literature over the correlation between ice crystal size and the sensory perception of iciness. The rate of product melting (Koeferli et al., 1996), fat content (Roland et al., 1999) and product thickness (Prindiville et al., 2000) have all been identified to impact on the perception of iciness and thus product formulation has a

large influence over iciness perception. Goff & Hartel (2013) have reported that although many factors influence the perception of iciness, the critical ice crystal size for detection is 50 µm. If the majority of ice crystals are smaller than 50 µm then the product is perceived as smooth and lacking iciness. In contrast, Russell et al. (1999) has reported on the detection of ice crystals at 30 µm in an 8% fat ice cream. However, the perception of iciness may not only be down to the mean ice crystal size but also the distribution of crystal sizes (Baer et al., 1999).

Table 5.5 Mean ice crystal size (µm) in fresh and temperature abused ice cream samples. Measured at -20 °C.

	Fresh	**7 day abused**	**14 day abused**
AQ+ CF	26. 46 ± 10.74	94.19 ± 23.62	104.99 ± 30.85
FibreGel CF	33. 81 ± 13.26	101. 70 ± 30.53	120.47 ± 34.58
Psyllium	23. 47 ± 11.78	88.02 ± 25.47	99.76 ± 28.77
Sugar Cane	24.16 ± 12.53	92.34 ±27.87	100.28 ± 27.73
Standard	20. 23 ± 9.865	86.76 ± 27.07	103.77 ± 26.35

± indicates the standard deviation in µm.

The mean size of ice crystals as determined using light microscopy of the fresh and temperature abused samples assessed in sensory evaluation are reported in Table 5.5. The results show that in general, FibreGel CF stabilised samples have a larger mean ice crystal size in comparison to all the other formulations across all three abuse treatments. However, this difference is marginal and it would be difficult to justify the differences in the perception of iciness purely based on differences in ice crystal size. In fresh samples, Psyllium was described as the least icy sample with a mean ice crystal size of 23.47 µm. FibreGel CF ice crystals were found to be

only around 10 µm larger in mean size than Psyllium samples and it is difficult to expect panellists to be able to detect a 10 µm difference, although this would be dependent on the distribution of sizes. Thus it is most likely that other factors have a part to play in this higher perception of iciness. FibreGel CF samples were found to be the fastest melting, thinnest and least mouth coating, all attributes according to the literature that can impact on the perception of iciness. Controversially to the critical ice crystal size of 50 µm proposed by Goff & Hartel (2013), however in agreement with Russel et al. (1999), panellists were able to detect product iciness at 33 µm and thus product formulation and the impact of other sensory properties further make it difficult to develop a one size fits all approach to determining a critical ice crystal size. AQ+ CF and the Standard stabilised samples were identified to have similar ice crystal sizes in 14 day abused samples, but received different sensory responses, with iciness intensity scores of 4.60 and 6.69, respectively. However, AQ+ CF was found to be thicker and more mouth coating offering further support that these two parameters have a large impact on the perception of iciness. Additionally, the mean ice crystal size of Psyllium samples were found to be only marginally smaller than that of the Standard and so it is suggestive the lower perception of iciness in Psyllium Fibre samples is not solely down to control over ice recrystallisation.

As previously detailed, fresh FibreGel CF samples had a larger mean ice crystal size than the other samples. This shows that FibreGel CF has a poor ability to prevent ice recrystallisation during the early stages in ice cream hardening and during storage. This further reiterates its poor suitability as an ice cream stabiliser.

The mechanisms by which plant fibres control ice crystal growth is still unknown. Studies by BahramParvar & Goff (2013a) found that Basil seed gum (BSG), an arabinogalactan with both glucomannan and xylan fractions, was able to reduce the rate of ice recrystallisation by 30 – 40% in comparison to a commercially stabilised ice cream. It was proposed that the difference

in control was due to the water binding capacity and network formation properties of BSG. The formation of a gel-like structure surrounding ice crystals enhances melt-regrow kinetics rather than diffuse-regrow recrystallisation. Upon thawing, melting ice is absorbed by the highly hydrophilic xylose residues. Upon cooling, freezing water is redeposited onto existing crystals and therefore allows the preservation of small ice crystals and a narrow size distribution. This is in agreement with Soukoulis et al. (2009) who proposed that the control offered by plant fibres was due to their water binding capacity, network and gel forming capabilities. However, as the exact mechanism by which plant fibres offer cryoprotection still requires investigation.

5.3.4 Transmission electron microscopy

The postulated explanation as to why Psyllium fibre behaves better than the other fibres in controlling structural and sensory deteriorations with heat shock is due to its freeze-thaw stability. Repeated freeze-thaw cycling may cause compression of the fibres causing a drop in functionality. In Chapter 3 in the assessment of homogenised dispersions of fibres under freeze-thaw conditions, confocal imaging identified that Sugar Cane Fibre, AQ+ CF and FibreGel CF exhibited a poor ability to rehydrate after compression from ice crystal growth. This was supported by a large reduction in water binding capacity after the first freeze-thaw cycle, demonstrating freeze-thaw susceptibility. In Chapter 4, confocal assessment of plant fibres in ice cream formulations did not exhibit such a large change in structure after a single freeze-thaw cycle due to the less extreme dehydration conditions as a result of an unfrozen phase. However, it was proposed that with repeated freeze-thaw cycling *in-situ* in ice cream, the effect of cycling, loss of moisture due to sublimation and serum drainage would result in a loss of functionality of the freeze-susceptible fibres similar to that seen in Chapter 3. To investigate if

this is in fact the case, transmission electron microscopy imaging was used to assess fibres *in-situ* in fresh and temperature abused ice creams which is reported in Figure 5.10.

From these images ice, air and fat can be identified by their conformation, outline and size. Casein (black) can be seen within the matrix phase and on the surface of fat droplets. Fat droplet can be seen on the surface of air cells and as clusters of fat droplets due to partial coalescence. A fine whey network (white) is also formed within the matrix phase and is associated with the plant fibres (grey).

In all four fresh formulations, homogenised plant fibres form dispersed clumps within the matrix phase. However, after heat shock, a change in structure and a drop in volume occupancy can be seen as a result of fibre compression. In the case of Psyllium fibre, this loss of original structure appears to be less extreme as in AQ+ CF, FibreGel CF and Sugar Cane. This provides further evidence that Psyllium is the most freeze-thaw stable plant fibre.

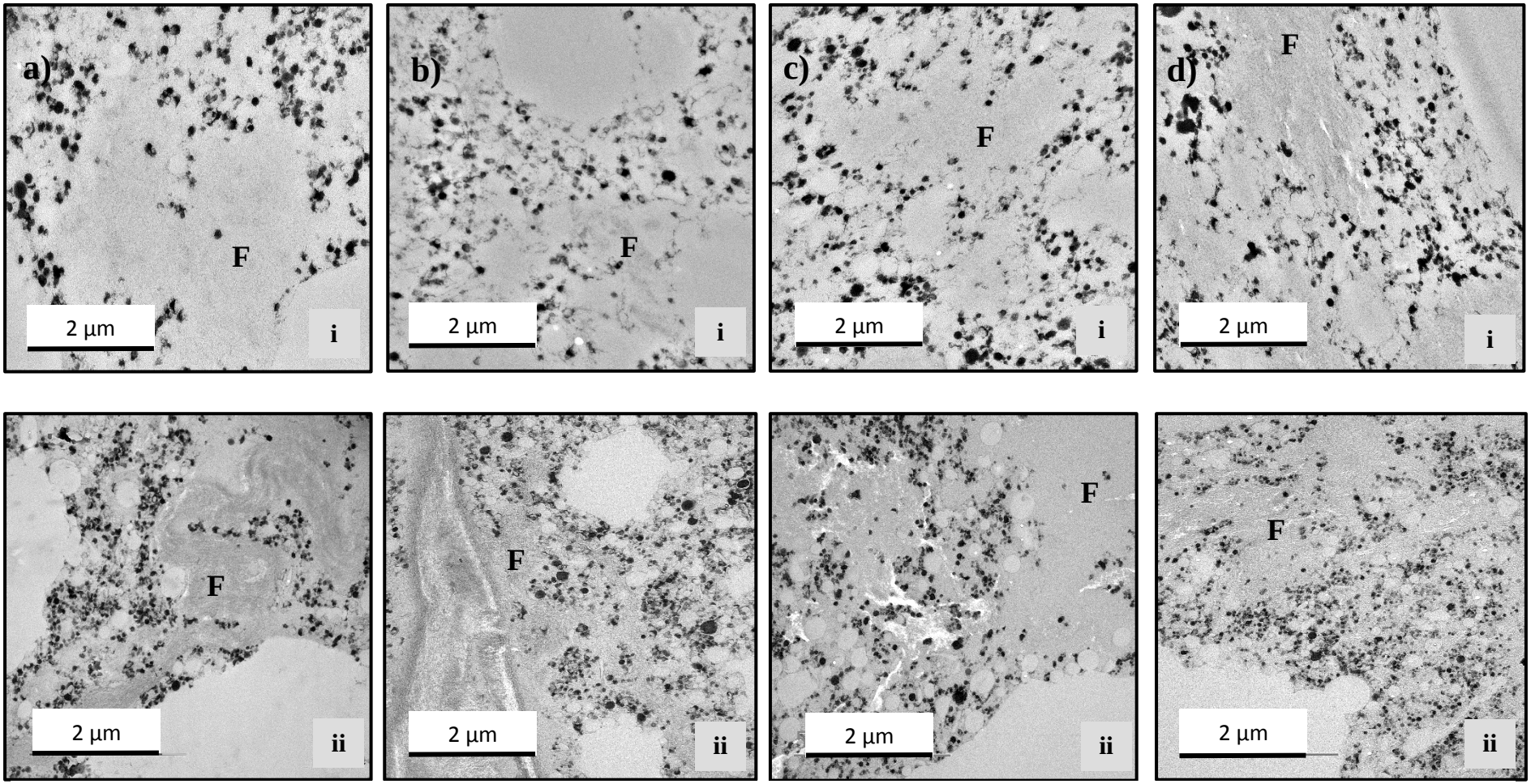

Figure 5.10 Transmission electron microscopy (TEM) images of (i) fresh and (ii) 14 day temperature abused ice creams containing different plant fibres (a) AQ+ CF (b) FibreGel CF (c) Psyllium (d) Sugar Cane. F indicates the position of plant fibres.

5.3.5 Perception of powderyness

The presence of plant fibres in formulations has been found to impart the perception of powderyness on the tongue due to the physical particles of insoluble cellulose in the molten ice cream. It is hypothesised that the denser or larger the particle, the higher the perception of powderyness. TEM imaging (Figure 5.10) identified that with temperature abuse, all plant fibres show some degree of compression which may result in them becoming denser particles or by forming clumps of particles like that seen in Chapter 3, which may result in the sensation of powderyness being perceived more easily on the tongue. Therefore, the impact of temperature cycling on the perception of powderyness was investigated (Figure 5.11).

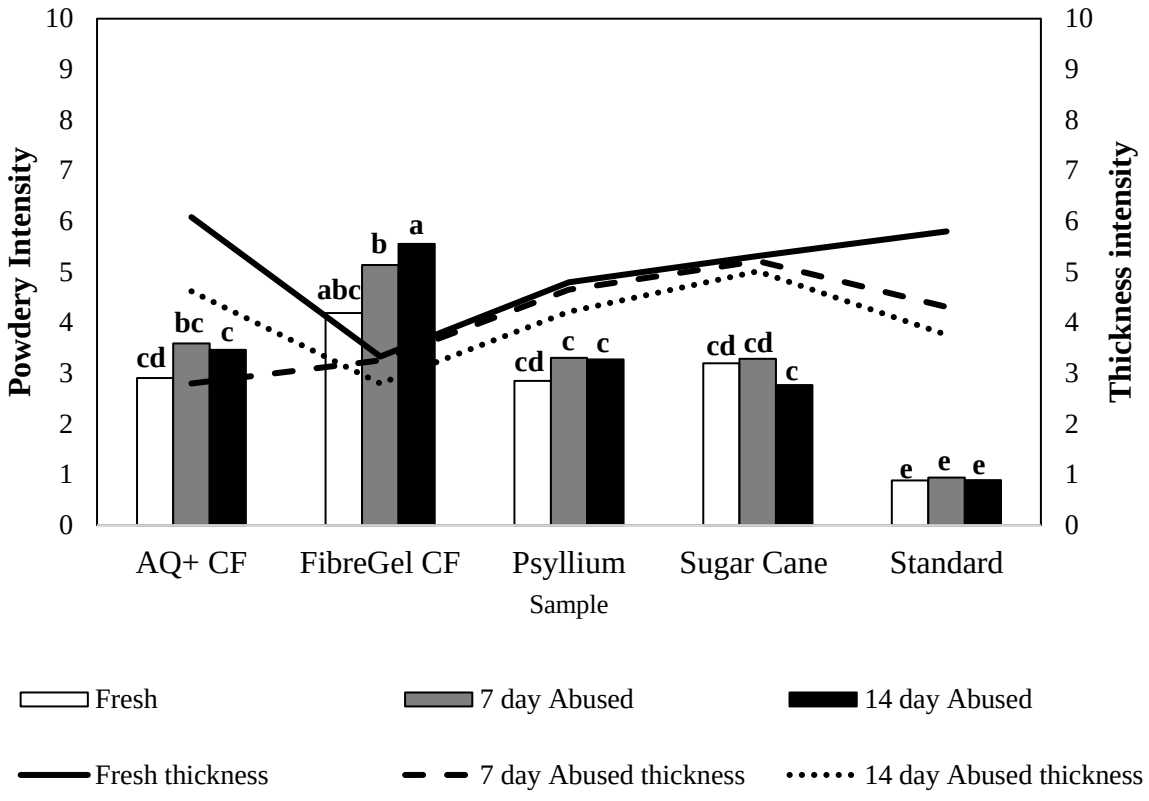

Figure 5.11 The impact of temperature abuse on the perception of powderyness in plant fibre stabilised ice creams and the relation with product thickness.

The results show that in only FibreGel CF stabilised samples, the sensation of powdeyness significantly increased with increasing temperature abuse thus suggesting that FibreGel CF compresses the most creating denser particles as temperature abuse proceeds. However, FibreGel CF was not the only fibre that demonstrated compression in TEM assessment so interactions between other sensory attributes should also be considered. As with the perception of ice crystals, which are also particles, thickness and viscosity have a large part to play in their perception. By indicating the corresponding changes in thickness with perception of powderyness as an effect of temperature abuse, we can see that generally thickness decreased with increasing temperature abuse. However, in all formulations changes in thickness were not identified to be statistically significant, therefore the increasing perception of powderyness in FibreGel CF is not an effect of decreasing thickness but is in fact due to fibre compression. However, the perception of powderyness was found in all samples containing plant fibres as all fibres contain a proportion of insoluble fibre. Engelen et al. (2005) investigated the size (2 – 230 μm) and type (silica dioxide and polystyrene spheres) of particulates added to vanilla custard on the perception of roughness. It was found that particle addition, even at 2 μm increased the sensation of roughness while decreasing smoothness. Particle size analysis of plant fibre stabilised pre-mixes in Chapter 4 identified that for all four fibres, the fibre particle sizes were between 10 and 1000 μm so it is easy to suggest that the presence of these particles is imparting the perception of powderyness.

5.4 Conclusions

The effect of plant fibres on the sensory properties of ice cream were assessed and differences in the sensory properties of formulations containing different plant fibres was identified. In addition, differences in the ability of plant fibres to prevent sensory quality deteriorations with heat shock varied between fibre type.

Increases in firmness, hardness on spooning in, icy, dry and crumbly were identified to be the main quality deteriorations associated with ice creams subjected to heat shock conditions. After 14 days of temperature abuse, all samples demonstrated a large loss of sensory quality whilst after 7 days of temperature cycling, AQ+ CF, Psyllium along with the Standard exhibited the least change in sensory quality.

Analysis of variance identified that the incorporation of plant fibres within ice creams had a significant effect on the sensory quality of fresh ice cream. However, overall sweetness and vanilla flavour were not found to significantly vary with varying plant fibre type. Plant fibres were found to impart the unwanted perception of powderyness and mouth drying when included in an ice cream formulation. Psyllium was found to impart a cream colour and caramel flavour. However, with the exception of FibreGel CF, AQ+ CF, Sugar Cane Fibre and Psyllium were all able to impart the correct mouthfeel properties required of a suitable stabiliser. On the contrary, due to the low total fibre content of FibreGel CF, suggesting a lower concentration of the effective stabiliser – fibre, FibreGel CF stabilised samples were found to be watery, low mouth coating, thin and fast melting which are all attributes associated with the use of an inappropriate stabiliser or too low a concentration of stabiliser. This shows that the suitability of a plant fibre as an ice cream stabiliser is determined by its compositional make up, primarily its purity.

However, although the sensory properties of the fresh samples is acceptable, the sensory properties after samples have been subjected to heat shock is poor and improvements need to be made for plant fibres to be considered as alternative ice cream stabilisers. Plant fibres were identified to provide no further control over increases in hardness on spooning in, firmness, dry and crumbly. As all these attributes are associated with the foam structure, improvements in the foam structure post heat shock need to be made as so far this appears to be hindering plant fibres in retaining sensory quality with heat shock. However, plant fibres were found to improve quite an important quality deterioration in ice cream – increases in iciness. Psyllium was found to have the best control over changes in the intensity of ice perception with temperature cycling. Transmission electron microscopy assessed the structures of fibres *in-situ* and found, as hypothesised throughout this thesis that the higher freeze-thaw stability of Psyllium allowed it to behave as the most suitable ice cream stabiliser. Additionally, this paired with its ability to form cohesive gels with temperature abuse, a possible cryo-gelation capacity, are likely to offer further advantage to controlling microstructural deteriorations with heat shock.

CHAPTER 6: GENERAL CONCLUSIONS

6.1 Summary

The main novel finding of this thesis is the identification of a suitable plant derived ice cream stabiliser that can be exploited in clean label products. It was identified that Psyllium Fibre possess the best functional properties needed to be an effective stabiliser as it aids in melt resistance and microstructural stability during heat shock as well as being able to impart the correct sensory mouthfeel. AQ+ CF was also found to possess promising properties as an ice cream stabiliser for these same reasons with the exception that its stability in freeze-thaw conditions requires improvement.

The structure of these plant fibre stabilisers have been imaged and studied *in-situ* in ice cream which has aided in their selection and is not something that has been previously reported in the literature. Such an in-depth study into the functional properties of the plant fibres and how this enhances their suitability as alternative ice cream stabilisers has also not been previously reported. This research has found that the key criteria for the selection of a suitable fibre is a high water binding and water holding capacity, a high total fibre content and therefore a high concentration of the effective stabiliser and finally the plant fibre and its functional properties must be freeze-thaw stable to survive heat shock conditions.

It was found that plant fibres have no impact on the rate of ice cream melting, nor the final mass loss but does inhibit the onset of ice cream melting, which is beneficial in terms of structure stability as a fast melting product is more susceptible to heat shock. It was found that this enhancement of the melting properties was due to not only to a viscosity enhancement of the ice cream pre-mix but also due to the ability of the plant fibres to act as ‘sponges’; holding onto the water from melting ice and hindering the rate of drainage by the formation of a fibrous network within the matrix phase of ice cream. It was found in isothermal conditions that Sugar Cane Fibre could provide the best control over the rate of ice recrystallisation due to a higher

water binding capacity. However, due to the high freeze-thaw instability of this fibre this was not reflected under temperature cycling conditions.

As previously mentioned, conclusions have been able to be drawn between plant fibre composition and their suitability as an ice cream stabiliser. The low total fibre composition of FibreGel CF seems to have a domino effect on the desirable properties of fibre stabilised ice creams. The low fibre content resulted in a low pre-mix viscosity, which resulted in rapid meltability in meltdown assessment. The poor pre-mix viscosity and higher meltability has resulted in FibreGel CF not being able to impart the correct mouthfeel characteristics during sensory testing.

Sensory analysis of the plant fibre stabilised samples provided a beneficial tool which allowed all the results to be tied together, in particular the links between the ice cream microstructure and the impact this has on the sensory properties of ice cream. It was found that although the microstructure of the fresh samples is good as plant fibres have little/no effect on the correct microstructure formation in ice cream in addition to a good ability to impart the correct mouthfeel properties in ice cream, the sensory properties post-heat shock still requires some improvement.

Psyllium and AQ+ CF have a good ability to prevent the development of an icy texture in ice cream subjected to heat shock conditions. However, the results of sensory analysis are not fully conclusive and a large amount of work is still needed to improve the post-heat shock sensory properties. One of the largest factors hindering sensory quality with temperature cycling is the poor foam structure. This has resulted in plant fibres providing no additional control over the increase in hardness on spooning in, firmness in mouth, dry and crumbly which are all sensory attributes that the foam structure can have an impact on.

6.2 Future work

Further research should be carried out to improve the stability of the foam structure in plant fibre stabilised ice creams. The presence of particles of plant fibres seem to be behaving as anti-foams. However, in the case of the gum of Psyllium husk, the gum seems to be less destructive to the foam structure. Therefore, further research into the use of mucilage gums as stabilisers should be conducted. The literature has found that Basil seed gum has the ability to not only stabilise ice cream but has also been found to be able to stabilise the foam structure in bread (Israr et al,. 2016). Therefore, a comparison of the gums that can be obtained from a variety of sources (Psyllium, chia, basil, flaxseed, yellow mustard seed etc.) should be performed.

There are also a number of emerging cultivars of Psyllium husk (Phan et al, 2016). Further work should be performed to assess the functional properties of these new cultivars as some of these may possess optimum functional properties e.g. higher water binding capacity and enhanced freeze-thaw stability.

Psyllium proved to be an effective stabiliser due to its freeze-thaw stability. The reason for its enhanced freeze-thaw stability is not known. However, it could be proposed that as the mucilage is obtained from a seed, it is this origin that requires it to be able to hydrate and bind water to allow germination of the seeds as well as to be able to cope with times of drought. Based on this biological requirement to be able to bind water and survive drought conditions an interesting sources of plant fibre could be provided from the variety resurrection plants that are usually native to the desert environments of North Africa. These types of plants are able to survive severe dehydration for many months or years and are then able to revive within a few hours once the desert rains return. Such a source of plant fibre may have an increased ability

due to its biological origin to retain structure in desiccate-rehydrate cycles, much like the freeze-thaw cycles experienced in ice cream.

Finally, consumer studies and the consumer acceptability of plant fibre stabilised ice creams should be performed to check that formulating ice creams with plant fibres has no impact on consumer liking.

APPENDIX

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ORIGINAL RESEARCH

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Consumer-orientated development of hybrid beef burger and sausage analogues

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Innovate UK

Abstract

Hybrid meat analogues, whereby a proportion of meat has been partially replaced by more sustainable protein sources, have been proposed to provide a means for more sustainable diets in the future. Consumer testing was conducted to determine consumer acceptability of different formulations of Hybrid beef burgers and pork sausages in comparison with both meat and meat-free commercial products. Acceptability data were generated using the 9-point hedonic scale. Check-all-that-apply (CATA) questioning was used to determine the sensory attributes perceived in each product as well as information on the attributes of consumers' ideal products. It was identified that Hybrid products were generally well liked among consumers and no significant differences in consumer acceptability ($p > .05$) were identified between Hybrid and full meat products, whereas meat-free products were found to be less accepted. However, Hybrid sausages received higher acceptability scores (6.00–6.51) than Hybrid burgers (5.84–5.92) suggesting that format may have a large impact on consumer acceptability of Hybrid products. Correspondence Analysis (CA) indicated that Hybrid products were grouped with meat products in their sensory attributes. Penalty analysis found that a "meaty flavor" was the largest factor driving consumer acceptability in both burgers and sausages. Cluster analysis of consumer acceptability data identified key differences in overall acceptability between different consumer groups (consumers who only eat meat products and consumers who eat both meat and meat-free products). The Hybrid concept was found to bridge the acceptability gap between meat and meat-free products; however, further product reformulation is required to optimize consumer acceptability.

KEYWORDS
Acceptability, CATA, consumer studies, hybrid meat analogs, preference mapping

1 | INTRODUCTION

Global meat consumption and production has dramatically increased over the years raising growing concerns among governmental bodies, academics, and industry leaders (Cordts, Nitzko, & Spiller, 2014; Graça, Calheiros, & Oliveira, 2015; Speedy, 2003; Tilman, Balzer, Hill, & Befort, 2011). Such a demand is unsustainable and has been identified as the cause of many environmental, health and sustainability related issues (Cordts et al., 2014). The significant challenge of feeding 9 billion people by 2050 poses concerning questions as to how we can meet the predicted demand, sustainably (de Bakker & Dagevos, 2012). It has been suggested by the FAO that we will have to double

the production of meat if we are to deliver on the predicted demand for 2050 (Steinfeld et al., 2006). This is alarming, as already competition for agricultural land and resources as well as the unknown impact of climate change on agriculture suggests that we cannot achieve the future protein demand using current practices (de Bakker & Dagevos, 2012). Thus, feeding the future population is a concern that needs addressing sooner rather than later (Godfray et al., 2010; Steinfeld et al., 2006). Besides this, a diet high in animal proteins has been linked to negative health effects of obesity, type 2 diabetes, and an increased risk of heart disease and some types of cancer (Chao et al., 2005; Mann, 2002; Walker, Rhubart-Berg, McKenzie, Kelling, & Lawrence, 2005).

Growing eastern economies and other developing countries have placed further pressure on the supply and demand of meat with China's demand almost doubling its consumption between 1992 and 2002 (Naylor, 2005). Livestock production is a relatively inefficient process as around 7 kg of grain are required for 1 kg of beef, 4 kg of grain for 1 kg of pork, and 2 kg of grain for 1 kg of poultry (Aiking, de Boer, & Vereijken, 2006). Thus, it is difficult to justify such large use of crops to feed livestock rather than directly to humans.

Converting predominantly meat eaters to a meat-reduced diet is a societal transition that will require careful strategic planning if we are to shift to more sustainable diets. Although media coverage of the negative side effects of meat consumption have been found to play a major role in reducing meat intake (Burton & Young, 1996; Cordts et al., 2014; Rickertsen, Kristofersson, & Lothe, 2003), only minimal success has been achieved through other nongovernmental organizational campaigns (Laestadius, Neff, Barry, & Frattaroli, 2013). One of the pathways of transition is to achieve partial substitution in the diet of animal proteins with more sustainable proteins such as plant protein (Schösler, de Boer, & Boersema, 2012), although achieving long-term transitions rather than phases in consumption behavior are key to success (Hoek et al., 2013). The requirement for meat substitution is a topic that has been largely discussed (de Bakker & Dagevos, 2012; Lea, Crawford, & Worsley, 2006), however few studies have quantified what is required by consumers in order for them to change their behaviors (Elzerman, Hoek, van Boekel, & Luning, 2011; Hoek et al., 2011; Schösler et al., 2012). It has been found that to create an effective dietary change, new practices must be somewhat similar to the previous behavior of the consumer (Ryan & Deci, 2000). Convenience and minimal skill in cooking techniques have also been identified as a major factor in hindering consumer transition to alternative protein sources (Schösler et al., 2012). The proposed method in this study to achieve meat substitution is by a built-in meat reduction in products by partially replacing animal proteins with more sustainable protein sources. Such a strategy would bridge the gap between meat and meat-free products, provide convenience, and allow consumers to continue using products as they conventionally would.

Meat is an expensive commodity and supermarkets offer a wealth of low-cost products with low percentages of meat whereby replacement has been achieved through cheap fillers and bulking agents as a means to cut costs. Such fillers have little nutritional bonus and usually consist of cereals, starches, and breadcrumbs (Gunter & Peter, 2007). In this study, a proportion of meat has been replaced with ingredients that contain a high amount of protein as a means to a more sustainable way to include alternative proteins within the diets of consumers.

The meal context in which meat substitutes are used has been found to have a significant impact on consumer acceptability (Elzerman et al., 2011; Schösler et al., 2012). Schösler et al. (2012) assessed current consumer behaviors regarding meat substitution and identified that meal formats played a key role in finding pathways to transition. By combining a meat substitute with a food format familiar to the consumer (mince or pieces), it was proposed that meat substitution in convenience foods, whereby meat as an ingredient is already less visible, posed a suitable method for substitution. Hoek et al. (2013) identified that repeated exposure to meat substitutes increased consumer acceptability. It was suggested that focus should be made on increasing willingness to try meat substitutes and creating positive initial product experiences.

It has previously been identified that nonvegetarian consumers generally judge the overall sensory quality of meat substitutes lower than that of meat (Hoek et al., 2013). The special status of meat within society, and its taste and texture are highly valued by many consumers, especially the juiciness and tenderness (Elzerman et al., 2011). Current meat substitutes are likely to be perceived as less complex than meat as they do not possess the sensory attributes in order to be accepted by meat eaters. Taste and texture have been identified as important characteristics for the acceptance of meat substitutes (Hoek et al., 2013). Although it has been identified that consumers prefer a meat-like meat substitute (Hoek et al., 2011), mimicking meat—a highly complex product—is a large technological challenge. Thus, in order to create successful meat alternatives, a consumer-orientated approach to product development is required. One way to achieve this is through developing products with consumer preferences in mind (Grunert and Valli, 2001; Stewart-Knox and Mitchell, 2003).

Check-all-that-apply (CATA) questions offer an alternative to conventional Quantitative Descriptive Analysis (QDA) methods which are comparatively more expensive and time consuming due to the requirement of trained panels (Meilgaard et al., 1999). CATA questioning has been described as a reliable, quick, and cost effective method of consumer testing and has been gaining popularity for sensory characterization of food products over recent years (Ares, Barreiro, Deliza, Giménez, & Gámbaro, 2010; Ares, Dauber, Fernández, Giménez, & Varela, 2014; Bruzzone et al., 2015; Da Conceição Jorge et al., 2015; Dooley, Lee, & Meullenet, 2010). In this method, consumers are presented with a list of sensory terms and are asked to select all the terms they consider appropriate to describe a sample (Ares et al., 2014). Da Conceição Jorge et al. (2015) used the application of CATA questions to evaluate and characterize samples of "Mortadella," an Italian pork sausage eaten cold. Ares et al. (2014) used Penalty Analysis on samples of yogurts and apples to link consumer acceptance with a product's sensory characteristics; thereby, identifying the terms that positively or negatively contributed to a products acceptance.

In this study, two meat products (pork sausages and beef burgers, two meal formats familiar to UK meat consumers) with partial

meat substitution were tested against commercial meat and meat-free products in order to determine consumer acceptance in relation to the two categories. Products in which part of the meat is replaced by more sustainable protein sources is not a novel concept and have been termed Hybrid meat analogues. Hybrid sausages, hamburgers, and mince have already entered the Dutch food markets and have created a means whereby eating sustainable products gradually becomes more accessible (de Bakker & Dagevos, 2012). Caparros Megido et al. (2016) assessed the sensory liking of Hybrid insect-beef burgers. Their studies found that overall liking varied between genders as Hybrid products were preferred by men more than women. Food neophobia (reluctance to try novel foods) was a large contributor to acceptance. However, to the best of our knowledge, no studies have been conducted that assess the sensory attributes and consumer acceptance of hybrid products by meat eaters. In this study, a consumer-generated lexicon of the sensory terms was produced. Consumers indicated their liking of each product and CATA questioning was used to determine the sensory attributes that characterize the products. Consumers were also asked to indicate the sensory attributes that characterize their ideal pork sausage or beef burger. The combined analysis of liking and CATA allows the identification of drivers for liking and consumer acceptance. Penalty analysis enabled an indication of the penalty on liking when undesirable attributes are present or the sample is different from the ideal. Assessed together with the ideal, directions to aid in product reformulation are outlined.

2 | MATERIALS AND METHODS

A variety of alternative proteins (textured soya, mycoprotein, insect protein, and pulses) were assessed for hybrid formulations before two were selected. Two concept formulations of Hybrid beef burgers and two formulations of Hybrid pork sausages were produced at pilot scale (DuPont, Denmark), frozen and transported to the United Kingdom and stored frozen (−18 °C ± 2°C). Commercial meat and meat substitutes (Table 1) were purchased from a local supermarket. All samples for consumer testing were prepared on the day of testing, served within 30 min of cooking, and kept warm in slow cookers (75°C ± 4°C). All commercial samples were prepared as per the manufacturer's guidelines. Diluted lime cordial (1:5 lime to water, Rose's Lime Juice Cordial) and mineral water (Evian) were used for palate cleansing before and between samples.

2.1 | Sensory evaluation

All sensory analysis was performed after approval by The University of Nottingham Faculty of Medicine and Health Science Research ethics committee. A consumer-generated lexicon of sensory attributes for the CATA questions was first defined. Consumers (n = 12; M = 5, F = 7), aged 18–60 years who consume both meat and meat substitutes, were recruited from the campus population via email advertisement to attend a 1 hr session. In sensory booths, each consumer received three pairs of sausage samples and were asked to write down differences in sensory attributes relating to texture, flavor, and appearance. After a 5-min rest break, consumers were presented with three pairs of burger samples and asked to do the same. Sample pairs were selected to represent the extremes in differences in sensory attributes as well as to illustrate all attributes within the sample set of Meat, Hybrid, and Vegetarian products. Frequency tallies were performed and the most recorded terms (Table 2) were used to develop the CATA questionnaire.

In a second stage, consumers (n = 94; M = 43, F = 51) were recruited from the campus population via email and poster advertisements to attend one 30-min session. Consumers were selected based on their meat consumption behavior and divided into two groups: only meat eaters and do not consume meat substitutes (n = 49); most commonly eat meat products but sometimes eat meat substitutes (n = 45), and their interest and availability to participate. Consumers received each of the five burger samples and each of the five sausage samples and were asked to consume no more than a quarter of each sample. Samples were presented monadically, on white paper plates labeled with random three digit codes and served at 75°C (±5°C). The order of presentation of samples and tests followed a randomized balanced design.

For each sample, consumers were first asked to score their overall liking using a vertical 9-point hedonic scale anchored at "dislike extremely" (1) and "like extremely" (9). Next, they completed a

TABLE 1 Concept and commercial samples

Burgers	Products	Cooking method
Concept Formulation	Hybrid 1%–37% Beef	Oven cooked
	Hybrid 2%–37% Beef	Oven cooked
Commercial Products	Beef burger–77% Beef	Oven cooked
	Vegetarian burger 1–Mycoprotein based	Pan fried
	Vegetarian burger 2–Soya based	Oven cooked
Sausages		
Concept Formulation	Hybrid 1%–30% Pork	Pan fried
	Hybrid 2%–30% Pork	Pan fried
Commercial Products	Meat sausages–61% Pork	Pan fried
	Vegetarian sausage 1–Mycoprotein based	Pan fried
	Vegetarian sausage 2–Soya based	Pan fried

TABLE 2 Consumer-generated sensory attributes relating to texture, flavor, and appearance describing the sample set

	Burger products	Sausage products
Texture	Juicy	Dry
	Dry	Fibrous
	Granular	Soft
	Greasy	Hard
	Easy to cut	Easy to cut
	Difficult to cut	Difficult to cut
	Hard	Greasy
	Soft	Poor mouthfeel
		Moist
Flavor	Sweet	Meaty
	Peppery	Wheaty
	Smokey/Grill	Herby
	Off-flavor	Peppery
	Meaty	Off-flavor/ Unpleasant aftertaste
	Wheaty	
Appearance	Dark brown color	Dry
	Light brown color	Coarse
	Dry	Visible herbs
	Oily	Pale color
	Processed	Fatty
	Uneven color	

CATA questionnaire with the 20 terms related to the sensory attributes of the samples (Table 2). Consumers were asked to try the sample and then check all the terms they considered appropriate to describe each sample. Consumers were also asked to complete the CATA questionnaire to describe their ideal pork sausage and beef burger.

All testing was performed in separate, purpose-built sensory testing booths, under Northern Hemisphere lighting and under controlled air, temperature, and humidity conditions.

2.2 | Data analysis

Overall acceptability scores were analyzed using ANOVA one-factor analysis of variance. Tukey's honestly significantly different (HSD) post hoc analysis of the difference categories with a confidence of 95% was used to identify significant groups in acceptability between samples. Agglomerative Hierarchical Cluster (AHC) analysis was performed in order to identify consumer groups with different preference patterns.

Frequency of use of each sensory attribute in the CATA questionnaire was determined by counting the number of consumers that used that term to describe each sample. Cochran's *Q* test was carried out to identify the significant differences between samples for each of the terms included in the CATA questionnaire.

Correspondence analysis (CA) was used to generate a biplot representing the samples and the relationship between samples and the terms from the CATA questioning.

Penalty analysis was carried out on consumer responses to determine the drop in overall acceptability associated with deviation from the ideal for each of the sensory attributes in the CATA question.

Multiple factor analysis was used to investigate the relationship between responses to the CATA questions and the consumer groups identified in the cluster analysis.

A significance level of 0.05 was chosen and statistical analysis was performed using XLStat–Pro (Addinsoft, France).

3 | RESULTS

3.1 | Consumer evaluation of beef burger products

3.1.1 | Overall liking

Significant differences in acceptability between beef burger samples were identified ($F = 53.636$, $p < .0001$). As shown in Table 3, acceptability scores of vegetarian and meat-containing products were varied among consumers. The Vegetarian burger 2 had the lowest acceptability score of 2.85 and was disliked very much by meat-eating consumers. Tukey's test identified this as significantly different from the other samples. This was followed by Vegetarian burger 1 which received the second lowest acceptability score (5.38), then the Hybrid 2 and Hybrid 1 with mean acceptability scores of 5.84 and 5.92, respectively. Receiving the highest acceptability score of 6.34, corresponding to 'liked slightly' was the Meat burger. According to Tukey's test, no significant difference in acceptability was identified between the full meat burger and the two Hybrids; however, a significant difference in acceptability was identified between the meat-free samples and the meat only sample.

3.1.2 | CATA questionnaire

3.1.2.1 | CATA counts

The frequencies by which consumers checked an attribute for a particular sample are shown in Table 4. As can be seen, samples vary largely in their sensory attributes and significant differences in 19 out of the 20 attributes were identified between samples ($p < .05$). No significant difference in "peppery flavor" was identified between the five samples tested ($p > .05$). The Vegetarian burger 2 was described as having an "off-flavor", "processed appearance," "wheaty flavor," "hard

TABLE 3 Mean acceptability scores of burger samples evaluated.

Sample	Mean acceptability score
Vegetarian burger 2	$2.85^{a} \pm 1.60$
Vegetarian burger 1	$5.38^{b} \pm 2.29$
Hybrid 2	$5.84^{b,c} \pm 1.80$
Hybrid 1	$5.92^{b,c} \pm 1.79$
Meat burger	$6.34^{c} \pm 1.66$

Mean acceptability scores with different superscripts are significantly different according to Tukey's HSD test with a confidence level of 95%.

TABLE 4 Frequency by which consumers used the terms of the CATA question to describe the burger samples tested and their ideal products. Cochran's Q test identifies significant differences between samples

	Sample						
Attribute	*p*-value	Vegetarian burger 2	Vegetarian burger 1	Hybrid 2	Hybrid 1	Meat burger	Ideal
Juicy	<.001	1	57	31	25	26	82
Dry Texture	<.001	66	15	32	53	50	1
Granular	<.001	41	6	44	37	38	11
Greasy	−.001	9	27	23	12	11	15
Easy to cut	<.001	31	87	50	68	57	78
Difficult to cut	<.001	35	2	26	12	18	3
Hard	<.001	43	0	24	19	21	6
Soft	<.001	17	85	41	46	41	67
Dark brown color	<.001	50	53	68	50	16	63
Light brown color	<.001	28	24	9	29	58	18
Dry appearance	<.001	50	33	41	59	61	12
Oily appearance	<.001	21	16	30	6	6	25
Processed appearance	<.001	58	50	28	31	37	7
Uneven color	−.001	27	26	10	16	31	2
Sweet	−.004	13	27	15	11	21	16
Peppery	−.072	24	10	19	18	22	47
Smokey Flavor/Grill	<.001	40	81	15	18	17	51
Off-flavor	<.001	56	18	12	11	6	0
Meaty flavor	<.001	3	40	66	59	69	91
Wheaty flavor	<.001	38	18	19	20	16	3

texture," "dry texture," and being "difficult to cut." The Vegetarian burger 1 was described as being "juicy," "easy to cut," "soft," having a "processed appearance," and a "smokey-grill flavor." The Hybrid 2 burger was described as "granular" in texture, "easy to cut," "dark brown" in color, and "meaty" in flavor. The Hybrid 1 and Meat burger were found to be similar in the sensory attributes and were described as "meaty" in flavor, "easy to cut" but having a "dry appearance". The ideal burger was described as "juicy," "easy to cut," and "dark brown" in color with a "meaty flavor."

Correspondence Analysis (CA) is a statistical technique that can be used to generate a biplot showing the relationships between samples and the terms used in CATA questioning. The outcomes of the correspondence analysis of CATA data are shown in Figure 1. The five burger samples were sorted into three areas according to their sensory attributes. The first area comprised the meat-containing samples; the Meat burger and the two Hybrid products are separated from the non-meat products along dimension 2. The two Hybrid samples also shared similar formulations and the only contributing factor to differing sensory attributes would have come from the meat replacer used. The Vegetarian burger 1 and the Vegetarian burger 2 have very different formulations; thus, large differences in sensory attributes were identified among consumers and were separated along Dim1. These two samples were separated by the Vegetarian burger 2 product having a "wheaty flavor" and the Vegetarian burger 1 being softer and "juicy," and having a "smokey flavor."

3.1.2.2 | Penalty analysis

Penalty analysis (PA) is a method of determining the penalty or reward on liking scores associated with the presence or intensity of sensory attributes. It is commonly used with liking scores and data from Just-About-Right or intensity scales; however, recent studies have utilized this approach with the binary responses (checked or unchecked) from CATA questions (Ares et al., 2014; Plaehn, 2013). PA can also be used to identify directions for product improvements in terms of reformulation if a consumer's "ideal" product is included in the questionnaire (Ares et al., 2014). PA determines the mean drop in consumer acceptability when consumers select an attribute for the ideal products but is not described for the test sample. This data can be used to prioritize product development areas to those which are subject to the highest penalty if not deemed by the consumer to be correct. The results of the penalty analysis to determine the sensory attributes that drive consumer acceptability in burgers are shown in Figure 2. As can be seen, the absence of a "meaty flavor" is found to be the largest contributor to a decrease in consumer acceptability with a drop of 2.20 in acceptability and is related to 47% of consumer responses. The Meat burger received the most counts for

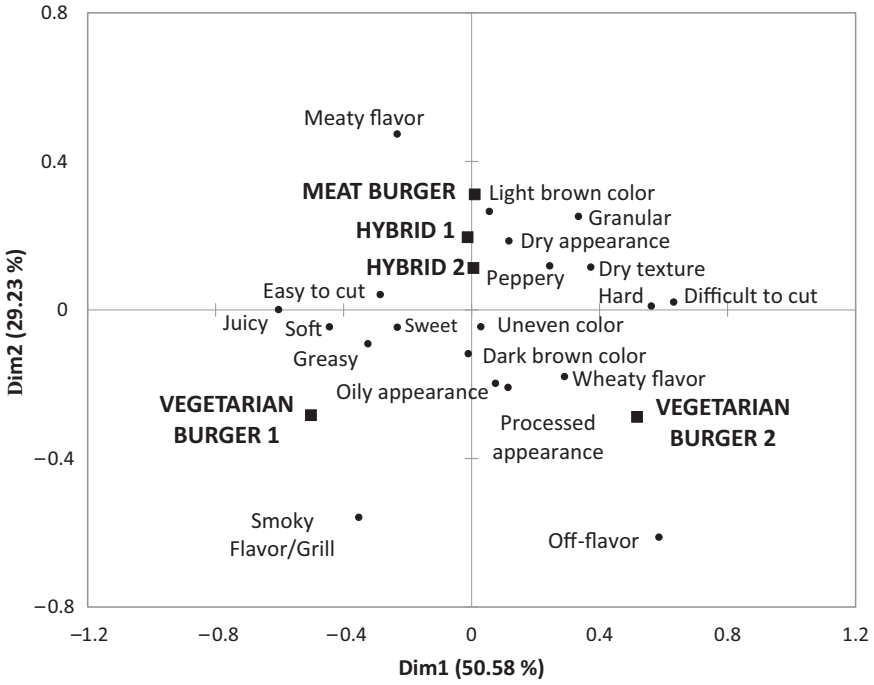

FIGURE 1 Representation of burger samples and their related terms from the CATA question. First and second dimensions of the correspondence analysis

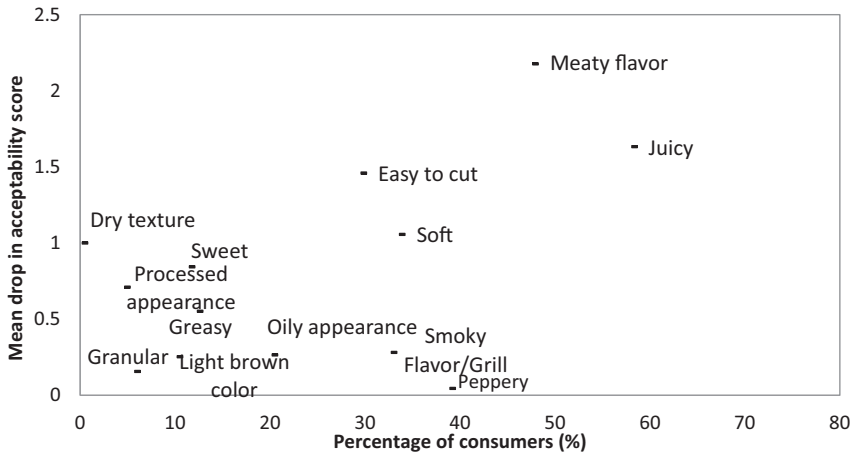

FIGURE 2 Mean drops in overall acceptability when a sensory attribute was described in a consumer's ideal but when not present in a particular sample, consumer acceptability significantly decreased

"meaty flavor" (Table 4) which would offer an explanation as to why this burger achieved the highest acceptability score. Also, "juicy," "easy to cut," and "soft"— all had a large influence in consumer acceptability with mean drops of 1.65, 1.50, and 1.05, respectively. The Vegetarian burger 1 received the most counts for "juicy," "easy to cut," and "soft", thus, increasing its acceptability among consumers. However, the low counts for "meaty flavor" may have prevented the Vegetarian burger 1 from achieving a higher acceptability score. As shown in Table 4, the two hybrid samples and the Meat burger received similar counts for "juicy," "easy to cut," and "soft" but the Meat burger received a higher count for "meaty flavor." Therefore, in order to improve consumer acceptability of the hybrid concepts, reformulation may involve the development of a meatier flavor closer to a consumer's ideal count.

Figure 3 details the sensory attributes that a sample must not have; otherwise, consumer acceptability significantly decreases. These are

TABLE 5 Mean acceptability scores of sausage samples evaluated

Sample	Mean acceptability score
Vegetarian sausage 2	$4.39^{a} \pm 2.07$
Vegetarian sausage 1	$5.10^{a} \pm 1.97$
Hybrid 2	$6.00^{b} \pm 1.52$
Meat sausage	$6.39^{b} \pm 1.78$
Hybrid 1	$6.51^{b} \pm 1.52$

Mean acceptability scores with different superscripts are significantly different according to Tukey's test with a confidence level of 95%.

the sensory attributes that consumers did not mention in their ideal but when present in a sample, acceptability significantly decreased. "Off-flavor" "processed appearance," and "dry texture" were identified as resulting in the largest mean drop in acceptability score with drops

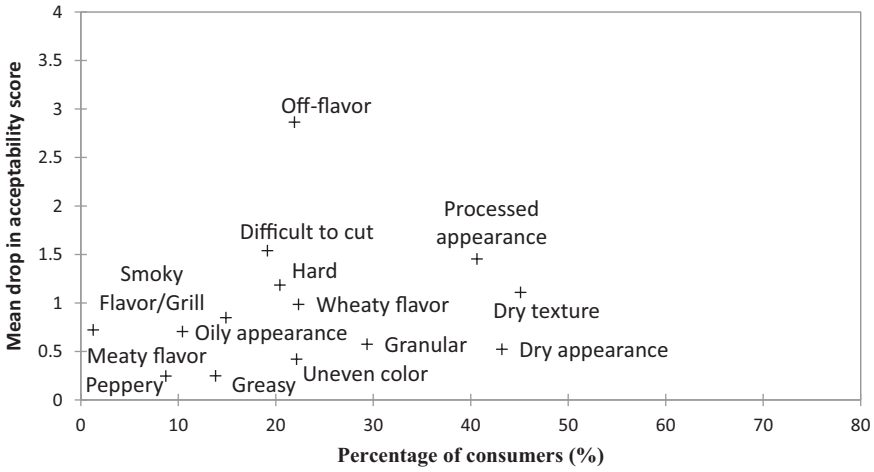

FIGURE 3 Mean drop in overall acceptability when a sensory attribute was not described in a consumer's ideal and when present in a sample

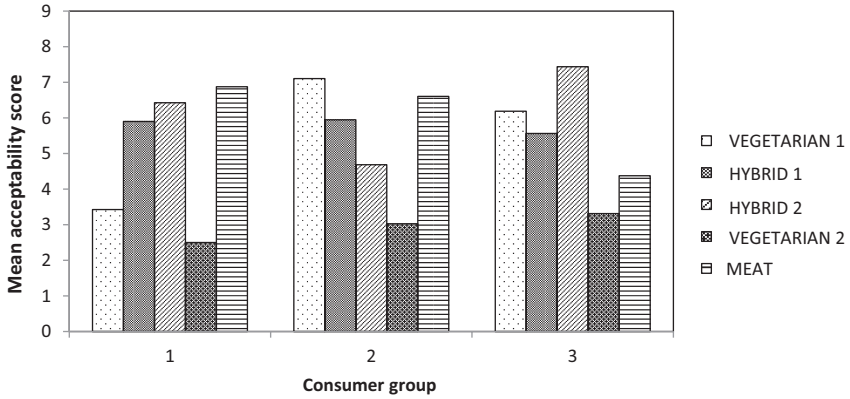

FIGURE 4 Preferences of the consumer groups identified from the cluster analysis

of 2.90, 1.40, and 1.10, respectively. The Vegetarian burger 2 received the most counts for all three of these attributes and would provide an explanation as to why this product received such a low consumer acceptability score.

3.1.2.3 | Multiple factor analysis

Cluster analysis was used to identify trends in consumer responses and three significant groups in terms of consumer preferences were identified, their acceptability profiles for the product set are shown in Figure 4. Consumer group 1 (n = 40) had a higher preference for the meat-containing samples, especially the Meat burger, and rejected both Vegetarian burgers. Consumer group 2 (n = 38) had a higher preference for both meat and meat-free products, especially Vegetarian burger 1 and the Meat burger. Consumer group 3 (n = 16) were found to have a preference for the two Hybrid samples and Vegetarian burger 1. By identifying individual panelist numbers within each consumer group, group 1 was identified as predominantly the consumers who only eat meat products and do not eat meat substitutes. Group 2 was identified as consumers who most commonly eat meat but sometimes eat meat substitutes. Group 3 was identified as a mixture of the two.

Multiple factor analysis was used to investigate the relationship between responses to the CATA questions of the consumer groups identified in the cluster analysis (Figure 5). This suggests that the preferred attributes for consumer group 1 (the meat eaters) include "light brown color" and "meaty flavor", whereas consumer group 2 have a higher preference for the attributes "easy to cut," "juicy," and "soft".

3.2 | Consumer evaluation of pork sausage products

3.2.1 | Overall liking

Significant differences in acceptability scores between pork sausage products were identified (F = 53.636, p < .0001). As shown in Table 5, acceptability of meat-free and meat-containing products were varied among meat-eating consumers. The Vegetarian sausage 2 received the lowest mean acceptability score of 4.39. The Vegetarian sausage 1 received the second lowest mean acceptability score of 5.10. According to Tukey's test, these two meat-free products were identified as significantly different in acceptability from the meat-containing samples. The Hybrid 2 sausage received a lower acceptability score than the Meat sausage of 6.00 and 6.39, respectively. The Hybrid 1 sausage received the highest mean acceptability score of 6.51 and was 'liked slightly' by consumers. However, according to Tukey's test, was not identified as significantly different in acceptability to the Meat and Hybrid 2 sausage.

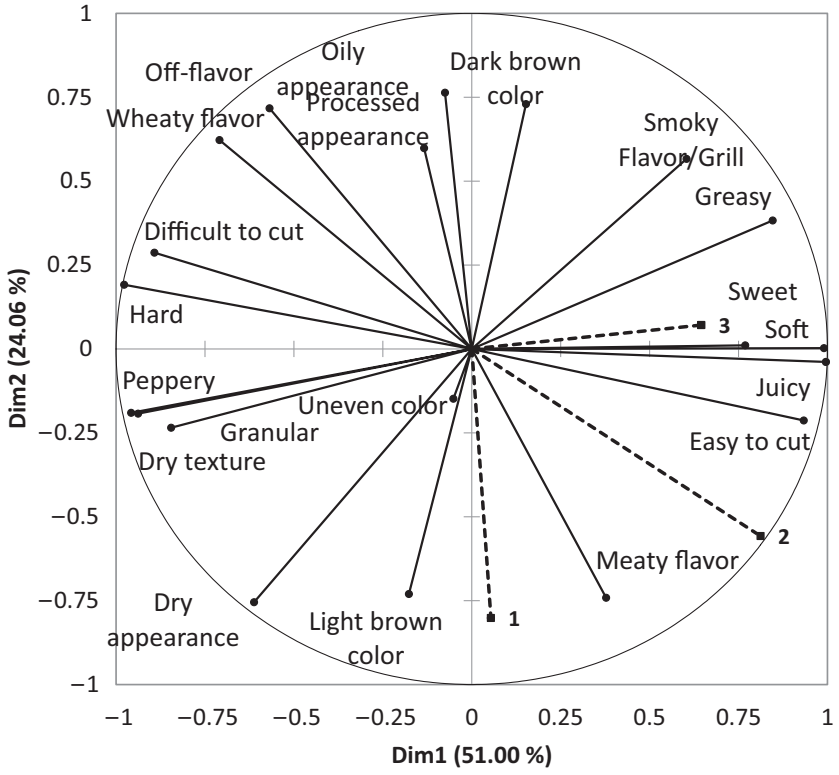

FIGURE 5 Multiple factor analysis of sensory attributes from CATA questioning and the consumer groups identified from cluster analysis

TABLE 6 Frequency by which consumers used the terms of the CATA question to describe the sausage products tested and their ideal products. Cochran's *Q* test identifies significant differences between samples

	Sample						
Attribute	*p*-value	Vegetarian sausage 2	Vegetarian sausage 1	Hybrid 2	Meat sausage	Hybrid 1	Ideal
Fibrous texture	<.001	49	17	35	5	23	15
Dry texture	<.001	23	6	32	0	23	2
Poor mouthfeel	−.003	35	26	26	16	15	0
Greasy	<.001	6	20	14	62	14	16
Easy to cut	<.001	53	75	27	66	53	72
Difficult to cut	<.001	11	1	49	10	20	1
Hard	<.001	9	0	36	2	15	7
Soft	<.001	45	77	29	74	46	60
Moist texture	<.001	28	59	19	73	33	75
Coarse appearance	<.001	23	11	21	2	13	18
Dry appearance	<.001	32	29	30	0	27	9
Visible herbs	<.001	76	20	27	0	16	42
Pale color	<.001	34	38	18	47	19	2
Meaty color	<.001	18	21	51	39	57	78
Fatty appearance	<.001	3	13	18	52	14	15
Herby flavor	<.001	65	39	35	15	28	40
Peppery flavor	−.255	35	43	31	31	35	38
Off-flavor/unpleasant aftertaste	<.001	32	29	10	4	6	0
Meaty flavor	<.001	13	21	50	59	55	82
Wheaty flavor	−.109	23	29	24	14	23	2

3.2.2 | CATA questioning

3.2.2.1 | CATA counts

The frequencies by which consumers checked a sensory attribute for each of the sausage products including their ideal are shown in Table 6. Samples were all described very differently in their sensory attributes and 18 out of the 20 attributes were identified as being significantly different between samples ($p < .05$). "Peppery flavor" and "wheaty flavor" were identified as not significantly different ($p > .05$) between the five products. However, similarities were identified between the two Hybrid sausages. The Hybrid products were identified as having the "meatiest color" and a "meaty flavor" in line with the Meat sausage which received the highest counts for "meaty flavor". The two Hybrids were also described as having the "driest texture". These similarities would be expected as the recipes used for the two Hybrids are the same with the only differing factor being the meat replacer used. The Hybrid 1 sausage was described as "easy to cut," having a "fatty appearance," and received low counts for "off-flavors". The Hybrid 2 sausage was described as being "difficult to cut," "hard," received low counts for "greasiness," "off-flavor," and had a "pale color." The Vegetarian sausage 2 was described as having a "fibrous texture", "being easy to cut", "herby" in flavor and received the highest counts for an "unpleasant aftertaste and off-flavor"; however, in general, these counts were quite low. The Vegetarian sausage 1 was described as "easy to cut," having a "moist texture," and very "soft". The Meat sausage was described as "greasy," "fatty appearance," "easy to cut," "moist," having a "coarse texture" and "pale in color". The ideal sausage was described as "easy to cut", having a "moist texture", a "meaty color" and "meaty flavor".

The outcomes of the correspondence analysis of CATA data generated are reported in Figure 6. The correspondence analysis shows that samples were found to be very different in their sensory attributes; however, similarities between the two Hybrids were identified and thus were grouped together due to their similar formulations. The two Vegetarian sausages are shown to be very different in their sensory attributes which is due to their very different formulations and are separated along dimension 1.

3.2.2.2 | Penalty analysis

The results of the penalty analysis of the attributes that help to drive consumer acceptability in pork sausages are shown in Figure 7. It identified that "meaty flavor," "meaty color," and "moist texture" were important factors for consumer acceptability and account for a drop in overall consumer acceptability of 1.80, 1.30, and 0.80, respectively, with 47%, 46% and 40% of consumers agreeing with this trend, respectively. The Meat sausage was described as "meaty" in flavor with a "moist texture" but was described as "pale", whereas the Hybrid 1 sausage was described as having a "meaty flavor" and color, thus, showing the importance of a products color in influencing consumer acceptance.

Figure 8 reports on the sensory attributes that a sample must not have; otherwise, consumer acceptability significantly decreases. "Off-flavor/unpleasant aftertaste" and "poor mouthfeel" were identified as the most important sensory attributes resulting in a large drop in overall acceptability with scores of 2.50 and 1.90, respectively. The Vegetarian sausage 2 received the most counts for "off-flavor/unpleasant aftertaste" and "poor mouthfeel" which would explain its low acceptability score.

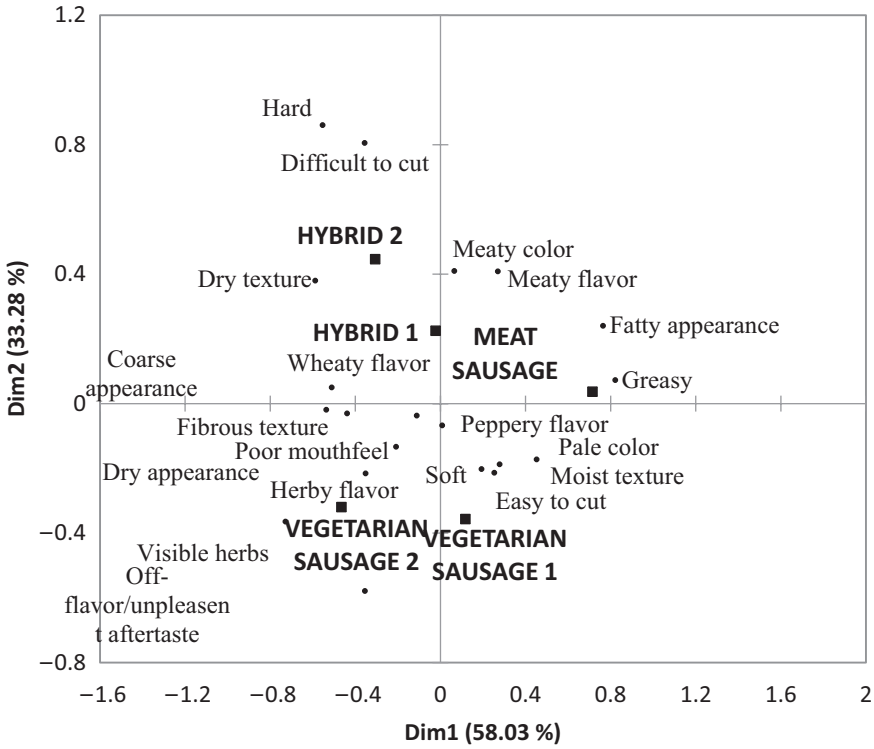

FIGURE 6 Representation of sausage products and their related terms from the CATA question. First and second dimensions of the correspondence analysis

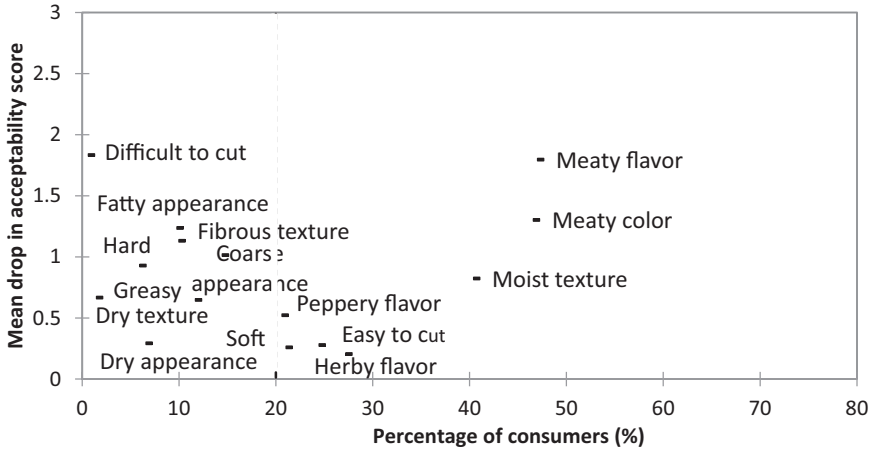

FIGURE 7 Mean drop in overall acceptability when a sensory attribute was described in a consumer's ideal but when not present in a particular sample, consumer acceptability significantly decreased

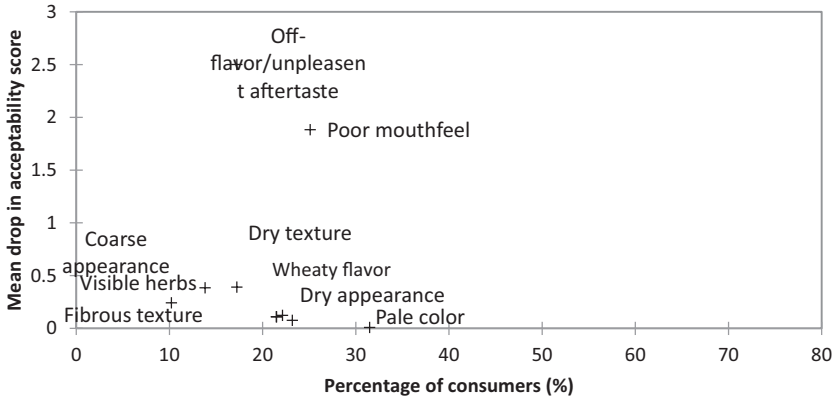

FIGURE 8 Mean drop in overall acceptability when a sensory attribute was not described in a consumer's ideal and when present in a sample

3.2.2.3 | Multiple factor analysis

Cluster analysis identified three significant groups in consumer behaviors in terms of preference (Figure 9). Consumer group 1 (n = 41) were identified as having a preference for both meat and meat-free products with the exception of the Vegetarian sausage 2. This group, however, had a higher preference for the Meat and Hybrid 1 sausage but Hybrid 2 and Vegetarian sausage 1 received similar acceptability scores. Consumer group 2 (n = 33) were found to have a higher preference for the meat-containing samples; the two Hybrids and the Meat sausage, due to the "meaty color" and "meaty flavor". Consumer group 3 (n = 14) were found to have a higher preference for the Vegetarian 2 and Hybrid 1 sausages.

By identifying individual panelist numbers within each group, group 1 were identified as predominantly the consumers who tend to like both meat and meat-free products. Group 2 were identified as predominantly pure meat eaters; they are the consumers who only eat meat products and do not consume alternatives or substitutes. Group 3 were identified as a mix of the two.

Multiple factor analysis (Figure 10) identified the relationship between responses to the CATA questions of consumer groups identified during the cluster analysis. This suggests that consumer group 1 has a higher preference for samples containing the sensory attributes "greasy," "fatty appearance," and "meaty flavor", whereas consumer group 2, the meat eaters, has a higher preference for products that have a "meaty flavor" and "meaty color."

4 | DISCUSSION

The importance of meat alternatives has been well documented (de Bakker & Dagevos, 2012; Lea et al., 2006). Modern day demand and consumption of meat is unsustainable and a need to reduce meat consumption has importance for both the environment and human health. Although novel protein alternatives are widely available on the market, the lack of acceptability of some meat substitutes with meat-eating consumers due to a perceived compromise in sensory attributes, has hindered consumer transitions to more sustainable diets (de Bakker & Dagevos, 2012). A means to create a stepping stone between meat and meat-free is through Hybrid meat analogues, creating products with greater consumer acceptability but reduced meat content. This should aid in lowering the impact on both human health and the environment.

In this study, a consumer-generated lexicon of the sensory attributes that compromise the products was generated for two sets of products; beef burgers and pork sausages. This sensory lexicon in a consumer's language was used in Check-all-that-apply (CATA) analysis.

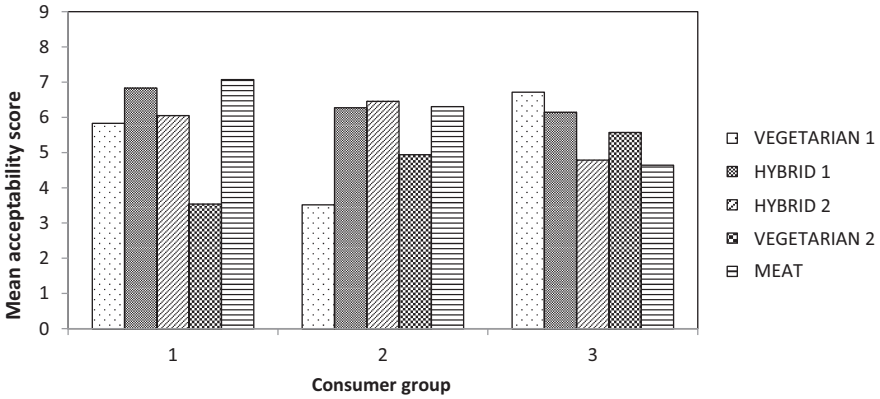

FIGURE 9 Preferences of the consumer groups identified from the cluster analysis

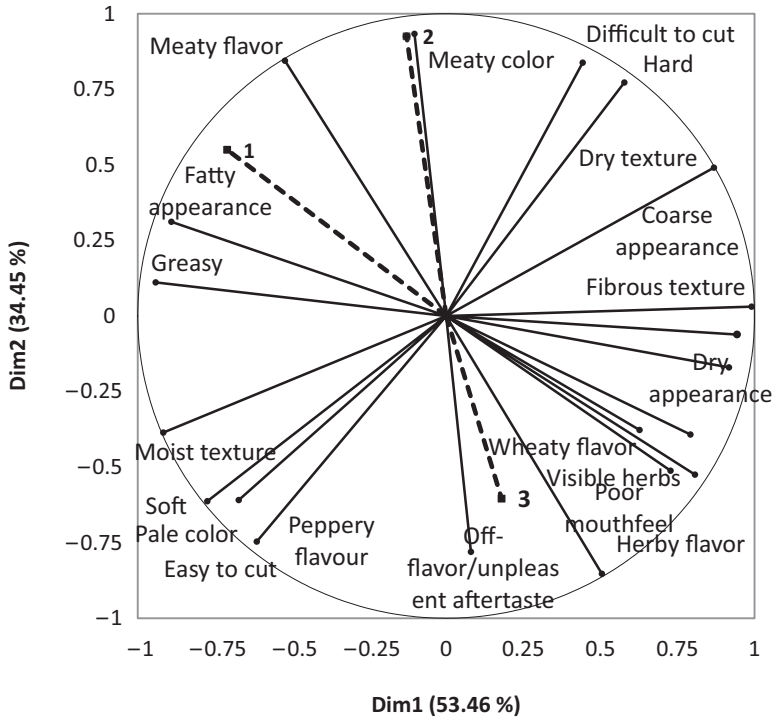

FIGURE 10 Multiple factor analysis of sensory attributes from CATA questioning and the consumer groups identified from cluster analysis

Consumers were presented with samples of commercial meat, meat-free, and Hybrid products and scored overall liking. Using the CATA questionnaire, they identified the sensory attributes they perceived to be present in each product as well as indicating the attributes of their ideal product. The results found that Hybrid products are generally well liked among consumers. However, it was found that Hybrid sausages had a higher overall acceptability in comparison with Hybrid burgers suggesting that the format of the product may have a large impact on consumer acceptability.

No significant differences in consumer acceptability ($p > .05$) could be identified between meat and Hybrid products, whereas consumer acceptability of meat-free products was significantly lower than the meat-containing products ($p < .05$).

Correspondence analysis showed that the Hybrids were grouped together with the full meat products indicating that they possess similar sensory attributes. By clustering acceptability data it was also identified that significant differences in acceptability of the products tested existed between different consumer groups. Predominantly meat eaters who do not eat meat substitutes have a higher preference for the meat-containing products. Consumers who most commonly eat meat but also eat meat substitutes were found to have a broader preference for both meat-containing and meat-free products suggesting that familiarity to vegetarian meat substitutes increased their acceptability among this consumer group. As has been previously suggested (Hoek et al., 2011, 2013), in this study, multiple factor analysis suggests that replicating a "meaty flavor" and "meaty color" in Hybrid products is key to increasing their acceptability among predominantly meat consumers. However, in terms of converting the three consumer groups to a meat-reduced diet, it is encouraging to see that at least one of the Hybrid formulations is prominent within each group. Thus,

it could be proposed that by creating a positive initial experience and replicating the flavor and texture of meat within a substitute and repeated exposure of Hybrid products among meat consumers will aid in the transition to more sustainable diets.

The novel approach used in this study of combing Penalty analysis with CATA data helped to uncover, in a consumer language, the key attributes that drive consumer liking and disliking in meat-containing and meat-free products. This can provide information and focus for product reformulation. CATA questioning is a relatively novel consumer analysis technique and offers an alternative to conventional Quantitative Descriptive Analysis (QDA) (Meilgaard, Carr, & Civille, 2006). CATA questioning provides a rapid and easy method of sensory analysis using consumer language. Using appropriate analysis techniques, a wealth of information can be generated to help drive product reformulation. However, a disadvantage of CATA questioning is related to the fact that information about an attributes intensity and degree of difference between a product and the ideal cannot be generated (Ares et al., 2014).

The results generated from this study indicate that the Hybrid concept helps to bridge the acceptability gap among predominantly meat eaters between meat and meat-free products. It is possible that the Hybrid concept could be used as a stepping stone in the transition of converting meat eaters to a meat-reduced diet, increasing their familiarity with meat substitution. The Hybrid concept does not provide the sole means to solving the protein issue but should be used among various other strategies to move consumers to more sustainable protein diets. Although the Hybrid products were found to be acceptable, this does not mean that the consumers have any intention to buy and further studies should be conducted to determine this type of consumer behavior.

5 | CONCLUSIONS

Consumer testing has shown that the new concept products are generally well accepted by predominantly meat eaters. Acceptability scores are able to show that the Hybrid concept helps to bridge the gap between meat and meat-free products. No significant difference in acceptability could be seen between meat samples and Hybrid samples ($p > .05$). This can provide encouragement for the use of the Hybrid concept to reduce consumers' meat consumption and promote the substitution of meat in consumers' diets to more sustainable protein sources.

Hybrid sausages were found to have a larger impact on acceptability compared to burgers. Information on this difference is provided by the CATA questions as the acceptability of the burgers was reduced by the samples being too dry as fat and moisture were easily cooked out while in the sausages fat and moisture were retained within the skins. In future reformulations, this issue should be addressed to optimize acceptability.

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