Microstructural Engineering of Cakes

by

Amir Kasra Asghari

A thesis submitted to
The University of Birmingham
for the degree of
Doctor of Engineering

School of Chemical Engineering
College of Engineering and Physical Sciences
The University of Birmingham
November 2016

Declaration

Abstract

The objective of this thesis is to advance the current understanding of some pertinent formulation and processing interactions informing final cake microstructures. A primary concern is to understand how certain ingredients, fundamental to cake batter formation interact, in order to develop new methods and models for optimising and characterising these microstructures.

The motivation of this work stems from the empirical methods still prevalent within cake research. However an approach based on fundamental understanding of formulation and processing functions is necessary for both future innovation and eradication of some current challenges facing the cake baking industry, such as emulsifier reduction/replacement.

Our bottom-up approach begins by exploring the interactions of key structural components; starch and egg white protein (EWP) within wet-foam systems. It is demonstrated that firstly use of hydrophobic starch in conjunction with EWP can produce synergies capable of enhancing foam stability. Secondly heat-treatment of native starch granules could induce additional surface hydrophobicity which enhanced their interaction with EWP thus positively influencing foam stability.

Consequently the structure of the model system is further developed to resemble a foam based cake batter in which the influence of heat-treated (HT) flour is evaluated. It was established that in this regard the heat-treatment of flour and smaller flour particle sizes both act to produce cake batters with higher air-retention capacities facilitating a reduction in the EWP concentration. This chapter provided validated evidence that HT flour can act to maximise aeration capacity within cake batters and potentially increase final cake volumes.

In order to further evaluate the influence of flour particle size, quinoa starch with it's small granule (1-2 μm) and higher potential for adsorption at the air/water interface was trialled within the same model foam cake system. A relatively novel rheological characterisation tool; the 'weak gel model' was used to also assess the thermal behaviour of HT wheat and quinoa starches. It was observed that partial replacement of wheat flour with quinoa starch could produce cakes with increased volume. Heat treatment of quinoa significantly modified its functionality to allow better aeration and network formation within the foam batters. Here we also validated X-ray micro-computational tomography (x-ray micro-CT) as an effective tool for quantification of microstructural parameters which demonstrated good agreement with mechanical properties of the final cakes.

Finally the efficacy of the mixing mechanisms on emulsification by acoustic and high shear means was compared. Acoustic emulsification proved more efficient both in terms of emulsifier and energy consumption, particularly at elevated viscosities.

Acknowledgements

I would like to thank a number of important people who have assisted me in many areas during my research time at Birmingham. I'll start with my academic supervisors: Ian Norton, Thomas Mills and Fotis Spyropoulus. They have all been helpful: Ian has continued to ensure that I perform to the best of my ability, while Fotis has always endeavoured to provide context. Richard Greenwood, for his efforts to ensure that I remained focussed on the task in hand, no matter how often I attempted to stray from the path and Tom for his friendship and guidance.

We have been in close collaboration with Dr Peter Sadd from the Premier Foods, who has continued to provide constructive feedback on the direction of the research as well as a wealth of experimental knowledge and material. Also all of the staff at Birmingham, in particular Kathleen Hynes, John Hooper and Lynn Draper. I would also like to thank Paul Stanley and Theresa Morris for assistance with Cryo-SEM equipment, the EPSRC for their sponsorship of this work and Dr Sophie Cox for her assistance with the X-ray.

There are numerous other people who have made my EngD time pleasant: My family; the many new friends I have made as a result of this time; every inhabitant of 50 Symphony court; and the members of the old Birmingham gang. An especial thank you is in order for Maman, Baba and Rosa for their love, dedication and continued support throughout this period of growth. I doubt anyone will be as proud and jubilant in merely seeing this thesis as Maman Roohi and Baba Omid; I will always be in debt for your words of encouragement.

Finally, Emmanuelle for entering my life just when I needed her and for her continued affection and encouragement.

"Sell your cleverness and buy bewilderment."
Jalaluddin Rumi

Contents

List of Figures

List of Tables

Nomenclature

Emulsion

$d_{3,2}$	Sauter mean diameter
n_i	Number of droplets belonging to the i^{th} degree
d_i	Diameter of droplets belonging to the i^{th} degree
V	Volume of dispersed phase
A	Area of dispersed phase
ΔP	Laplace pressure differential
γ	Interfacial tension
r	Emulsion droplet radius
η_c	Continuous phase viscosity
We	Weber number
G	Velocity gradient
η_d	Dispersed phase viscosity
ρ	Density
g	Gravitational acceleration
v	Separation velocity
η_k	Kolmogrov's length scale
ν_ε	Kinematic viscosity
ϵ	Energy diisipation rate
N_P	Power Number
N	Rotor rotational speed
D	Rotor diameter
m	Mass of emulsion
φ	Oil-phase fraction

Foams

P_i	Pressure inside bubble
P_o	Pressure outside bubble
σ	Surface tension
V	Volume of gas
n	Number of gas moles
T	Temperature
R	Radius of bubble
π_h	Hydrostatic pressure
π_d	Disjoining pressure
A_0	Interfacial area of fresh foam
A_t	Interfacial area after time, t
ΔP_t	Net change in external pressure
ΔP_x	Pressure at infinite time after foam collapse
V_R	Rate of drainage
h	Film thickness
μ	Dynamic viscosity
θ	Contact angle
φ_A	Air-phase fraction
W_l	Weight of liquid
W_f	Weight of foam
ϕ_l	Weight of liquid
ϕ_l^*	Critical liquid fraction
φ_{max}	Maximum air-phase fraction

Rheology

G'	Elastic/storage modulus
G''	Viscous/loss modulus
$G*$	Complex modulus
δ	Shift phase angle
η_{app}	Apparent viscosity
$\dot{\gamma}$	Shear rate
η^*	Complex viscosity
A_F	Gel strength
z	Interaction factor
ω	Angular frequency
γ	Stress
τ	Shear stress
τ_o	Casson yield stress
k or K_c	Consistency coefficient
n	Flow behaviour index
A	Area of drop
E'	Dilatational elastic modulus
E''	Dilatational loss modulus
η_d	Dilatational viscosity

Acoustic

P_{co}	Cohesive pressure
P_{th}	Sound pressure amplitude
I_{th}	Cavitation threshold intensity
P_0	Hydrostatic pressure
v_s	Sound velocity

I_a	Acoustic intensity
P_a	Acoustic power
S_A	Surface area of the sonotrode tip
$\delta T/\delta t$	Change in temperature with respect to time

Mechanics

F	Compression force
A_0	Sample area in contact with probe
δ_E	Engineering stress
ϵ_E	Engineering strain
δ_T	True stress
ϵ_H	True strain
E	Young's/elastic modulus
K	Bulk modulus
L	Height of test specimen
D	Width of test specimen
P	Force per unit area
V	Original volume
μ	Poisson's ratio
ρ/ρ_s	Relative density
E/E_s	Relative compressive elastic modulus
C, m or n	Geometry specific constants
φ_c	Crumb porosity
t_c	Cell wall thickness
l	Cell edge length

List of abbreviations

EDS	Emulsion drop size
US	Ultrasound
HS	High shear
A/W	air/water
CO_2	Carbon dioxide
EWP	Egg white protein
GMS	Glycerol monostearate
HLB	Hydrophilic lipophilic balance
PPI	Pea protein isolate
IFT	Interfacial tension
HT	IHeat treated
HTQ	Heat treated quinoa
HTW	Heat treated wheat
HTF	Heat treated flour
WF	Wheat flour
o/w	oil/water
OSA	sodium octenyl succinate
WPI	Whey protein isolate
SG	Specific gravity
TPA	Texture profile analysis
SEM	Scanning electron microscopy
G.T	Gelatinisation temperature
CLSM	Confocal scanning laser microscopy
X-ray CT	X-ray micro-computational tomography
TA	Texture analysis
ES	Emulsifier concentration

1 | Introduction

1.1 Introduction

The changing trends within food and nutrition remain food industry's main source of innovation and progress. Current global challenges in food science and technology are the alignment towards sustainability in terms of sourcing of ingredients and use of energy. Depending on the socio-economical background of the consumer, individual diets especially within Europe are now based on food products that are partially or entirely processed (Fischer and Windhab, 2011). In this regard, the consumer driven preferences in recent years have been mainly in the context of nutritional value and cleaner more organic labeling (Zink, 1997). Decomposition and subsequent re-composition of food materials allows tailored design of foods according to nutritional trends through addition/elimination of ingredients for enhancing nutritional benefits as well as sensory preferences. Consequently food science and engineering has devoted significant research to the role of individual ingredients (for example, stabilizing agents) along with processes that can facilitate innovation in food microstructure design (Fischer and Windhab, 2011).

1.1.1 Challenges Facing Cake Industry

Clean label is no longer a trend, it's the rule, according to 'Innova Market Insights, Arnhem'. The ultimate clean label is now considered to reflect the organic nature of the product emphasising the consumer trend to go back to basics (Watrous, 2015). In the past couple of years, multi-national conglomerates such as Kellogg, Nestlé and many other major food and beverage companies have committed to removing artificial ingredients from popular products. However additives such as emulsifiers have a critical role within the functional properties of cakes, thus their reduction or elimination requires a conscious move away from the current empirical nature of cake research, *i.e.* to combine its artisan nature with fundamental underpinning science (Cauvain and Young, 2006). The basic ingredients are an area where a more concerted effort is required for understanding the specific roles. Therefore continuous research is necessary to provide the ability for more accurate prediction of functionality based on formulation and processing steps, in order to reduce wastage and produce quality products consistently; the 'artisan' component of technology is going to have to be reduced.

1.1.2 Trends and Opportunities

Health concerns over sugar consumers are now the precedent; overtaking from 'public enemy number one' fat (Mintel, 2016). However recent market data (2015-2016) suggests that consumption within this category are still largely unwilling to cut indulgent treats such as cakes from their diet; with the general consensus that they are acceptable as part of a balanced diet (Mintel, 2016). The cake market remains a billion pound industry in the UK, with further growth forecast for the next five years (Fig. 1.1).

Consumers are not satisfied with the presence of additives in their food products however the use of nutraceuticals or functional ingredients in formulations poses an opportunity. Exploration of healthier formulations, due to significant unmet demand for products in the context of 'superfood' ingredients such as ancient grains, are forecast to provide a huge opportunity in tapping into current focus on 'positive nutrition' (Mintel, 2016). Another important and stimulating area for development is hybrid products, products that do not readily fall into any particular category: a good examples of these would be Jaffa cakes, but there is substantial room for further innovation in this category.

subsectionCake Microstructure Cake is enjoyed for its sweet taste and tender eating quality. Its macro-porous semi-solid structure is critical to quality and consumer ac-

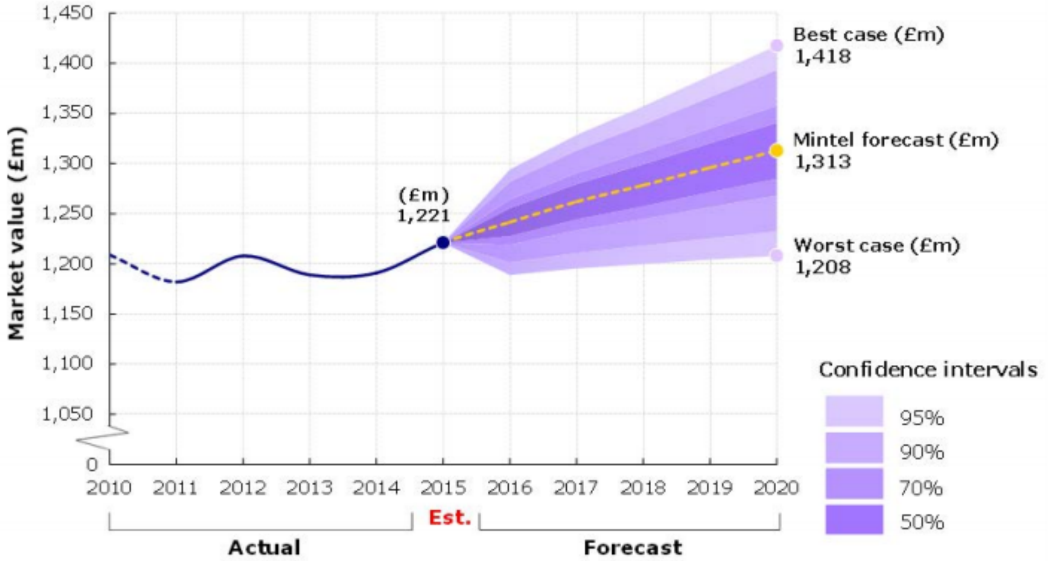


Figure 1.1: The Cake market forecast according to Mintel (2016)

ceptability. The development and solidification of this structure requires a fine balance between the processing and formulation that composes it. The aeration process, batter preparation and baking are all stages paramount to the final quality. The traditional demand for sweet and moist cakes, particularly in the UK and USA, has led to increased proportions of sugar and liquid in commercial cake recipes (Cauvain and Young, 2006). Such recipes are termed 'high ratio' and are defined as those containing a ratio of sugar-flour and/or liquid-flour, in excess of 1 (Sumnu and Sahin, 2008). High ratio recipes tend to be sweeter, moister, tenderer and generally possessing longer shelf lives. The disadvantage however, is that the large proportions of sugar and liquid put considerable strain on the structure-building components in particular flour and eggs (Sumnu and Sahin, 2008).

1.1.3 Role of Emulsifiers

In this regard, the emulsifier serve an important function; addition to the formulation aids batter aeration, thus creating the desirable light and porous foam-structure during processing that is more resilient to the gravitational and mechanical stresses until the structure sets upon baking (Sumnu and Sahin, 2008). In cake baking, emulsifiers aid the incorporation and subdivision of air into the liquid phase to promote foam formation.

Emulsifiers provide necessary aeration and gas bubble stability during the processing until the cake structure solidifies into a cellular solid (Sahi and Alava, 2003). Emulsifiers also serve several other functions in;

1. aiding dispersion of fat phase
2. reducing mixing time
3. reducing the amount of egg required within the formulation

Apart from the undesirable labeling considerations, another important factor when using emulsifiers in baking formulation is their fluctuating cost (Sumnu and Sahin, 2008). Therefore, the capacity to change formulations for more organic stabilisers without compromising the quality is considered essential to the baking industry's longevity and sustainable innovation. This requires a scientific understanding of the individual components and processes in relation to the final quality characteristics. On this basis, the use of a microstructural engineering approach has emerged and proved to be a successful tool for bridging the gap between fundamental science and industrial application.

1.2 Strategy

The premise of this approach (Fig. 1.2) is the use of underpinning scientific knowledge to understand the inter-relationships between both the process and formulation and how they combine to inform the final microstructure (Fig. 1.2). This approach has been used as the fundamental basis for food microstructure design of new functional foods. It also provides the flexibility in allowing change in the formulation whilst maintaining the desirable characteristics of the final product.

Two important relevant fields of food microstructural design that this approach has already been applied to are emulsions and foams, both of which are integral components in cake batter systems. The interplay of formulation and process design for creation of nano-emulsions (Dickinson, 2010) or the use of particles for stabilisation of foams and emulsions (Murray et al., 2011) are all important and pertinent examples of the success of this approach in the field of food engineering.

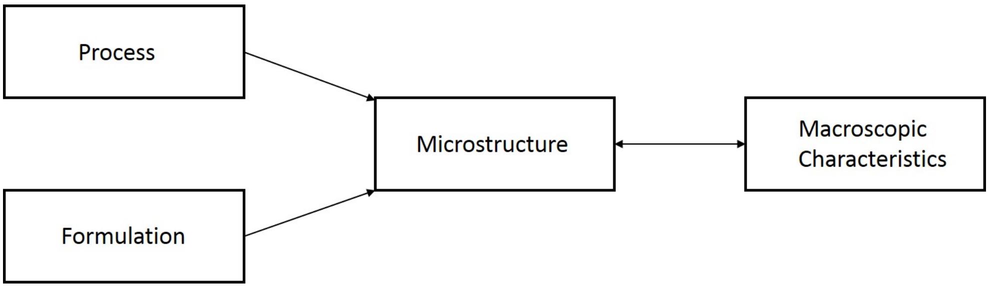


Figure 1.2: Microstrcutural engineering approach. Adapted from (Farrés, 2015).

1.3 Objectives of Research

Considering the current challenges and disposition of research, there remains afflictive areas of missing knowledge. A subsequent need for finding more organic methods of stabilising cake batters and thus controlling the destructive dynamic forces during baking exists to achieve the desired final cake structures. Both of which remain considerable challenges due to presence of meta-stable systems, and ones that are currently alleviated by addition of thickeners and stabilisers. This broad context of research is narrowed by focus on two dominant aspects; (1) enhancement of starch granule functionality as both a stabilising agent during foam formation and a water sink during the baking process, (2) utilisation of more innovative processes for a more efficient use of emulsifiers. The filtered aims of this body of work are funnelled into research on the incremental building of a model cake structure in order to investigate;

- The use of organic ingredients for inducing additional stability of wet-foams
- Use of trending nutritious grains such as quinoa with for better air-bubble stabilisation within the batter and volume maximisation in the final solid sponge
- Exploring innovative processes for emulsion creation as a basis for more efficient use of emulsifiers and energy
- Development of novel quality and character analysis techniques for translatable evaluation of cakes and cake batters

Research by Binks and Horozov (2005); Dickinson (2010); Yusoff and Murray (2011) along with recent investigations by Rayner et al. (2012) have presented particles and in particular starch as functional sources for stabilisation of metastable systems such as foams and emulsions. It has been shown the addition of particles of correct size and hydrophobicity can enhance the stability of protein stabilised wet-foams (Murray et al., 2011). Thus the experiments were designed for use of a variety of starch species with different physiochemical characteristics for determination of the properties that could induce synergy. The results are analysed and discussed in terms of foaming and interfacial properties of these systems in order to obtain the mechanism by which synergy may be achieved. Secondly the evidence of additional hydrophobicity imparted by heat-treatment of all flour components was investigated as a preliminary basis for highlighting the influence of heat-treatment on rheological properties of batters and mechanical properties of cakes, in a representative cake formulation. The proceeding chapter assesses the use of quinoa starch with small granules (1-2μm) in the same representative batter formulation in order to investigate the effect of heat-treatment on it's functionality at ambient and elevated temperatures. Finally high power, low frequency ultrasound is compared in its efficacy as an emulsification technique to conventional high shear mechanical agitation for evaluation of it's performance in viscous systems in terms of energy and emulsifier consumption.

1.4 Relevance to Premier

Premier Foods Plc the industrial collaborator for this project is a British food manufacturer head quartered in St Alban's, Hertfordshire. Premier Food maneuvers only within the UK and Irish food markets. It has an annual revenue of $\sim$ £767m and employs over 3600 people across 13 sites. The business is structured in terms of two strategic units; 'Grocery' and 'Sweet treats' (Premier-Foods, 2016). The sweet treats business unit, which accounts for 28% of the annual revenue stream is composed of three power brands in; 'Mr Kipling', 'Lyons' and 'Cadbury', the combination of which account for $\sim$ 25% of the

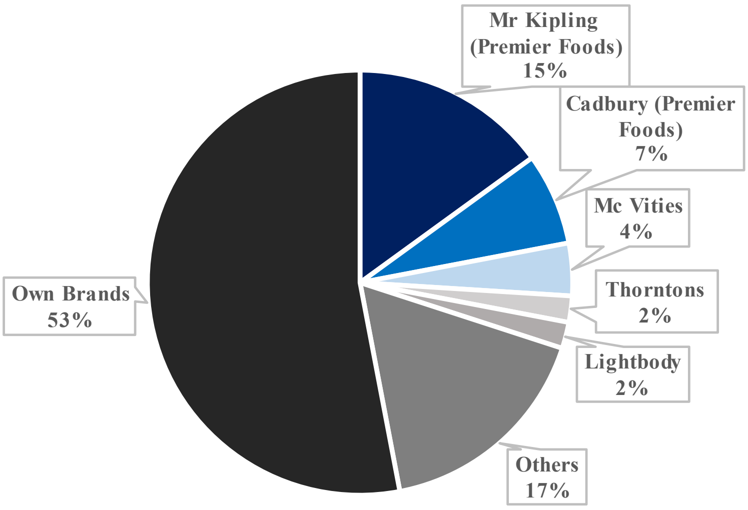


Figure 1.3: The market share for premier foods in the UK cake market according to Mintel (2016)

UK cake market (2015-2016).

The increasingly health conscious consumer, poses a number of challenges for this business unit. Amongst other important challenges is the aging population in which the usage of cakes is lower; 76% for over-55's, compared with 89% of under-25's (Mintel, 2016). Thus nutrition and health is now an integral strategic model for the growth in the Premier Foods strategy. The enhancement of individual ingredient's functionality for development of novel formulations can provide additional nutritional benefits and also meet the category's requirement for a more organic labeling. This is desirable for sustainable and consistent product development, which also served to provide the commercial edge in a highly competitive market (Premier-Foods, 2016). The underpinning science of the processes and ingredient functionality is important for facilitation of this. Moreover the untapped market in designing products for those of special dietary requirements such as sufferers of Coeliac disease is an opportunity for further growth. Additionally this thesis has aimed to explore the potential of novel 'superfood' ingredients with additional nutritional benefits such as the quinoa grain.

1.5 Thesis Layout

In order to guide the reader, this section is designated to provide an overview of the different studies conducted and their context in this body of work. The core structure of the work comprises of an introduction, a relevant literature review within the subject area, four results chapters presented as individual research papers and finally concluding remarks and considerations for future work. The work is broadly divided based on a progression through the formation of the cake microstructure starting with formation of a protein wet-foam and ending with the solid sponge (Fig. 1.4). The four individual results chapters have been styled as independent pieces of research formatted as journal articles, and subsequently include an introduction, discussions and conclusions specific to the material used within that particular chapter. The rationale for this format is that; firstly it allows a smoother progression through the various themes discussed, secondly that it is the division lines for submission and publishing of the individual pieces of research.

The first research chapter explores the synergy of hydrophobic starch particles within protein wet-foams, inducing additional stability.

The second research chapter is a preliminary investigation into the influence of heat-treatment within a more representative batter system and it's influence on aeration and potential for facilitation of EWP reduction; thus acting as prelude to the third experimental chapter.

The third chapter is composed of an investigation into the ambient and thermal behaviour of quinoa starch (and it's heat-treatment) in the cake batter and its potential as a substitute for wheat and comparative effects of heat-treatment on this grain. The focus of the chapter remained the influence of formulation, thus the batter processing (*i.e.* mixing and baking) conditions were kept constant.

Penultimately the fourth experimental chapter explores processing routes as an area for minimising of energy and emulsifier expenditure. Finally, the main findings of the study are drawn together in Chapter 7, and recommendations for future work are discussed.

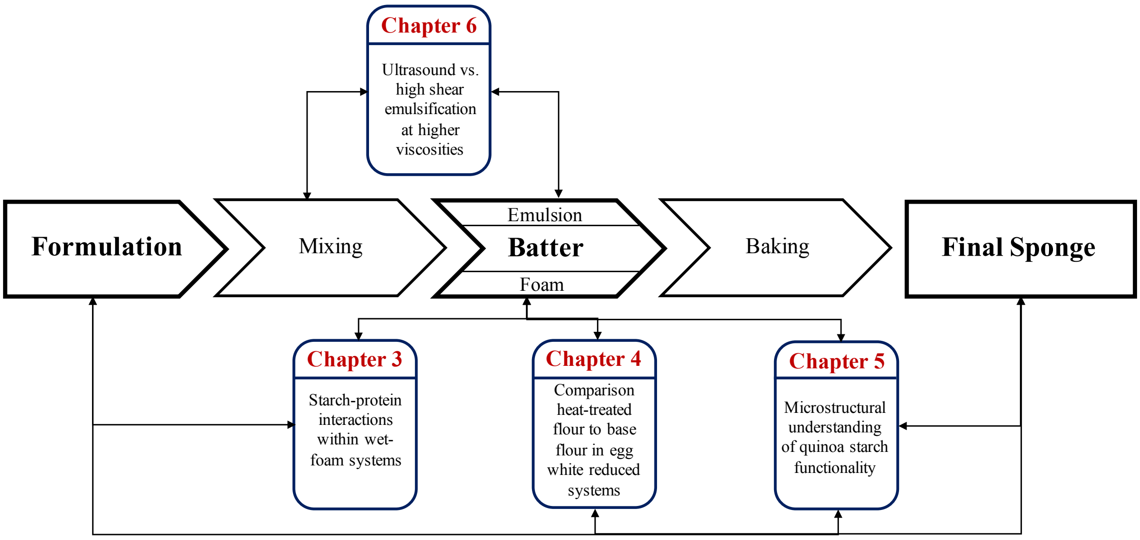


Figure 1.4: The arrangement of the experimental chapters presented in the context of structural evolution of cakes.

1.6 Communication of Results

The findings of the work have been published or presented as follows;

Presented;

A.K. Asghari, T. Mills, F. Spyropoulus and I.T. Norton Use of hydrophobic starch for stabilisation of protein foams. 12th International Hydrocolloids Conference, Taipei, 2014.

A.K. Asghari, T. Mills, F. Spyropoulus and I.T. Norton Fabrication and subsequent dehydration of protein-starch stabilised foams for food applications. 2nd UK food hydrocolloids symposium, Birmingham 2015.

A.K. Asghari, T. Mills, F. Spyropoulus and I.T. Norton Fabrication of protein-starch stabilised foams for food applications. 2015 Rideal Meeting, London 2015.

Published;

Asghari, A. K., Norton, I., Mills, T., Sadd, P.,& Spyropoulos, F. (2016). Interfacial and foaming characterisation of mixed protein-starch particle systems for food-foam applications. *Food Hydrocolloids, 53* , 311-319

1.7 References

List of References

Binks, B. P. and Horozov, T. S. (2005). Aqueous foams stabilized solely by silica nanoparticles. *Angewandte Chemie*, 117(24):3788–3791.

Cauvain, S. and Young, L. (2006). *Baked Products: Science, Technology and Practice*. Wiley.

Dickinson, E. (2010). Food emulsions and foams: Stabilization by particles. *Current Opinion in Colloid & Interface Science*, 15(12):40 – 49.

Farrés, I. F. (2015). Fluid gel production and tribological behaviour of alginate and agar.

Fischer, P. and Windhab, E. J. (2011). Rheology of food materials. *Current Opinion in Colloid & Interface Science*, 16(1):36 – 40.

Mintel (2016). Cakes and cake bars - uk - march 2016. resreport, Mintel.

Murray, B. S., Durga, K., Yusoff, A., and Stoyanov, S. D. (2011). Stabilization of foams and emulsions by mixtures of surface active food-grade particles and proteins. *Food Hydrocolloids*, 25(4):627 – 638. Food Colloids 2010: On the Road from Interfaces to Consumers.

Premier-Foods (2015-2016). Annual report. Technical report, Premier Foods Plc, St Albans, UK.

Rayner, M., Sjöö, M., Timgren, A., and Dejmek, P. (2012). Quinoa starch granules as stabilizing particles for production of pickering emulsions. *Faraday discussions*, 158(1):139–155.

Sahi, S. and Alava, J. M. (2003). Sponge cake batters. In Sumnu, S. and Sahin, S., editors, *Food Engineering Aspects of Baking Sweet Goods*, chapter Cake Batter Rheology, pages 99–120. CRC Press.

Sumnu, S. and Sahin, S. (2008). *Food Engineering Aspects of Baking Sweet Goods*. Contemporary Food Engineering. CRC Press.

Watrous, M. (2015). Trend of the year: Clean label.

Yusoff, A. and Murray, B. S. (2011). Modified starch granules as particle-stabilizers of oil-in-water emulsions. *Food Hydrocolloids*, 25(1):42 – 55.

Zink, D. L. (1997). The impact of consumer demands and trends on food processing. *Emerging infectious diseases*, 3(4):467.

2 | State of the Art

2.1 Introduction

It is important to establish the identity of cakes in terms of their microstructure and expected characteristics. Therefore it is necessary to first determine the position of cakes in the context of baked products, . This is especially relevant as throughout this thesis, there will be instances where studies conducted on other baked products such as breads are used as the basis for comparison. Therefore we begin by firstly defining baked products. Encompassing a broad scope of food products, including breads, cakes, pastries, cookies and many others, they are difficult to unify with a single definition, due to the arbitrary nature of any attempted definition. A common link can either be established through the formulation or the process by which baked products are made (Cauvain and Young, 2006b). However both of these attempts fall short; the unifying ingredient is usually identified as wheat flour however this definition excludes a number of baked products based on other cereal grains. (Cauvain and Young, 2006b). The use of processing *i.e* baking as a common thread for consolidation is also inadequate, as in most cases no differentiation is afforded between a cooked product. A composite definition of baked products has been attempted by Cauvain and Young (2006a);

"Baked products are foods manufactured from recipes largely based on or containing significant quantities of wheat or other cereal flours which are blended with other ingredients, they are formed into distinctive shapes and undergo a heat-processing step which involves the removal of moisture in an oven."

2.1.1 Historical context

The primary forms of baked products are thought to have been developed for the first time in ancient Middle East, the home of domesticated cereal-grain production (Oliver, 2015). Babylonians then passed on the art of baking to the ancient Egyptians who developed the first organised bakeries.

Although the Oxford English dictionary traces the English word 'cake' back to the 13th century ('kaka'), the precursors of modern cakes were first baked in Europe in the 17th century (Oliver, 2015) primarily due to advances in technology (more reliable ovens and manufacture of food molds) and ingredient availability (refined sugar). The first cakes were very different from the modern cake. They were more bread-like and sweetened with honey. Nuts and dried fruits were often added.

In France, Antonin Careme (1784-1833) is considered the premier historic chef of the modern pastry/cake world (Kelly, 2009). However it was not until the middle of the 19th century that cake as we know it today was developed. The Cassell's New Universal Cookery Book (London, 1894) contains a recipe for layer cake, American (Heritage, 1894).

Modern developments have also been important in the evolution of modern cake characteristics. In the 1950s and 1960s the quality of ingredients available for the manufacture of cake products changed, so that along with chlorine treatment of flours came the feasibility of manufacture of 'high-ratio' cakes (Cauvain and Young, 2006a; Oliver, 2015). However since then health concerns over chlorinated wheat flour have opened the door to a number of ingredient and processing alternatives.

2.1.2 Classification of Baked Products

There still exists no coherent nomenclature for baked products mainly due to the vast number of cultures associated with it. A poignant example arises in the term 'biscuit', which in English refers to a low moisture, hard, sweet and thin product with a long shelf life, whilst in France the term *biskuit* refers to a low moisture, dry-eating, long-shelf-life,

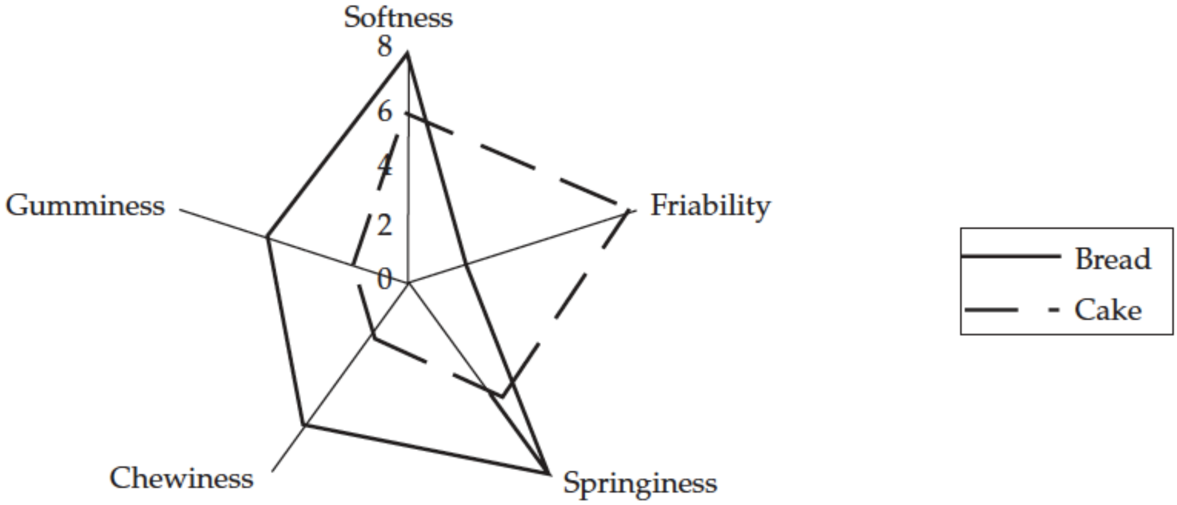


Figure 2.1: Comparison of bread and cakes in terms of textural characteristics represented in a radar plot (Taken from(Cauvain and Young, 2006b)).

sponge-type cake with an aerated structure resembling a British sponge cake (Cauvain and Young, 2006b). The emotive aspect of baking has mainly prevented the scientific community from developing a common descriptive language. Defined boundaries of these products are paramount when developing rule sets which determine the final quality and especially relevant for delivery of a consistent product. Additionally it will aid the scientists to develop the appropriate underpinning scientific knowledge of the formulation and the processing technology applied in their manufacture (Cauvain and Young, 2006b). Currently after much study, there is still a lack of understanding as to what determines ingredient performance and also no coherent way of testing and quality control. Thus one of the greatest challenges facing the baking industry currently is development of coherent nomenclature for baked products along with quality rule sets ensuring their consistency and troubleshooting.

2.1.2.1 Current Classifications

Firstly it is important to identify the differences between cakes and breads in terms of macrostructural properties *i.e.* the consumer perception. fig. 2.1 shows the difference in the mechanical properties which are interrelated to the microstructure of each system.

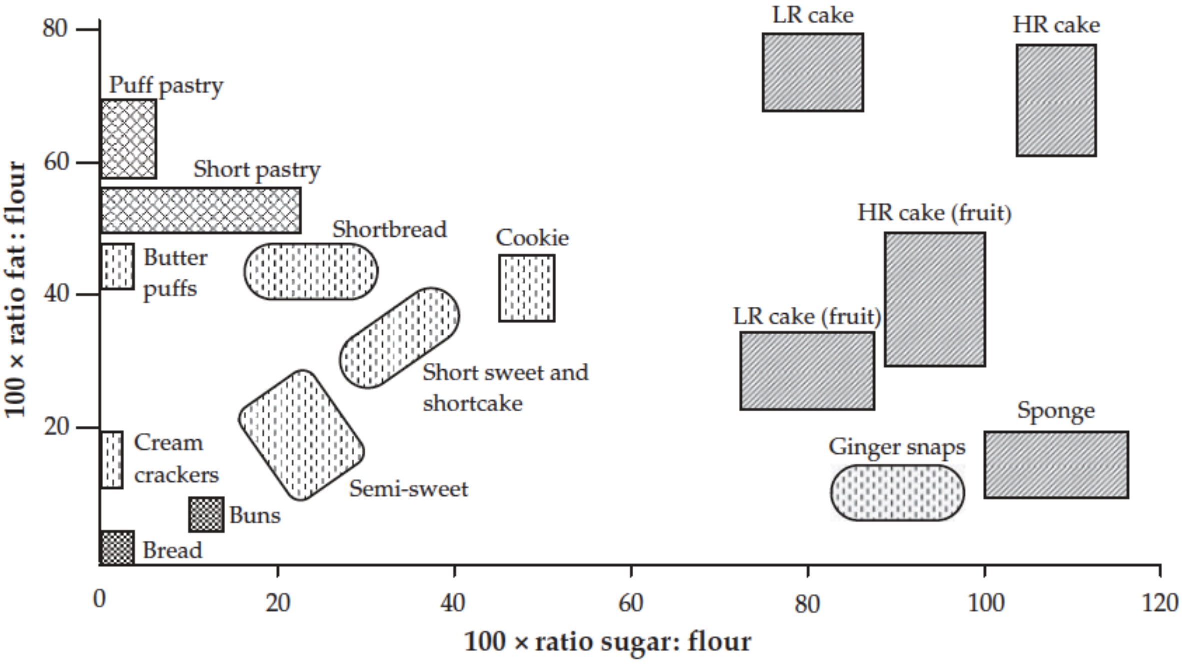


Figure 2.2: Two dimensional representation of bakery products based on their sugar and fat content (Taken from (Cauvain and Young, 2006b)).(LR refers to low-ratio and HR to high ratio cakes.)

Primary differences are lower moisture content of cakes along with presence of sugar and fat which contribute differently to the final microstructure (Cauvain and Young, 2006b); Sugar will limit the formation of a gluten network whilst fat acts as as tenderizer (Sahi, 2008) indicating that cakes have a soft and friable eating quality. Fig. 2.2 shows the position of each product in the context of this broad food category as characterized by their formulation.

2.1.3 Cakes

Cakes, like breads, are examples of macro-porous materials generated *insitu* by raising the temperature of aerated emulsions or foams (Sahi, 2008). Cake batters are distinguished by high amounts of sugar and variable levels of flour, eggs, fat, baking powder and salt. Other commonly used ingredients include emulsifiers and milk powder (Sahi, 2008). These ingredients are dissolved or suspended in a continuous liquid phase through which air is mechanically dispersed producing a complex aerated system. The incorporation of air cells during mixing generates a wet foam which is converted into a solid foam through baking (Sahi, 2008; Cauvain and Young, 2006a).

During baking a combination of starch gelatinisation, protein denaturation and generation of carbon dioxide (CO_2) from the baking powder provide its final structure(Sahi, 2008). Physical and structural changes that occur in cake batter during processing are very important in determining the performance and quality of the final cake (Indrani and Rao, 2008). Fig. 2.3 shows the generic steps required for the formation of the final cake structure.

Having roughly defined the boundary within which cakes sit, it is now necessary to define the different types of cakes differentiated through their balance of ingredients, mixing and baking method. Commercially available cakes can be generalised into three different types (Miller, 2016);

2.1.3.1 Batter cakes

Batter cakes such as pound cake are commercially the most common. They can be characterised by the high levels of sugar and fat. The consistency of their batter is dependent on the ingredients used and the amount of air whipped in during mixing. Leavening products are very important in these systems for final structure development (Miller, 2016).

2.1.3.2 Foam cakes

Angel food cakes are an example of foam cakes. These systems depend mainly on the capacity of egg whites (EW) for their final structure and texture. These are low fat alternatives to batter type cakes; indeed presence of fat is detrimental to their structure. These cakes are often used as building blocks in other more complex creations, such as the ladyfingers used in Tiramisu (Miller, 2016; Palav, 2016).

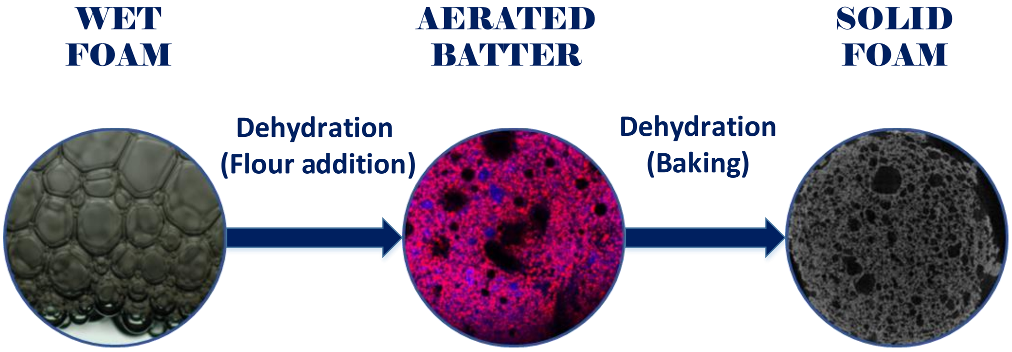


Figure 2.3: Representation of the cake microstrcuture development; starting from wet foam formation (left - microscopy image), batter formation (centre - confocal microscopy image of fat (red) and starch (blue) stained batter) and final sponge (right - X-ray CT cross-sectional image of cake microstrcture).

2.1.3.3 Chiffon cakes

Chiffon cakes are a combination of batter and foam cakes. Aeration in these cakes depends on EW and chemical leavening. These cakes contain high amount of oil. Sponge cakes can also fall within this category. In sponge cakes, whole eggs or yolks contribute to the structure of the cake (Miller, 2016).

2.2 Functionality of major ingredients

2.2.1 Wheat Flour

Sweet baked products made from wheat flour encompass a wide variety of textures, flavours, nutritional values and shelf-lives, which include different types of cakes, cookies, pastries, and many more. Flour quality is in turn, influenced by the wheat genotype, growing environment, and processing (Indrani and Rao, 2008). The genotype and growing environment determine the amount and characteristics of the wheat components, including proteins, carbohydrates and lipids, whilst processing includes the proper milling (Sugden and Osborne, 2001; Webb and Owens, 2003) and utilisation of the correct post-milling

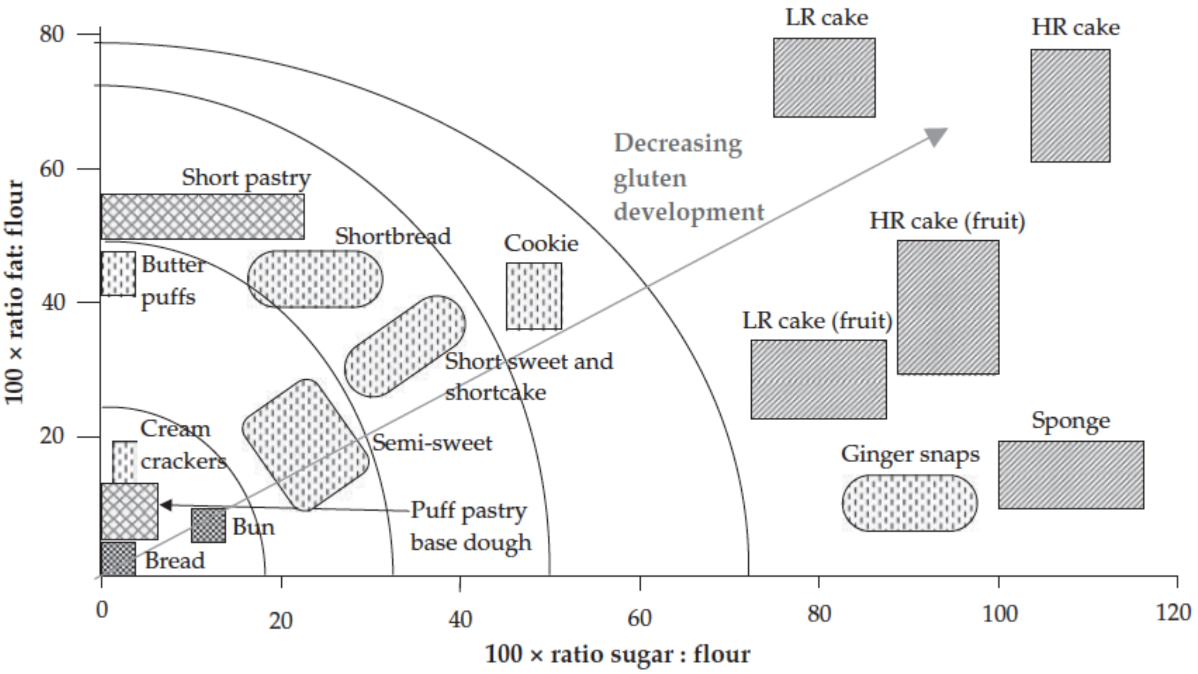


Figure 2.4: Two dimensional representation of degree of gluten development in bakery products (Taken from (Cauvain and Young, 2006a)).(LR refers to low-ratio and HR to high ratio cakes.)

processing; chlorination or heat-treatment (Indrani and Rao, 2008).

2.2.1.1 Major Constituents

The influence of wheat flour depends on its composition and important physiochemical properties, such as particle size and protein content and quality (Cauvain and Young, 2006a). The most important flour constituents in relation to its functionality include proteins, starches, pentosans (non-starch polysaccharides), and lipids (Indrani and Rao, 2008; Palav, 2016).

Proteins

The role of wheat proteins is the formation of gluten structures that are of paramount importance in breads but not so important in many types of cake. More tender products such as cakes and cookies generally require soft wheat flours which are low in protein content (8 to 10%) and are comprised of proteins weaker in strength (Indrani and Rao, 2008). Higher protein content in flour was shown to produce cakes of substandard quality

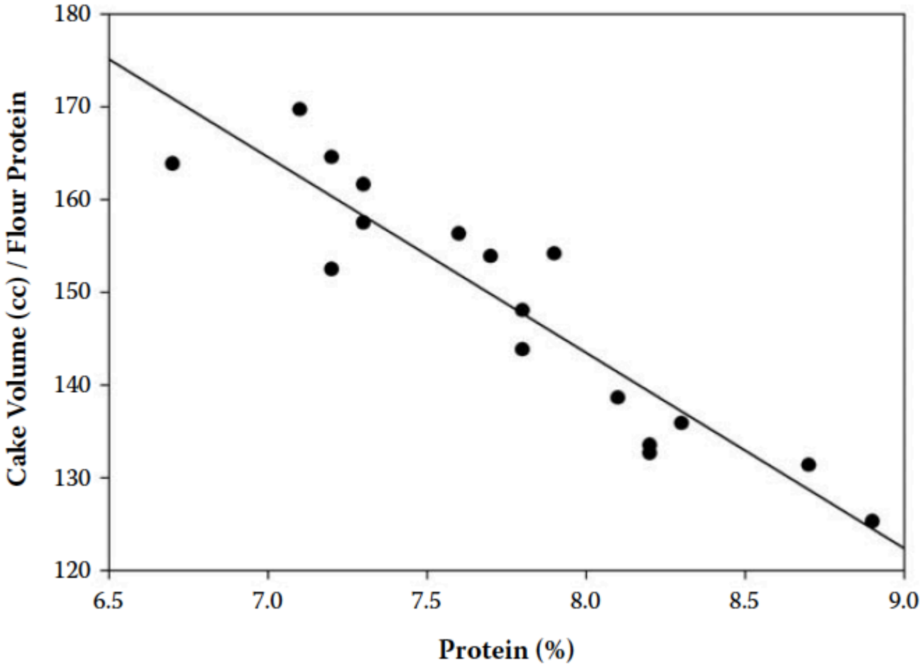


Figure 2.5: The negative influence of protein content on Japanese sponge cake volume per unit flour protein in cakes as reported by Yamamoto et al. (1996)

by Kaldy and Rubenthaler (1987), who demonstrated that higher protein content in the flour resulted in cakes with lower volumes and coarser structures due to gluten disruption of the foam structure in the cake batter.

Yamamoto et al. (1996) also reported a negative correlation between gluten content and Japanese sponge cake volume (Fig. 2.5). Gaines and Donelson (1985) reported that the volume and texture of white layer cakes remained unaffected by protein content, whereas a reduction of over 2% protein was enough to see a significant increase in angel food cake volumes, demonstrating the attainable discrepancies based on formulation. However Howard et al. (1968) reported that despite the adverse effects of excess flour protein, soluble proteins (both from the flour and from eggs) were still needed for thermal stability of the cake foams during baking.

Starch

Generally wheat flour contain over 70% starch that is composed of approximately 25% amylose and 75% amylopectin (Sollars and Rubenthaler, 1971). Amylose is primar-

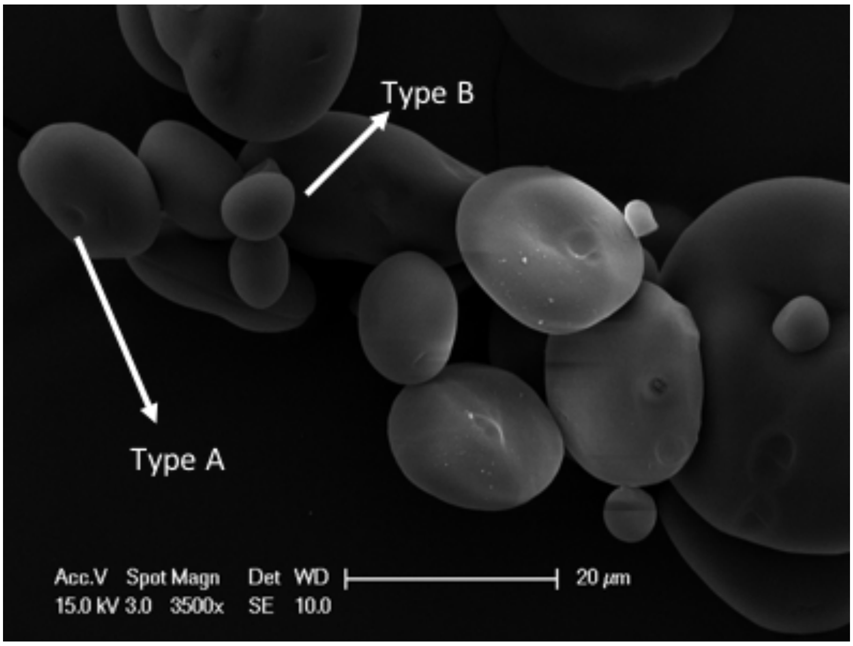


Figure 2.6: Figure highlighting the bi-modal size distribution of wheat starch and its two different granule types

ily a straight-chain polymer of α-1,4-linked D-glucopyranose molecules (Indrani and Rao, 2008). Amylopectin is a branched polymer of α-1,4-linked glucose connected by α-1,6-linked branch points. Wheat starch granules come in two forms: oval type A granules about 15-40 μm in diameter, and round type B granules approximately 1-10 μm in diameter (Indrani and Rao, 2008). The most important property of starch is its capacity to swell and absorb water when it is heated in excess water. The swelling causes an increase in viscosity of the starch-water slurry until the eventual breakdown of the starch granule, thus releasing amylose followed by amylopectin. Upon cooling the amylose re-associates, forming a gel (Wilderjans et al., 2010).

The swelling and breakdown of the starch granule are generally referred to as gelatinization and pasting, respectively. These properties are generally quantified through observing the viscosity of a starch-water slurry as it is heated and subsequently cooled (Indrani and Rao, 2008). Starch granules can be physically damaged during the milling process, which in turn can increases their water-absorbing capacity. This was first reported by Greer and Stewart (1959), where they found 2 grams of water was absorbed by each gram of starch compared to the 0.44 g absorbed by its native counterpart.

However high levels of starch damage has been shown to lead to loss of volume in breads. Cauvain and Young (2009) discussed the effect of excessive damaged starch in the context of the *"Chorleywood Bread Process"*, where it lead to a more open cell structure and graying of bread crumb.

Functionality in Cakes

High-ratio cakes demand more from the flour as the structure building component. Therefore the wheat flour used, usually undergoes further processing steps (Indrani and Rao, 2008). Chlorination of flour was a common procedure within the baking industry until its discontinuation in 1960's (Cauvain and Young, 2006a). Chlorinated flour has been reported to improve texture and volume of cakes (Donelson et al., 2000), with Seguchi and Matsuki (1977) reporting improved mouth-feel; drier and less 'gummy' cakes. However current processing methods are restricted to heat-treatment of wheat flours. Cake batters made with heat-treated flours have been reported to observe higher viscosities compared to their untreated counterparts (Indrani and Rao, 2008). Meza et al. (2011) reported that heat-treatment of flour facilitated the formation of stronger gels during baking while Seguchi and Yamada (1988) hypothesised that the heat-treatment process; i) increases the amount of protein adsorbed on the surface of the starch granules and ii) causes denaturation of the surface proteins rendering them more hydrophobic. It was suggested that this phenomena increases their capacity to bind to oil and air which in turn will increase the batters capacity to incorporate air. However the exact mechanism by which heat-treatment affects flour performance is still not fully understood.

Lipids

The flour lipids are important for quality attributes of cakes, mainly volume (Indrani and Rao, 2008). Within whole grain flours lipids exist at around 2-4% (Morrison, 1978). Lipids in flours can be separated into either starch lipids (present in the amylose inclusion complexes) or non-starch lipids. Starch lipids due to their protected environment are deemed less functionally important (Morrison, 1978). The non-starch lipids can be further classified in to two types; free lipids, or bound polar lipids (phospholipids and glyco-lipids).

Clements and Donelson (1982) reported that extraction of free flour lipids resulted in white layer cakes with lower volumes and inferior textures. They also observed that whilst their reintroduction restored cake quality, the use of different wheat lipid varieties did not affect the results, signifying the mere importance of their presence rather than their source. Takeda (1994) confirmed this result by a similar extraction, which resulted in reduced sponge cake volumes. The fractionation of the lipids into polar and nonpolar fractions enabled the observation that polar lipids (monogalactosyl and digalactosyl diglycerides) could restore cake volumes while the non polar fractions had no significant influence.

Flour Particle size

Measurement of soft wheat flour particle size distribution by laser diffraction showed that a larger percentage of its particles were below 41 μm compared with hard wheat flour (Hareland, 1995). Yamamoto et al. (1996) in using different wheat flours demonstrated that particle sizes of soft wheat flours observed a negative correlation to cake volume. In addition to reduction of particle size produced by milling, research has shown that further reduction of particle size through post milling processing (*i.e.* pin-milling and air-classification) can also improve cake volumes (Chaudhary et al., 1981). However in reducing particle size it is important to limit starch damage, as damage levels greater than 5% have been negatively correlated to cake quality (Indrani and Rao, 2008; Cauvain and Young, 2006a).

2.2.2 Sugar and sweeteners

Sugar is available in a number different crystalline forms (*i.e.* caster, granulated, icing, etc.) the main effect of particle size is the rate of solubility (Cauvain and Young, 2006a). Within cake formulations the most commonly used type is caster due to its high solubility. In high-ratio cake formulations addition of sugar results improves air incorporation leading to more viscous and stable batter foams (Paton et al., 1981). However the most important functional property of sugar in modern cakes is its role as a tenderiser; it restricts formation of a gluten network and its presence increases the temperature of starch

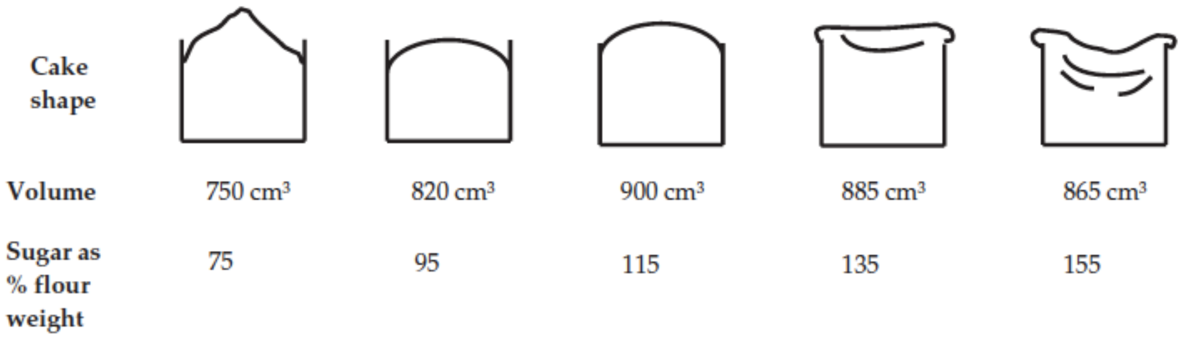


Figure 2.7: The influence of sugar concentration on the volume of sponge cake (Adapted from (Cauvain and Young, 2006a)).

gelatinisation and egg protein denaturation thus contributing to volume (Indrani and Rao, 2008). In delaying the temperature at which starch gelatinises during baking, addition of sugar allows for air bubbles to expand more due to gas generation before the setting of structure (Perry and Donald, 2002). At the concentration used in cakes (55 to 60%), sugar delays the gelatinisation of starch from 57 to 92 °C, which allows for the formation of the desired cake structure (Spies and Hoseney, 1982). Sugar's ability to limit the water available to the starch is thought to delay gelatinisation (Derby et al., 1975). According to Spies and Hoseney (1982), there are two independent mechanisms responsible for this delay; (i) lowering the water activity of the solution (ii) interacting with the starch chains to stabilise the amorphous regions of the granule. Fig. 2.7 shows how the structure of cake changes with increasing levels of sugar whilst keeping the other components in the formulation constant. As the volume of the cake increases the shape of the cake changes until the weight can no longer be supported and the product sinks (Indrani and Rao, 2008). The other important function of sugar is flavour. Other sweeteners such as dextrose and corn syrup can be used for more sweetness intensity (Indrani and Rao, 2008).

2.2.3 Fat

Recent advances and a fundamental understanding of fat chemistry has led to development of different fat compounds which are commonly used in the manufacture of all baked products (Cauvain and Young, 2006a). The use of fat within foam type cakes can be

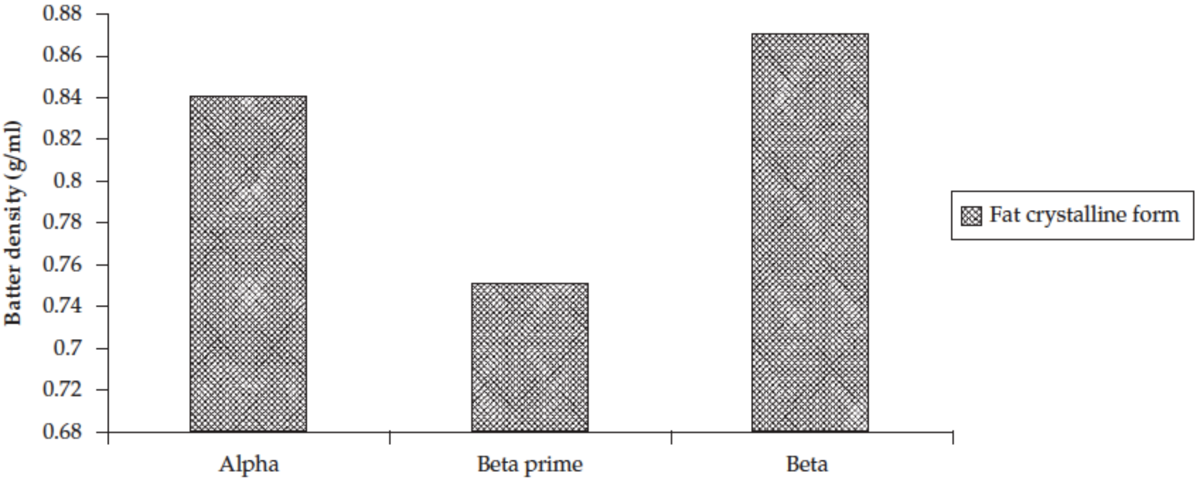


Figure 2.8: The influence of different fat crystalline forms on the level of batter aeration (Adapted from (Cauvain and Young, 2006a)).

detrimental (Miller, 2016). However it does have some functional properties within batter type cakes. Firstly fat acts as a tenderiser (enhanced mouthfeel) and helps extend the shelf-life (Cauvain and Young, 2006a; Palav, 2016; Indrani and Rao, 2008). However another major function of fat is to entrap air during the creaming stage of batter formation. Differences in fat crystalline form have been found to show different capacities for air incorporation (Cauvain and Young, 2006a). A comparison between three fat polymorphs in terms of their capacity to incorporate air is shown in fig. 2.8. During batter structure formation by mixing, the fat dispersed through the aqueous phase adsorbs at the newly formed air-water interfaces and coagulates to entrap the air bubbles. When the air cells begin to expand during baking, the fat crystals melt releasing fat/water interfaces and thus facilitating the expansion of bubbles (Brooker, 1993a,b). Before the negative publicity surrounding trans-fats, they were the major source used within cake formulations, however since then, these hydrogenated shortenings have successfully been replaced by liquid fats (Indrani and Rao, 2008)

2.2.4 Eggs

Egg White Protein (EWP) contains many functionally important components. Ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), ovomucin (3.5%), and lysozyme

(3.5%) are among the major proteins composing EWP. They mainly contribute to the structure and flavour of cakes. During mixing egg whites play an effective role in maximising the air entrapment capability of a cake batters. They have the capacity to form foams that are stable enough to support large quantities of sugar and flour (Indrani and Rao, 2008). In sponge cakes and foam cakes, the egg whippability is paramount in determining the final cake quality. The foams must have the ability to structurally support the other ingredients until a stable protein matrix is formed through heat coagulation (Indrani and Rao, 2008); It has been reported by Mine (1995) that three main constituents of EWP function as follows;

- Globulins help the incorporation of air during the mixing stage by lowering the surface tension.
- As the bubbles break-up during mixing and consequently maximising surface area, the ovomucin fraction undergoes surface denaturation to form a solid foam increasing the volume of foam.
- Finally the ovalbumin fraction that is readily heat-coagulable can support many times its weight in sugar and flour.

This particular multi-functionality of EW has made replacing them in cake formulations very difficult (Foegeding et al., 2006). Numerous studies for finding alternative protein sources, which can act as replacers of EWP have been made (Berry et al., 2009; Yang et al., 2009; Pernell et al., 2002). Egg can also act as a toughener partially due to its contribution to the gelitinisation of starch and development of gluten. While egg whites may act as tougheners, the fat content of the egg yolks make them tenderisers. It is also a rich source of emulsifiers such as lecithin and can also facilitate air incorporation (Pyler et al., 1973). Eggs contribute to leavening action through the emulsification of fat and incorporation of air (Cauvain and Young, 2006a).

2.2.5 Leavening agents

Leavening agents in baked products can be categorised as; mechanical, biological or chemical leavening agents (Indrani and Rao, 2008). Mechanical leavening implies incorporation of air by mechanical agitation of the eggs and sugar (and fat). Biological leavening *i.e*, yeast is only relevant for bread. Chemical leavening suggests use of chemicals such as baking soda, ammonium bicarboanate and baking powder. Baking powder and its variations contain an alkali typically sodium bicarbonate, and an acid together with starch to keep it dry. Upon dissolving in water the acid and the alkali react to emit CO_2 which expands the bubbles. Baking powders can be either single-acting and double acting. Some varieties which only contain low-temperature acid salts (*e.g.* cream of tartar, calcium phosphate and citrate) are classed as single acting (Cauvain and Young, 2006b). Double acting versions contain two acid salts (*e.g.* calcium aluminium phosphate); one which reacts at room temperature, and a second which is activated at higher temperatures causing further expansion during baking (Indrani and Rao, 2008). Generally in cakes three distinct leavening actions occur; (i) mechanical, incorporation of air during mixing. (ii) chemically, generation of CO_2 during the bake. (iii) Vapour pressure created by water resulting in enhanced volume (Indrani and Rao, 2008). It must be noted that the level of baking powder in cakes has a direct influence on cake volume and crumb structure, indeed as fig. 2.9 shows the inclusion of too much baking powder can result in baking cake collapse and therefore a more coarse final structure.

2.3 Batter Structure

Cake batters made with fat or oil coupled with the dispersed air bubbles incorporated during mixing, constitutes a complex aerated emulsion system. The gas phase has been demonstrated to be covered either by emulsifiers or proteins (Sahi, 2008). Heating of the batter during baking causes rapid expansion of the bubbles, which means that the viscoelastic properties of the films surrounding the gas cells are paramount in preserving

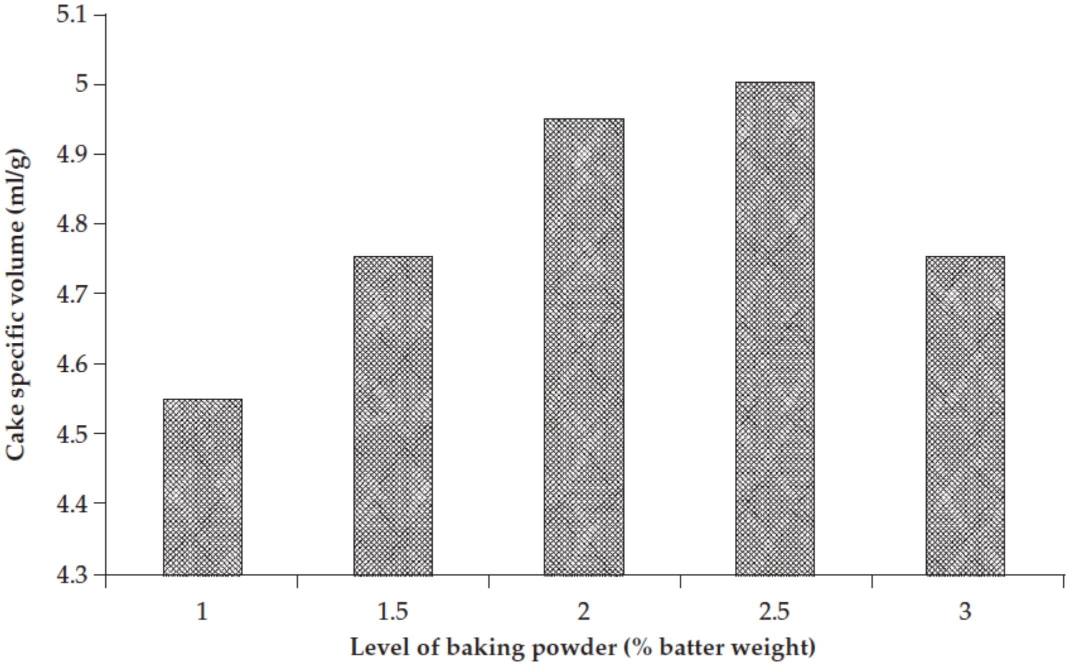


Figure 2.9: The influence of baking powder concentration on the specific volume of sponge cake (Adapted from (Cauvain and Young, 2006a))

.

their integrity. Expansion ceases when the cake structure is set and the discrete foam systems break to form an open network sponge (Sahi, 2008). At present, there is no established theory for understanding the contribution of ingredients present in batters to cake emulsion and foam stability (Sahi, 2008). There are a number of fundamental factors that govern emulsion and foam formation and stability. These invariably aid the stability of the dispersed fractions in a cake batters to some degree. It is the purpose of this section to explore foams and emulsions as independent systems and to discuss the major factors controlling their formation and stability.

2.3.1 Emulsions

An emulsion is a colloidal dispersion of one immiscible phase into another (McClements, 2005; Binks, 1998a). The two immiscible constituents, commonly oil and water can be in the form of oil-in-water (*e.g.* milk) or in a reverse arrangement (*e.g* margarine) (Indrani and Rao, 2008). The properties of such systems (stability, rheology *etc.*) are mainly dependent on the size of the droplets composing them (McClements, 2005). The Sauter mean diameter, $d_{3,2}$ is commonly used to portray emulsion droplet size and is defined as the ratio of the third to the second moment of the probability density function (Pacek et al., 1998);

$$d_{3,2} = \frac{\Sigma n_i d_i^3}{\Sigma n_i d_i^2} = 6\frac{V}{A} \tag{2.1}$$

where n_i and d_i, V and A are the number and diameter of droplets belonging to the i^{th} class, and the total volume and area of the dispersed phase, respectively.

2.3.1.1 Formation

Formation of an emulsion is regarded as a dynamic two step process: First step comprises of the break-up of the immiscible phase into small droplets. This initial break-down results in a considerable increase in interfacial area, thus yielding a thermodynamically unstable system (McClements, 2005). This is generally afforded by expenditure of mechanical energy. After breakdown potential re-coalescence of the newly formed droplets can occur through collision of interfaces with insufficient coverage (Vankova et al., 2007). However if the newly created interfaces are stabilsied in time by surface-active materials, commonly referred to as emulsifiers this process is largely avoided (McClements, 2005). Emulsifiers consist of molecules that possess amphiphillic properties (McClements, 2005; Binks, 1998a). This is possible as the constituting molecules are composed of both hydrophilic and hydrophobic parts. The hydrophobic part of the molecules consists of a fatty acid, the length of which can range from 12 to 18 carbon atoms. Whilst the hydrophilic part may consist of glycerol, sucrose, or other chemical groupings (Indrani and Rao, 2008). The type and concentration of emulsifier along with energy density co-determine the size of the formed emulsion droplets (Lee et al., 2013).

The boundary between the two immiscible phases *i.e.* the interface, is of paramount importance in determining the interactions of the molecules between the two phases (McClements, 2005). The energy required to deform the shape of a given interface by a set distance is defined as the interfacial tension (N/m). Interfacial tension describes the energy gradient between liquid-liquid interfaces (relevant for emulsification), whilst surface tension refers to that of gas-liquid interface (foams). The interfacial tension is influenced by a number of factors such as the temperature of the two phases forming the boundary as well as the presence of solutes (Binks, 1998a). Surface active solutes such as low molecular weight or high molecular weight surfactants (in the form of non-ionic surfactants or proteins respectively) have the biggest influence on the interfacial tensions. Although other components such as salt and sugar tend to have small effects also. Sugar is thought to have very little to no effect upon interfacial tension, whilst salt is known to slightly increase it (Gaonkar, 1992). It is the interfacial force which maintains the spherical shape of the newly formed droplets and resists droplet break-up. Thus the emulsifier in reducing the interfacial tension also facilitates easier break-up of the droplets. This is adequately described by the pressure differentials between the inside and the outside of the emulsion, which can be summarised by the Laplace equation (Walstra, 1993);

$$\Delta P = \frac{2\gamma}{r} \tag{2.2}$$

where γ is the interfacial tension between the two immiscible phases and r is the emulsion droplet radius. The criteria for break-up is therefore sufficient energy expenditure to overcome the interfacial tension forces (Binks, 1998b; Walstra and Smulders, 1998). Thus the feasibility of emulsion formation is resolved through a balance of the disruptive forces (*i.e.* fluid's inertia) acting on a given droplet and the interfacial forces maintaining its shape (*i.e.* interfacial tension), this can be quantified by the Weber Number We;

$$We = \frac{Inertial Forces}{Interfacial Forces} = \frac{\eta_c G d_{3,2}}{\gamma} \tag{2.3}$$

where η_c is continuous phase viscosity ($Pa.s$), G the velocity gradient i.e. shear rate (s^{-1}) and γ the interfacial tension. The break-up of the droplets is dependent on the hydrodynamic conditions, which can be categorised as either laminar or turbulent.

Turbulent Break-up

Turbulent break-up occurs generally when emulsification process is undertaken within inviscid continuous phases and turbulent eddies transmit the necessary stresses required for droplet deformation and break-up (Walstra, 1993). The Reynolds number of the system determine the size of the eddies, based on Kolmogorov's theory with smaller eddies having larger velocity gradients (Walstra and Smulders, 1998).

Laminar Break-up

Within laminar conditions, the force exerted upon an emulsion droplet is equivalent to the disruptive forces of eqn. 2.3; $\eta_c G$. The criteria for break-up under laminar regime is described by the critical Weber number, We_{cr} (Walstra, 1993) which must be exceeded. Additionally, viscosity ratio (of the dispersed phase (η_d) with respect to the continuous phase (η_c)) plays a critical role in droplet break-up. Grace (1982) demonstrated that at $\eta_d/\eta_c > 4$ no droplet disruption can occur.

2.3.1.2 Stability

In emulsions and emulsion based products long-term stability (over a year) is often required. The key physical events that contribute to emulsion instability are creaming/sedimentation, coagulation, flocculation and coalescence of the dispersed phase droplets. The sequence of occurrence of these phenomena leading to phase separation of emulsions is depicted in fig. 2.10.

Creaming

If the emulsion system contains sufficient amounts of emulsifier, the likelihood of phase separation due to coalescence is minimised. However, the density differential between the two phases can still result in separation due to gravitational forces. This process is called creaming or sedimentation (Chanamai and McClements, 2000; Damodaran, 1997). This phenomenon can be described by Stoke's law, which predicts the rate of separation due to gravity for a given droplet diameter;

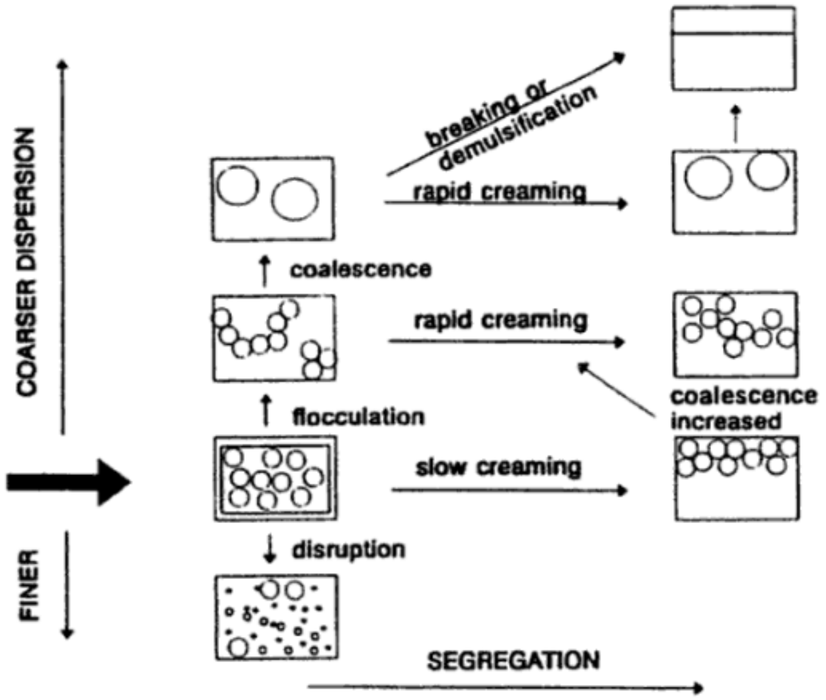


Figure 2.10: The various types of instabilities in oil-in-water emulsions (Adapted from (und P. Walstra, 1975)).

$$v = \frac{2d_{3,2}^2 \Delta\rho_d g}{9\eta_c} \tag{2.4}$$

where v is the separation velocity (m/s), $\Delta\rho_d$ is the density gradient between the dispersed and continuous phase (kg/m^3), g is the gravitational acceleration $(m.s^{-2})$ and η_c is the continuous phase viscosity $(Pa.s)$.

This phenomena can be prevented by controlling the physical properties of the two phases; modifying the viscosity of the continuous phase thus reducing the viscosity gradient between the two phases reduces the mobility of the droplets within the continuous phase, restricting the extent of creaming/ sedimentation. Moreover the quadratic relationship with droplet diameters as described by Stoke's equation (eqn. 2.4) means that reducing droplet sizes will also increase the stability of the emulsion.

Coagulation

Coagulation or droplet aggregation is generally caused due to the collisions of the droplets within the continuous phase. The dynamic nature of emulsion systems means that the droplets are in a constant state of motion due to either gravity or Brownian motion. The nature of these collisions and whether they lead to coalescence is generally determined by the physiochemical properties of the interfacial layer (McClements, 2005).

Flocculation

Flocculation describes the process of droplet aggregation where by the droplets retain their individual integrity. The formation of these 'flocs' results in an increase in the viscosity of a given emulsion system due to the associated increase in the effective hydrodynamic volume of the floc. Moreover the rate of separation due to gravitational forces is also increased as described by Stoke's equation (Eqn. 2.4).

Coalescence

Coalescence describes a process by which two smaller droplets merge to form a larger one (McClements, 2005; Indrani and Rao, 2008; Damodaran, 1997). This is inevitable due to the inherent thermodynamic instabilities associated with emulsions; whereby the system is minimising the surface area and thus the free surface energy. This process will also increase the rate of phase separation dramatically (McClements, 2005). Coalescence can be prevented by restricting the droplet movement for prevention of contact between the droplets either by increasing the viscosity of the continuous phase or by development of a thicker interfacial layer. Generally the latter can be achieved through use of sufficient emulsifier or by build up of layers of oppositely charged biopolymers (Damodaran, 1997; Dickinson, 2010).

2.3.1.3 Function of Emulsifiers in Cakes

The use of emulsifiers within cake systems is mainly due to promotion of foam and emulsion formation as well as for their stability within the batter (Sahi, 2008; Sahi and Alava, 2003). Emulsifiers can also coat the exterior of the fat drops thus preventing them from disrupting the protein films (Wootton et al., 1967). Emulsifiers used within cake formulations can generally be divided into two categories; lipid-based materials and proteins

(Sahi, 2008). Although in early days of cake production, the key emulsifiers were proteins from eggs, with further mechanization of the mixing process natural emulsifiers have been phased out for lipid-based ones (Sahi, 2008). This is primarily because emulsifiers such as GMS, polyglycerol esters, and lactic acid esters of monoglycerides facilitate better air-incorporation. When added to sponge batters (0.5 to 1.0% of batter weight), whisking time can be greatly reduced (Sahi, 2008). In also lowering the surface tensions they aid further bubble break-up in order to produce smaller more uniform cellular structures (Sahi and Alava, 2003). However instability of foams has readily been reported when the two types of surface-active materials are present together (Sahi, 2008; Damodaran, 1997). The egg is considered to be the foaming agent in sponge batters, and the presence of small amounts of oil or fat was found to reduce foam stability considerably (Sahi, 2008). Bubble coalescence was reported when cakes were baked with 0.25% GMS with coarser bubble structures, indicative of partial foam destruction during mixing. Whereas cakes made with no GMS or 0.75% GMS were of good volume. It was concluded that the protein and GMS could not combine together to stabilize interfacial films around the gas bubbles and that small quantities of GMS interfered with protein-protein interactions during baking reducing baking stability whereas at high concentrations, GMS displaced the protein and was able to saturate the air-liquid interface and maintain stability during the baking stage (Chorleywood-Food, 2008). An additional advantage of using lipid-based emulsifiers is the more stable batters produced with their incorporation which can better withstand the stresses of depositing and holding times during cake manufacture. Moreover GMS, polyglycerol esters, propylene glycol esters can be used to reduce the fat content of a cake through substitution with small quantities of vegetable oil. Although oil addition is considered to have adverse effects on the volume of the final cake, the correct emulsifiers blend can alleviate this problem (Sahi, 2008).

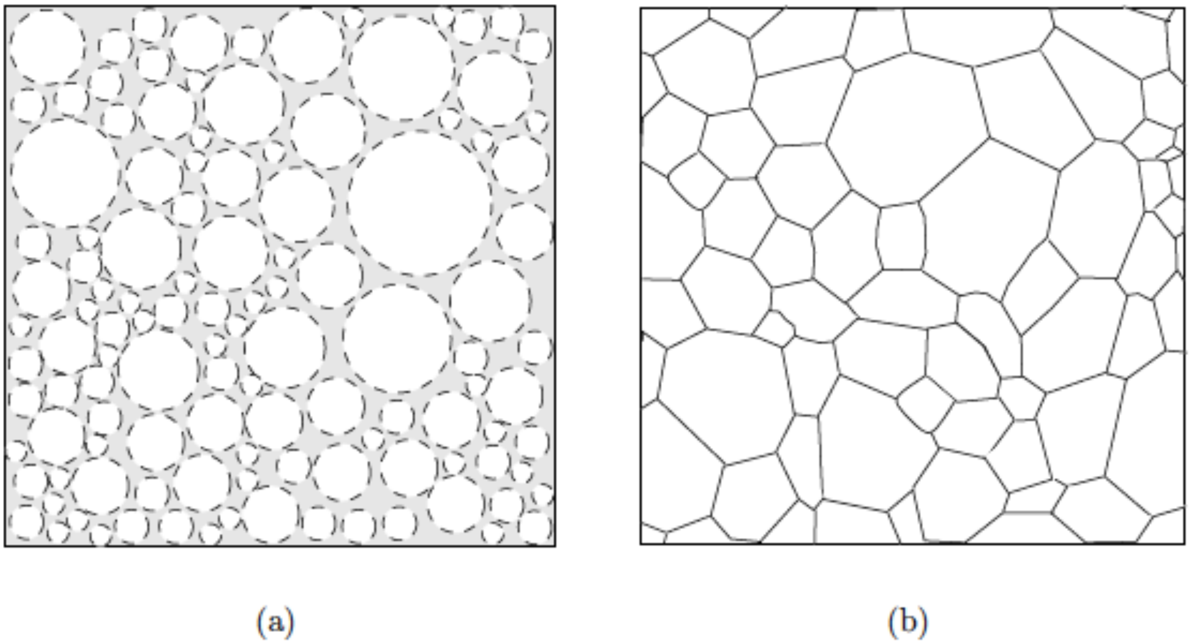


Figure 2.11: The schematic representation of (a) a wet-foam and (b) a dry foam. (Adapted from (Breward, 1999))

2.3.2 Foams

A foam is colloidal dispersion of air-bubbles in an aqueous continuous medium (Damodaran, 1997); in which the volume fraction of the liquid phase is small (Breward, 1999). Many processed foods such as whipped cream, ice cream and of course cakes are comprised of foams, the vast majority of which are protein stabilised (Damodaran, 1997). The volume fraction of liquid is an important parameter determining the size distribution of bubbles, structure and behaviour of foams. The broad classification of foams divides them into two distinct categories; 'dry' and 'wet' foams (Breward, 1999), depending on the volume fraction of liquid they contain (Cantat et al., 2013). In wet foams (where the portion of liquid is more than 10 %) the bubbles are approximately spherical, whilst in dry foams (where the volume fraction of liquid is less than 10 %), the bubbles are polyhedral in shape (Fig. 2.11).

Although at the point of creation foam bubbles are spherical, the aggregation of bubbles over the life span of foams eventually yields a polyhedral foam with a thin liquid film separating the bubbles (Damodaran, 1997).

Protein foams are created by bubbling, shaking or whipping of a protein solution. In this regard the evaluation of the foaming properties of a given protein depends on two main aspects; (i) their capacity to produce a large interfacial area for incorporation of bubbles (foaming capacity or overrun) and (ii) the ability to form viscoelastic interfacial films that can withstand the internal and external stresses (Damodaran, 1997). The ability of the protein to stabilise the structure of the foam against gravitational and mechanical stresses is often regarded as foam stability and is expressed as the time required for a 50% reduction in the foam volume or for 50% of the liquid to drain from the foam (Hailing and Walstra, 1981). A less empirical method for evaluating foam stability involves the determination of the initial interfacial area of the foam and its decay as a function of time (Nishioka and Ross, 1981). Laplace equation describes the state of a spherical liquid surface in a foam bubble;

$$P_i - P_o = \frac{2\sigma}{r} \tag{2.5}$$

where P_i and P_o are the pressures inside and outside of the bubble, σ is the surface tension and r is the radius of the bubble. Assuming that foam the foam bubble is obeying the ideal gas law;

$$P_i V = nRT \tag{2.6}$$

where V is the volume, $(Ar/3)$ and n is the total number moles of gas, T the temperature $(Kelvin)$ and R the ideal gas law constant. With the substitution of this term and the inclusion of the rate of change in pressure and thus interfacial area with respect to time, the following equation can be derived; (Damodaran, 1997).

$$\frac{A_t}{A_0} = \frac{(\Delta P_x - \Delta P_t)}{\Delta P_x} \tag{2.7}$$

where A_0 is the interfacial area of the freshly formed foam (i.e. the foaming capacity of the protein), A_t is the interfacial area after time t, ΔP_t is the net change in the external pressure and ΔP_x is the pressure at infite time when the foam has collapsed. Work by (Polyakov et al., 1986; Nishioka and Ross, 1981; Hailing and Walstra, 1981) used experimental determination of ΔP_t to investigate the foaming properties of food proteins.

2.3.2.1 Foam Formation

The criteria regulating the performance of given protein as a foaming agent are summerised below;

- It should be able to rapidly adsorb at the air-water interface during the creation of bubbles (either by whipping or by bubbling).
- The conformational changes and rearrangements should be rapid in order to reduce the surface tension.
- The final film formed around the interface should be cohesive and viscoelastic enough to withstand the mechanical and gravitational stresses.

The first two properties describe a protein with good foaming capacity where as the final criterion will have a direct influence on the stability of the foam (Damodaran, 1997). Graham and Phillips (1976) demonstrated that the most important parameter for foaming capacity is the rate at which the protein can reduce the surface tension during the process of bubble generation (Damodaran, 1997). Further to this work, Naofumi and Etsushiro (1988) reported that the equilibrium surface tension of the proteins was less significant in determining their foaming capacity than their rate of surface tension decay (Fig. 2.12).

2.3.2.2 Foam Stability

The two main processes that govern the kinetic stability of protein foams are liquid drainage from the lamella and film rupture (Morrison and Ross, 2002). The rate of these processes is dependent on the physical properties of the protein film and the lamella; which include film viscosity, shear resistance, elasticity and disjointing pressure between the films (Morrison and Ross, 2002). Any factor that will increase the rate of liquid drainage from

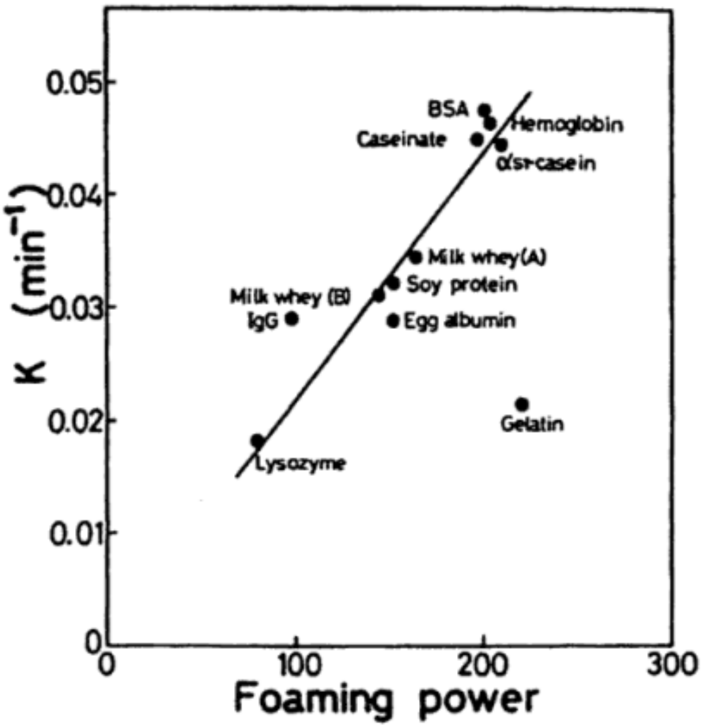


Figure 2.12: The relationship between foaming capacity and rate of reduction in surface tension (Taken from (Naofumi and Etsushiro, 1988))

the lamella will decrease the foam stability. The rate of drainage is described by the Reynold's equation (Manev et al., 1984):

$$V_R = \frac{dh}{dt} = \frac{(2h^3)}{3\mu R^2}\Delta p \tag{2.8}$$

where h is the film thickness, t is time, μ is dynamic viscosity and R is the radius of the bubble and $\Delta p = \pi_h$-π_d, where π_h is the hydrostatic pressure and π_d is the disjoining pressure between the two protein films. Since π_h is always greater than π_d, foams never reach thermodynamic equilibrium and the films drain continuously leading to thinning and bubble collapse (Marmur, 1984).

According to eqn. 2.8 the stabilities of the foams with similar bubble sizes depends on the disjointing pressure between the two adsorbed protein layers of the lamella separating them, which are related to the physiochemical properties of proteins forming them. Consequently they are influenced by steric repulsion, electrostatic attraction/repulsion, Van der Waals and hydration repulsion forces between the protein layers (Marmur, 1984).

As such it has been shown that Van der Waals and hydrophobic interactions between protein layers increase the rate of film thinning and drainage (Damodaran, 1997). On the other hand electrostatic interactions tend to have a positive contribution to the disjoining pressure, where charge repulsion between protein layers tends to oppose film thinning (Damodaran, 1997). The steric effects were also shown to positively influence disjoining pressure. Steric effects are dependent on the structural rigidity of the adsorbed layer. Graham and Phillips (1976) reported in their comparison of β-casein and lyzome that even though foaming capacity of the former was greater, the rates of drainage were considerably higher. This was attributed to the fact that β-casein is highly hydrophobic and the contribution of hydrophobic and Van der Waals forces negatively affected the disjoining pressure. Moreover the Lyzomes are rigid globular proteins and form thick rigid interfacial layers with significant amounts of steric, hydration and electrostatic repulsions and no attractive hydrophobic interactions between the proteins layer (Damodaran, 1997).

Surface Film Rigidity

Another important parameter affecting the stability of foams, is the rigidity of the protein films either side of the lamella film. The interfacial rheology of these protein films is dependent on the extent of molecular interactions within the adsorbed film. The composition of the film plays an important role in controlling it's properties. Clark et al. (1990) reported that lipid-stabilised films exhibit rapid drainage, whereas protein-stabilised films display slower rates of drainage (Clark et al., 1994). It was shown by Graham and Phillips (1976) that lyzomes form highly cohesive films with interfacial shear viscosities of 1000 mN/ms, where as β-casein films even with higher surface concentrations observed shear viscosities of only 1.0 mN/ms. Their research emphasised the importance of the protein species occupying the interfacial area in determining the surface film's properties

Film Rupture

The thickness of the adsorbed film is a critical parameter for controlling the creation of nucleating holes in the film which could to it's rupture (Damodaran, 1997). Prins (2005) suggested that presence of hydrophobic particles can cause film rupture by either making contact with both surfaces of the lamella (when the particle is larger than the lamella diameter and non-spreading) or by displacing the original surfactant stabilising the foam (spreading particles). However Kumagai et al. (1991) observed that hydrophobic SiO_2 particles used in conjunction with proteins, further enhanced stability of foams, whereas Teflon particles caused destabilisation. This was ascribed to their sizes ($SiO_2 \sim 0.7\ \mu m$ & Teflon $\sim 6.0\ \mu m$) as Teflon particles were larger than the thickness of the lamella, they could cause film breakage.

Disproportionation

As with Otswald ripening within emulsions the governing mechanism is the Laplace pressure differentials (Eqn. 2.5) between small and large bubbles (Damodaran, 1997). Because of the pressure gradient, gas diffuses from the smaller bubbles to the adjoining larger ones leading to their eventual collapse and disappearance. The rate of diffusion is dependent on the physical barrier composing the adsorbed interfacial layer, the surface dilatational modulus and the solubility of the gas in the lamella fluid (Damodaran, 1997).

2.3.3 Ultrasound Application in Mixing

2.3.3.1 Ultrasound overview

In recent decades application of ultrasound has been investigated for a vast number of industrial and scientific fields. Power Ultrasound (Low frequency, high power ultrasound) has especially gained an interest for its versatility in generating microstructures and its efficiency in aiding micro-mixing processes (Monnier et al., 1999; Povey and Mason, 1998). In general within this mode of ultrasound, the physiochemical modifications induced by the acoustic propagation are attributed to the 'cavitation' phenomena.

Cavitation

Sound is transmitted through any physical medium by waves that compress and stretch the molecular spacing of that particular medium (Fig. 2.13). Within a given liquid medium the average distance between the molecules will vary as they oscillate and when the negative pressure induced by the ultrasound is large enough, then the distance between the molecules of the liquid exceed the minimum molecular distance required to hold the liquid intact. This induces a break-down of the liquid and thus voids are created. These voids are the so-called cavitation bubbles (Mason, 1999; Santos et al., 2009).

Cavitation bubbles can be sub-categorised in to two different forms based on the ultrasonic intensities. At lower Ultrasound intensities (1-3 Wm^{-2}) stable cavitations are formed, where the bubbles oscillate in size about an equilibrium for many acoustic cycles. The second variety referred to as transient cavitations are formed at sound intensities in excess of 10 Wm^{-2} and describe creation of bubbles which expand in going through a few acoustic cycles before violently collapsing upon compression (Fig. 2.13).The collapse of each transient bubbles creates temperatures of several thousand degrees and pressures of higher than one thousand atmospheres, thus behaving as microreactors (Povey and Mason, 1998; Mason et al., 2015; Santos et al., 2009). Above the threshold for onset of transient bubbles, cavitation phenomena become stronger for higher external pressure and acoustic intensity values as well as in more viscous liquids (more violent bubble collapse) (Raso et al., 1999). However since viscosity of the liquid is a measure of its molecular interactions, higher viscosities can also increase the threshold for onset of transient cavitations. Briggs et al. (1947) described the term of cohesive pressure P_{co} as the liquid media's resistance to formation of cavities, and defined its relationship to viscosity, η and sound pressure amplitude $\hat{P_{th}}$ at cavitation threshold intensity, I_{th};

$$log\left(\frac{\eta}{\eta_0}\right) = -3.05\left(\frac{P_{co}}{atm}\right) \tag{2.9}$$

$$P_{co} = P_0 - \hat{P_{th}} = P_0 - \left(2I_{th}\rho.v_s\right)^{1/2} \tag{2.10}$$

where $\eta_0 \sim 0.13mPa.s$, P_0 is hydrostatic pressure, v_s is sound velocity.

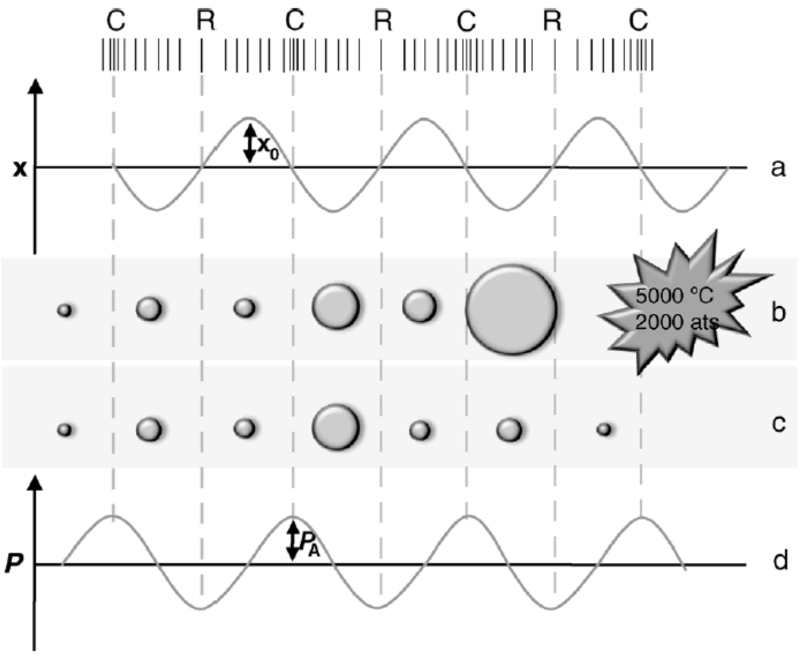


Figure 2.13: Creation of stable cavitation bubbles and creation and collapse of transient and stable cavitation bubbles. (a) Displacement (x) graph; (b) transient cavitation; (c) stable cavitation; (d) pressure (P) graph (Adapted from (Santos et al., 2009).

2.3.3.2 Applications of Ultrasound in Food Industry

Within the food industry emulsification and mixing by ultrasound has attracted great interest. Acoustic emulsification has been shown to produce stable droplets within the sub-micron range with very narrow distributions using a smaller emulsifier ratio with lower energy expenditure (Canselier et al., 2002). An example is the production of mayonnaise by sono-emulsification using polyoxyethylene sorbitan monooleate (Twin), where considerably more stable emulsion structures could be produced (Mason et al., 2015).

Emulsification and Mixing

Emulsification was one of the first applications of power ultrasound and the first related patent was taken out more than fifty years ago (Canselier et al., 2002).

According to Neduzhii (1965), a minimum acoustic intensity is required for the onset of cavitation and it is directly proportional to the viscosity of the liquid medium. The less viscous liquid generally will undergo cavitation more easily (Behrend et al., 2000). Li and Fogler (1978) proposed that irradiation time is the main parameter regulating the function of Ultrasound waves in emulsification as they proposed that;

Initially a combination of interfacial waves and Rayleigh-Taylor instabilities, lead to mixing of the two phases producing a coarse emulsion. The second step consists of breaking droplets up through cavitation near the interface; the intense effect (disruption and mixing) of shock waves yields small sub-micron droplets and during this stage the most influential parameters are those affecting cavitation phenomena (Li and Fogler, 1978).

The application of Ultrasound for emulsification has been trialled both in batch mode (Abismail et al., 1999) or within a continuous setup (Behrend et al., 2000). Within a batch set-up the sonotrode position and distance from the vessel wall is fixed and although local acoustic power is hundred times higher than in a ultrasound bath, the corresponding energy is only localised around the sonotrode tip. Within a continuous set-up, the medium is circulated into a small reactor where it is exposed to the ultrasound waves under very short times scales.

Tan et al. (2011) have extended the use of ultrasound as an mixing aid for cake batter processing; and found that application of 2.5 kW ultrasound assisted mixing resulted in more efficient sponge cake mixing during the batter formation stage thus producing batters with lower densities and higher viscosities at lower mixing times. This result also translated into a less hard and more springy final sponge after baking.

2.4 Rheology

2.4.1 Introduction

Rheology is defined as the science of deformation and flow (Sahin and Sumnu, 2007; Borwankar, 1992). Deformation pertains to solid materials and flow to liquid, hence in most simplistic terms elasticity is the property of interest in solids and viscosity in liquids. However most materials especially structures encountered in foods cannot be simply classified as solids or liquids. Thus they are neither viscous or elastic, they are viscoelastic (Sahin and Sumnu, 2007; Borwankar, 1992). Fig. 2.14 shows the classification

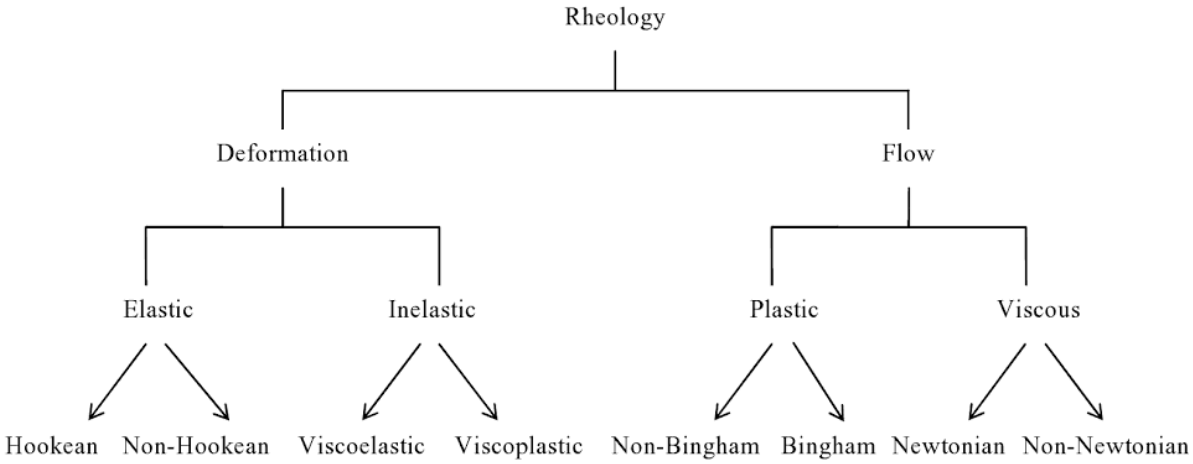


Figure 2.14: Classification of rheology (Adapted from (Sahin and Sumnu, 2007)).

of materials in terms of their rheological responses. Rheological properties are determined by measuring force and deformation as a function of time.

2.4.2 Rheology of Foods

Texture in foods can have many definitions. The International Organisation for Standardisation however defines *"texture as encompassing all rheological and structural attributes of a food product perceivable by means of mechanical, tactile and when appropriate visual and auditory receptors."* Bourne (2002a) . This implies that sensory perception of food products is related to texture and that this perception of texture occurs dynamically through the use of the product by the consumer (Borwankar, 1992). However it is the composition of a food product along with its structure which inform it's rheology (Borwankar, 1992).

Therefore one can see the importance of the study of food rheology for understanding the science of the material's composing it's structure and thus it's relevance to product development, process development (pipeline, pump design *etc.*) and for quality evaluation of the product (as demonstrated by fig. 2.15) (Sahin and Sumnu, 2007).

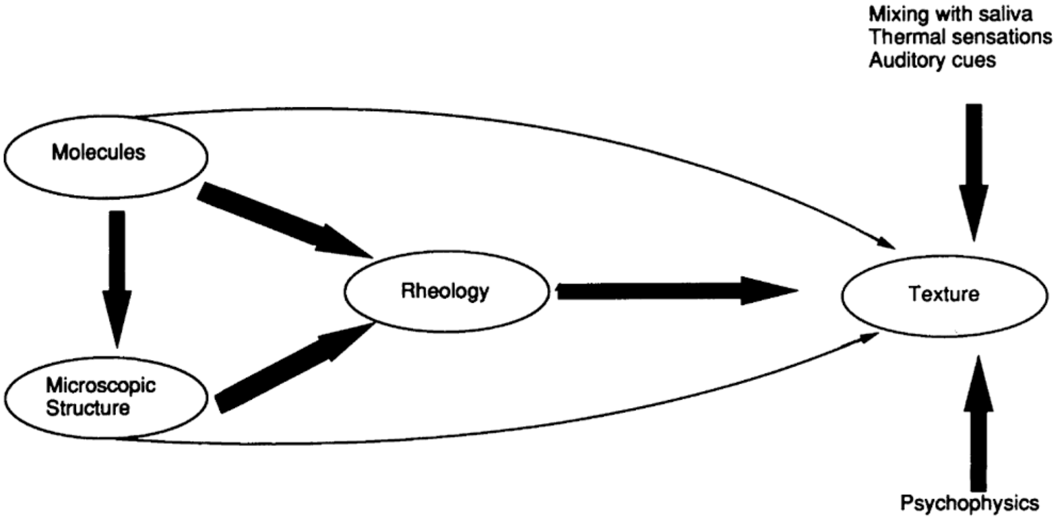


Figure 2.15: Interrelationship between food rheology, structure and texture (Adapted from (Borwankar, 1992).

2.4.3 Rheology of Cake Batters

Cake batter rheology is important since it is directly correlated to certain quality attributes of the final cake (*i.e.* volume and texture) (Fig. 2.15). Cake microstructure is one of great complexity and varies greatly between the different types of cake (Sahin, 2008). Some batters such as those of high-ratio cakes are considered to be complex oil-water emulsions with air-bubbles as the dispersed phase and dry ingredients suspended in the aqueous phase (Sahin, 2008). In simple sponges or angel cakes there can be no emulsion formed, with the batter having two distinct phases of air cells dispersed in a continuous phase of dispersed dry ingredients (Pernell et al., 2002). The interaction of the ingredients and structure development occurs during mixing and baking. Mixing creates a dispersed phase of air-bubbles and/or fat drops. A large number of small air cells must be created to provide maximum volume as first reported by Handleman et al. (1961). During the baking stage, the protein coagulation and starch gelatinisation as well as CO_2 generation give rise to a semi-solid porous gel-like structure. At this stage batter viscosity is thought to be a dominant factor in determining final cake volume. Firstly more viscous batters will be more stable against the coalescence of the entrapped air as drainage of the surrounding batter would be less likely (Handleman et al., 1961). Moreover the rate of bubble rise due to buoyancy force is inversely proportional to the viscosity of the batter (Sahin, 2008). This is due to the fact that in a less viscous batter, as the CO_2 evolves the water vapour cannot be trapped in the system and consequently the final cake will have a lower volume (Frye and Setser, 1992; Sahi and Alava, 2003). More importantly viscous batters help air-retention and stop bubbles from rising to the surface of the cake (resulting in an uneven crumb structure). In addition, the velocity gradient in the batter during baking can induce convection currents which is again dependent on the batters' viscosity, thus lower batter viscosities would result in more convection flow (Frye and Setser, 1992). However it must be mentioned here that if the batter is too viscous, although they hold the bubbles inside, their expansion during the baking stage will be restricted hence there is an optimum viscosity range for batters depending on their type and formulation (Sahi and Alava, 2003).

The literature is not short of studies in which the rheological properties of cakes are related to the final cake quality (Sahi and Alava, 2003; Lakshminarayan et al., 2006). The lower specific gravities of batters was observed to yield cakes of higher volumes by Shrestha et al. (1990) and conversely batters with low viscosity resulted in cakes with lower volumes and harder textures (Lakshminarayan et al., 2006). The increase in batter density was accompanied by lower storage (elastic) and loss (viscous) moduli in cake batter and consequently lower cake volumes (Sahi and Alava, 2003).

2.4.3.1 Rheological Methods

Studies of cake batters with different formulations (Baixauli et al., 2008, 2007; Sakiyan et al., 2004; Turabi et al., 2008) all described the behaviour as shear thinning (pseudo-plastic). The power law model (Eqn. 2.11) describes this behaviour;

$$\tau_{yz} = k\left(\frac{dv_z}{dy}\right)^n = k(\dot{\gamma}_{yz})^n \tag{2.11}$$

where k is the consistency coefficient ($Pa.s^n$), n is the flow behaviour index (n < 1 for shear-thinning fluids), τ_{yz} is the shear stress (Nm^{-2}), and $\dot{\gamma}_{yz}$ is the shear rate (s^{-1}). Baik et al. (2000) showed that the batters of different compositions observed yield stress behaviour and their shear stress-shear rate relationships fitted well with the Casson model:

$$(\tau_{yz})^{0.5} = (\tau_0)^{0.5} + K_c(\dot{\gamma}_{yz})^{0.5} \tag{2.12}$$

where τ_0 is the Casson yield stress (Nm^{-2}), K_c is the consistency co-efficient $(Pa.s)^{0.5}$.

Ideal elastic solids follow Hooke's Law;

$$\tau = G\gamma \tag{2.13}$$

where G is the shear modulus (Nm^{-2}), τ is the shear stress (Nm^{-2}), and γ is the shear strain. Thus this relationship describes a linear correlation between shear stress and shear strain. However in cake batters as with most complex food structures viscoelstic behaviour is observed. Different methods, including dynamic (oscillatory) test, creep recovery test and stress relaxation can be used to study the viscoelastic behaviour of cake batters (Sahin and Sumnu, 2007). In the study of Lee et al. (2004), the rheological properties of cake batters were studied using an oscillatory test. They reported that increased substitution of wheat flour with flaxseed caused decreased oscillatory storage and loss moduli.

In creep recovery tests, strain is measured as a function of time as a consequence of application of an instantaneous stress to the material (creep) and then the stress is removed whilst still recording the strain as a function of time (recovery). Therefore one can see that the ideal elastic material will show full recovery upon the removal of the stress, while an ideal viscous material will show no recovery and will thus be deformed indefinitely (Sahin, 2008).

Viscoelastic materials will demonstrate a behaviour in the middle, where partial recovery is observed upon removal of stress. In oscillatory rheology, the material is subjected to a harmonic stress with time, the consequent harmonic shear stress (*i.e.* deformation in the sample is then measured (Fig. 2.16)). Storage/elastic modulus G', which is high for

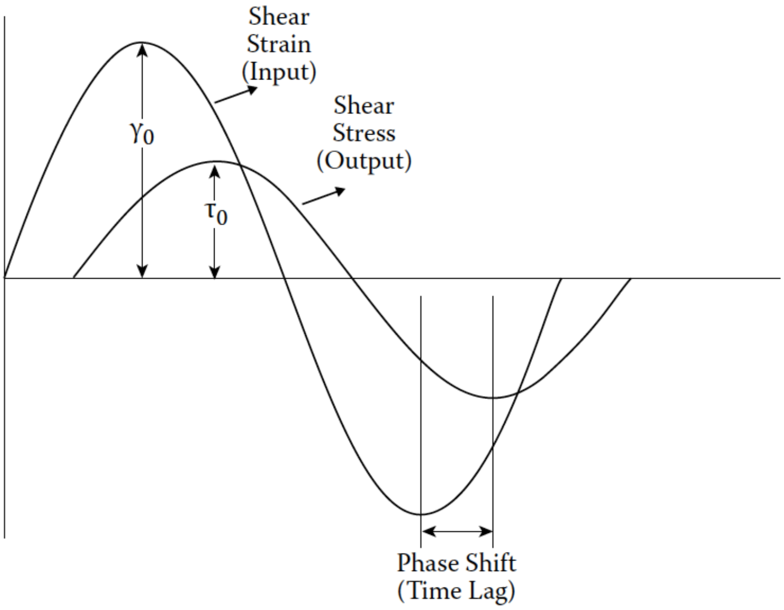


Figure 2.16: Harmonic shear stress vs strain for visocleastic materials in an oscillatory test (Adapted from (Sahin, 2008).

elastic materials, and loss/viscous modulus G'', which is high for viscous materials, are defined as follows:

$$G' = \left(\frac{\tau_0 \cos(\theta)}{\gamma_0}\right) \tag{2.14}$$

$$G'' = \left(\frac{\tau_0 \sin(\theta)}{\gamma_0}\right) \tag{2.15}$$

where τ_0 is the shear stress, γ_0 is shear strain and θ is the time lag (phase shift).

2.4.3.2 Influence of Ingredients

Most important factors that influence the rheological properties of cake batters are the formulation of the batter (*i.e.* ingredients used and their concentrations), the level of air incorporation (which depends on the mixing time and speed as well as the surface tension of the cake batter) as well as the temperature at which the rheological properties are measured. Here the effect of the main ingredients on batter rheology is briefly discussed;

Flour

Flour arguably has the most significant effect on cake batter rheology and thus final cake quality. Shelke et al. (1992) reported a direct correlation between flour age and the viscosity of the cake bater. Aged flour was found to have improved water binding capacity, consequently yielding more viscous batters at ambient temperature as well as during heating. There is a number of studies that focus on the influence of flour treatment on the final quality of the cake. Chlorine treatment of soft wheat flours was found to improve cake volume and produce more resilient crumb structures by Donelson et al. (2000). Huang et al. (1982) ascribed this to the oxidative depolymerization of starch which increased its water-binding capacity as a consequence of chlorination. Starch swelling power at high temperatures was said to increase after chlorination (Huang et al., 1982). Shelke et al. (1992) also observed that the chlorinated flour had higher viscosity than untreated flour. It was reported by Sinha et al. (1997) that hydrophobicity of proteins increases upon chlorination. However due to studies conducted on mice over the ingestion of chlorinated flours by Ginocchio et al. (1983), safety concerns led to their removal from the permitted list of food ingredients in the European Union for cake manufacture. Johnson and Hoseney (1979) observed that by using a starch-flour blend (12-43% of starch added) with untreated flours, very comparable pasting curves could be obtained to those of chlorinated flours. Currently heat-treated flours are seen as the alternative to chlorination. Heat-treatment has been shown to improve the performance of the flour (Seguchi, 1990).

Egg

Egg proteins can be a critical component for the structure of cake batters. Much of the cake's final structure depends on EWP to first facilitate incorporation of air into the batter and secondly for heat coagulation and setting during the baking stage. Lipoproteins in egg yolk assist in aeration and foaming (Shepherd and Yoell, 1976). Substitution of 10% of egg white in angel food cake by freeze-dried wheat water solubles was found to decrease batter viscosity (Maziya-Dixon et al., 1994). Whipping time increased as percent of substitution increased. Other studies by Berry et al. (2009), Yang et al. (2009) and Pernell et al. (2002) have trialled proteins such as whey as replacements for egg whites to no avail.

Emulsifiers

Emulsifiers are readily used within cake production; either within the shortening or through direct addition to the batter (Sahin, 2008). Their presence improves air incorporation into the batter and also aids dispersion of shortening within the system (Sahin, 2008). As discussed this is done through the lowering of interfcial tension thus facilitating incorporation and break-up of air cells to include higher number of small air cells. Air incorporation, volume and dispersion of ingredients were all found to be influenced by the concentration and type of emulsifier used in a study by Cloke et al. (1984). Dynamic oscillatory tests conducted on batters composed of varying concentrations of two different types of emulsifiers (glyceryl monostearate [GMS] and polyglycerol ester [PGE]) showed, that their addition increased both G' and G" of sponge cake batters (Sahi and Alava, 2003).

Lakshminarayan et al. (2006) observed that the addition of GMS or sodium steroyl lactate (SSL) in cake formulations with maltodextrin used as the fat replacer, the viscosity of the batter increased. It is stated that for best results a blend of emulsifiers is required with different hydrophillic-lipophillic properties. The effect of different types of emulsifier blends (*Purawave*® and *Lecigran*®) on the rheological behaviour of cake batters was studied by Sakiyan et al. (2004). Lipase enzymes have been used instead of emulsifiers by Guy and Sahi (2006). They concluded enzyme in producing surfactants from tri-glycerides had the capacity to increase the wheat protein's hydrodynamic volume which resulted in higher viscosities of the batter. Gujral et al. (2003) studied cake batter rheology after the addition of sodium lauryl sulfate (an anionic surfactant) to egg albumen during the mixing. They concluded that with increasing surfactant concentration the bubble diameter and specific gravity of cake batter were reduced.

The addition of Polysorbate-60 (PS-60) and sucrose esters to cake batters were compared by Kim and Walker (1992), who found PS-60 to be more effective at increasing the viscosity of batters. This was attributed to facilitation of incorporation of more air-bubbles. Consequently it was concluded that cakes with higher volumes and lower crumb firmness could be obtained with PS-60 addition. Jyotsna et al. (2004) reported that the use of emulsifier gels from sodium stearoyl-2-lactylate (SSL), distilled glycerol monostearate (DGMS), propylene glycol monostearate (PGMS), PS-60 and sorbitol monostearate (SMS) could produce batters with lower density, with more mono-dispersed air cell distributions which resulted in cakes of higher volumes and better textures.

Sugar

The addition of sugar reduces the available water for starch which affects the rheological properties, delays starch gelatinisation, retards structural development and modifies the heat-setting temperature of egg proteins during baking (Sahin, 2008). The addition of sugar was observed to increase the viscosity and elasticity of the interfacial films surrounding air bubbles (Yang et al., 2009) as well as increasing the viscosity of the batter (Berry et al., 2009). Sugar concentration was reported to increase batter viscosity at ambient temperature (Shelke et al., 1990). In the same study it was observed that sucrose was more effective than glucose and fructose in increasing the onset temperatures of batters during baking.

Effect of Temperature

Cake batters undergo important rheolgical changes during the baking process. The batter will decrease in viscosity initially and as they get towards the gelatinisation and coagulation temperatures of starch and protein respectively their viscosities will increase (Sahin, 2008).

2.5 Final Structure; Physical and Mechanical Properties

Sponges and cakes tend to have certain unifying characteristics that differentiate them from the rest of the baked goods category (Cauvain and Young, 2006c). They are classed as intermediate moisture products, with general moisture contents 10-20% lower than

Table 2.1: Typical moisture contents and densities for cake products (Adapted from (Cauvain and Young, 2006c)

Products	Properties	
	Moisture (%)	Density (g/ml)
Plain	22-30	0.30-0.40
Chocolate	18-28	0.35-0.45
White	26-34	0.25-0.35
Fruited	18-26	0.40-0.55
Plain Sponge	25-32	0.18-0.25
Chocolate Sponge	24-28	0.21-0.24

those in bread (Tab. 2.1). The cellular structure of cakes is often considered to be less defined compared to that of breads. However as it can be observed from tab. 2.1, there is great discrepancy between different cakes, with sponge cakes generally having a more defined cell-structure. A key attribute of cakes is the relatively longer shelf-life that they enjoy compared with that of bread. Both organoleptic and mould-free shelf-life are lengthened, mostly because of the lowering of water activity which restricts moisture losses and growth of micro-organisms (Cauvain and Young, 2009).

2.5.1 Texture

There is a great amount of variability in the definition of 'texture' which depends on the context and the culture in which it is used. However even without a coherent definition, Bourne (2002a) produced a set of characteristics that encompass food texture;

1. It is a group of physical properties that derive from the structure of the food.
2. It belongs under the mechanical or rheological subheading of physical properties.
3. Texture is sensed primarily by the feeling of touch, usually in the mouth, but other parts of the body may be involved (frequently the hands).
4. It is not related to the chemical senses of taste or odour.
5. Objective measurement is by means of functions of mass, distance and time only.

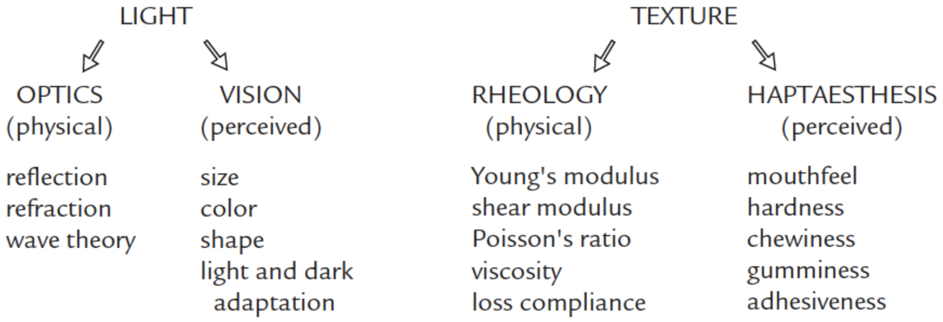


Figure 2.17: Comparison of physical measurement and human perception of light and texture (Adapted from (Bourne, 2002a)).

Muller (1969) claimed that *'texture'* as a descriptive term should be discarded as it creates confusion. He proposed two terms to replace it; (1) *Rheology*, a description of the physical properties of food; and (2) *haptaesthesis*, a branch of psychology that deals with the perception of the mechanical behaviour of materials. Muller makes this comparison with the concept of light, which he divides into two distinct branches; (1) *optics*, the objective physical properties of light; (2) *vision*, the subjective psychological responses to perception of light.

We are only concerned with the objective measurements of texture in this body of work. The sensory attributes stemming from the physical aspects of the cake structure will not be explored, only the physics which informs the mechanical responses of the material are of concern here.

2.5.2 Mechanics; Objective study of texture

From the material's response to deformation, a number of objectively quantifiable parameters can be deduced. Some of the fundamental tests that are used in the theory and practice of material science and are applicable to cake systems as food semi-solids are;

Young's Modulus

Young's modulus of elasticity is the ratio of stress to strain when an elastic solid material is compressed or extended and it is described by the equation:

$$E = \frac{Stress}{strain} = \frac{F/A}{\Delta L/L} \tag{2.16}$$

where E is Young's modulus (Pa), F is the applied force perpendicular to the area defined by the stress (N), A is the cross-sectional area of the test material (m^2), L is the length or height of the test specimen (m) and ΔL the change in length resulting from the application of force F. Young's modulus is equal to the slope of the stress-strain curve.

Shear Modulus

The ratio of shearing stress and the shearing strain;

$$G = \frac{ShearStress}{ShearStrain} = \frac{F/A}{\gamma/L} \tag{2.17}$$

where G is the shear modulus (Pa), F is the applied force parallel to the area defined by the stress, γ is the greatest distance of movement in the test material, and L is the length.

Bulk modulus

Bulk modulus is the ratio of the stress to the change in volume:

$$K = \frac{HydrostaticPressure}{VolumetricStrain} = \frac{P}{\Delta V/V} \tag{2.18}$$

where K is the bulk modulus (Pa), P the force per unit area (pressure) applied from all directions (isotropically), V is the original volume of the unstressed material and ΔV the change in volume resulting from the application of pressure P.

Poisson's Ratio

$$\mu = \frac{lateralstrain}{axialstrain} = \frac{changeinwidth}{changeinlength} = \frac{\Delta D/D}{\Delta L/L} \tag{2.19}$$

where μ is Poisson's ratio, D is the width of the test specimen and L its length or height, ΔD and ΔL the changes caused by the application of a stress. If the volume is unchanged when the stress is applied, Poisson's ratio is 0.5. However highly compressible

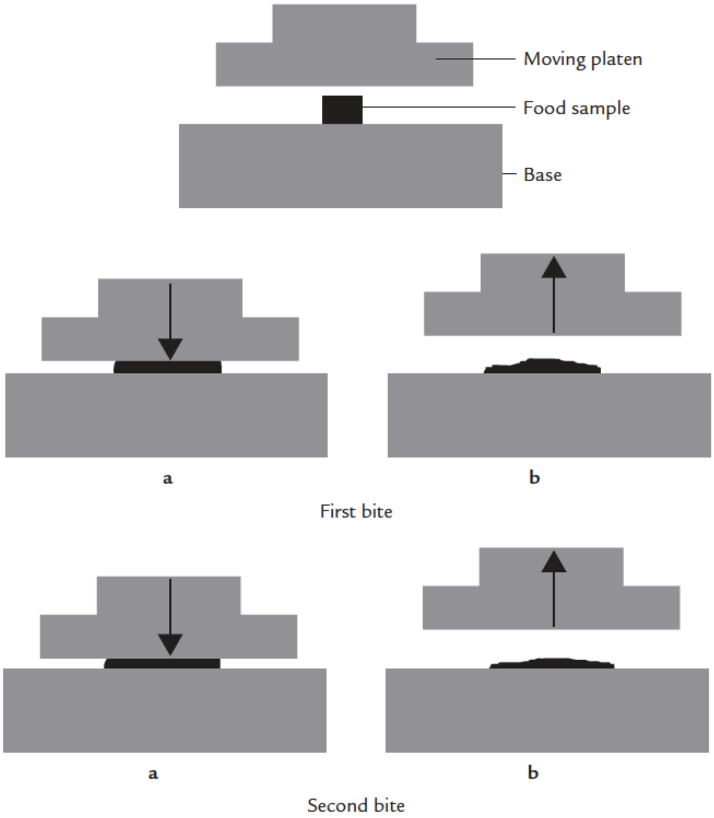


Figure 2.18: Schematic diagram of the two compressions required for the texture profile analysis test. (a) Downstroke actions during the first and second bites; (b) upstroke actions during the first and second bites (Figure adapted from (Bourne, 2002b)).

food materials such as cakes and bread have very low Poisson ratios. Individual cereal grains usually have a Poisson's ratio between 0.25 and 0.4. Researchers often assume a ratio of 0.3 for cereal grains if the actual Poisson's ratio is not known (Bargale and Irudayaraj, 1995).

Interrelations between moduli

E, G, K and μ are called 'material constants' because they are inherent to the material and their values should not be affected by the size and shape of the test specimen (Bourne, 2002b).

2.5.3 Texture Profile Analysis (TPA)

General Foods Corporation Technical Center pioneered TPA as a tool for imitation of the jaw during mastication (Bourne, 2002b). It compresses a piece of food twice in

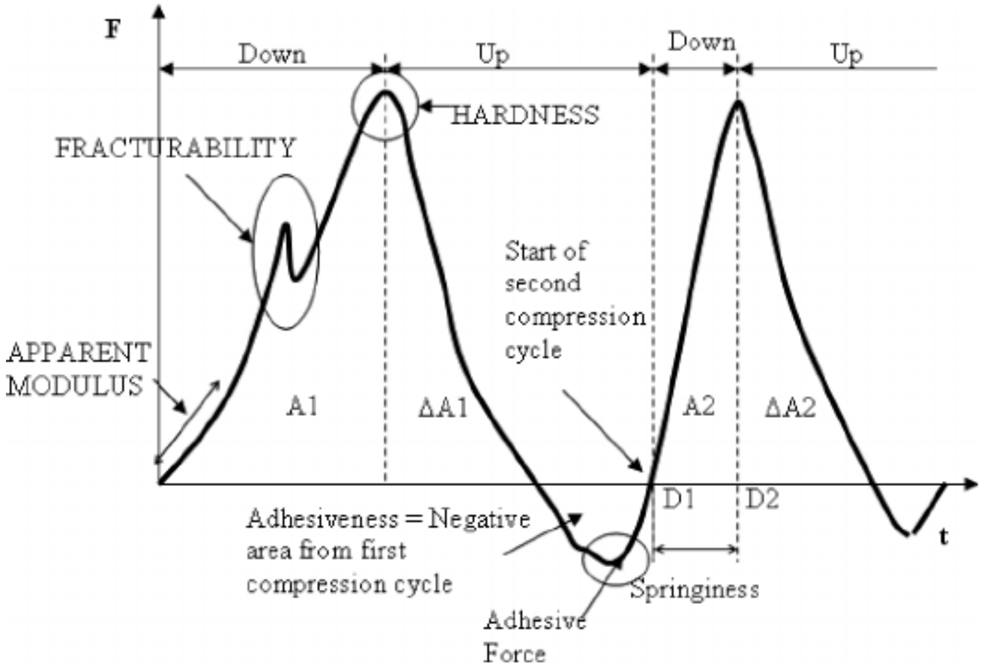


Figure 2.19: A typical TPA curve (Figure adapted from (Bourne, 2002b)).

a reciprocating motion and from the resulting force-time curve a number of textural parameters can be extracted that correlate to the sensory characteristics (Szczesniak, 1963). Fig. 2.18 shows a general TPA curve generated by a Texture analyser instrument

The maximum force reached on the first compression cycle is defined as hardness; Fracturability (originally called brittleness) which is not of relevance for cakes. The ratio of the positive force areas under the first and second compressions ($A2/A1$) was defined as cohesiveness. The negative force area represented the work necessary to pull the compressing plunger away from the sample and is defined as adhesiveness. The distance that the food recovered its height during the time that elapsed between the end of the first bite and the start of the second bite (BC) was defined as springiness (originally called elasticity) (Bourne, 2002b).

2.6 Mechanical Characterisation

The modernised baking industry requires accurate prediction of the product texture based on the materials and processes used (Liu and Scanlon, 2003b). As yet this is a field that

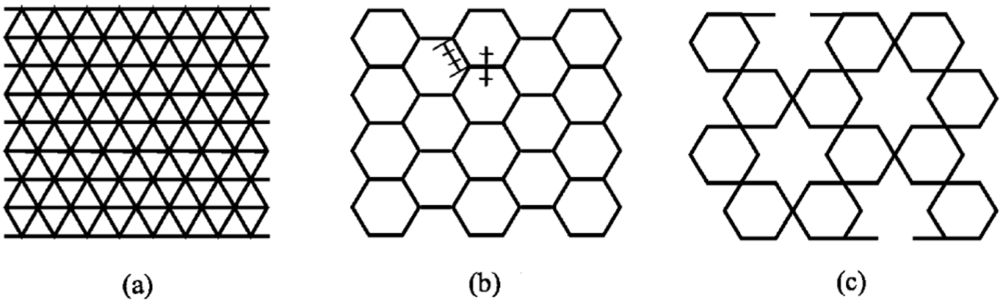


Figure 2.20: The structure of typical two-dimensional honeycomb models: (a) triangular; (b) hexagonal; and (c) mixed star and hexagonal (Figure adapted from (Gibson and Ashby, 1999))

is lacking in terms of fundamental understanding of how process and formulation can be used to predict the final mechanical properties of sponges (Attenburrow et al., 1989). In this regard the bread research is more advanced in understanding the hierarchical structures that are present from macroscale to microscale within bread crumb, and how they affect the mechanical properties of the bread crumb (Liu and Scanlon, 2003b). The cellular solids approach of Gibson and Ashby (1999) theory has been shown to predict the mechanical properties of bread crumb reasonably well (Liu and Scanlon, 2003b). The vast majority of studies in this field have assumed an idealised cellular structure constructed of simple regular geometries (Gibson and Ashby, 1999). Gent and Thomas (1963), the pioneers in attempting to predict the elastic modulus and critical stress, considered the usual textural classification of crumb firmness based on Gibson and Ashby (1999) models.

Most studies to date have assumed a two-dimensional honeycomb model with assemblage of uniform geometries (Christensen, 1995; Gibson et al., 1982) (Fig. 2.20). These idealised models, require certain pre-requisites to be met *i.e.* the relative density must be less than 0.3 and the cell wall thickness to cell wall length ratio must be less than 0.25 (Gibson and Ashby, 1999). If they are met then the relationship between relative compressive elastic modulus (E/E_s) and relative density (ρ/ρ_s) reduces down to;

$$\frac{E}{E_s} = C[\frac{\rho}{\rho_s}]^m \tag{2.20}$$

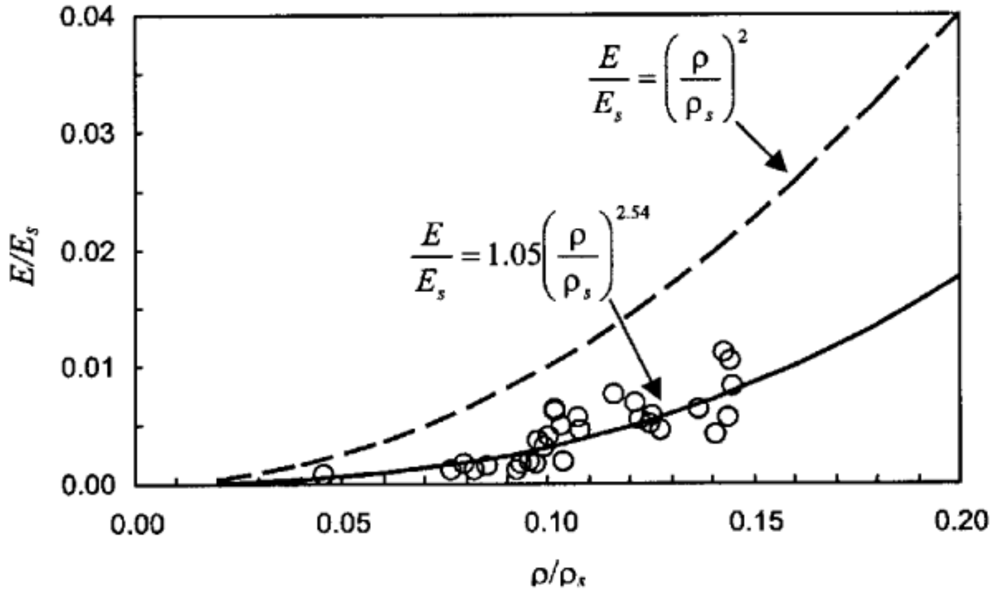


Figure 2.21: Relative Young's modulus as a function of relative density of bread crumb. (Figure taken from (Liu and Scanlon, 2003a)).

where C and m are geometry specific constants, ((0.33, 1) for triangles, (1.5, 3) for regular hexagons, and (0.56, 3) for the star shapes (mixed star and hexagon) (Christensen, 1995; Gibson and Ashby, 1999)). Liu and Scanlon (2003a) demonstrated that the bread elastic modulus does indeed conform to this model (Fig. 2.21). However the experimental points were below the predicted ones and thus they concluded that the deviation from the regular cellular solid power law relationship suggests that bread crumb is not a perfect open-cell foam. The presence of cell wall curvature and corrugations as well as missing cell walls (Zghal et al., 1999) in bread crumb will tend to create discrepancies compared to the power law theoretical model.

2.7 References

List of References

Abismail, B., Canselier, J., Wilhelm, A., Delmas, H., and Gourdon, C. (1999). Emulsification by ultrasound: drop size distribution and stability. *Ultrasonics Sonochemistry*, 6(1–2):75 – 83.

Attenburrow, G., Goodband, R., Taylor, L., and Lillford, P. (1989). Structure, mechanics and texture of a food sponge. *Journal of Cereal Science*, 9(1):61 – IN1.

Baik, O., Marcotte, M., and Castaigne, F. (2000). Cake baking in tunnel type multi-zone industrial ovens part ii. evaluation of quality parameters. *Food Research International*, 33(7):599 – 607.

Baixauli, R., Sanz, T., Salvador, A., and Fiszman, S. (2008). Muffins with resistant starch: Baking performance in relation to the rheological properties of the batter. *Journal of Cereal Science*, 47(3):502 – 509.

Baixauli, R., Sanz, T., Salvador, A., and Fiszman, S. M. (2007). Influence of the dosing process on the rheological and microstructural properties of a bakery product. *Food Hydrocolloids*, 21(2):230 – 236.

Bargale, P. and Irudayaraj, J. (1995). Mechanical strength and rheological behaviour of barley kernels. *International journal of food science & technology*, 30(5):609–623.

Behrend, O., Ax, K., and Schubert, H. (2000). Influence of continuous phase viscosity on emulsification by ultrasound. *Ultrasonics Sonochemistry*, 7(2):77 – 85.

Berry, T. K., Yang, X., and Foegeding, E. A. (2009). Foams prepared from whey protein isolate and egg white protein: 2. changes associated with angel food cake functionality. *Journal of food science*, 74(5):E269–E277.

Binks, B., editor (1998a). *Modern Aspects of Emulsion Science*. Royal Society of Chemistry.

Binks, B. P. (1998b). Emulsions - recent advances in understanding. In Binks, B. P., editor, *Modern Aspects of Emulsion Science*, pages 1–55. The Royal Society of Chemistry, Cambridge, UK, 1st edition edition.

Borwankar, R. P. (1992). Food texture and rheology: A tutorial review. In BORWANKAR, R. and SHOEMAKER, C., editors, *Rheology of Foods*, pages 1 – 16. Elsevier, Amsterdam.

Bourne, M. C. (2002a). Chapter 1 - texture, viscosity, and food. In Bourne, M. C., editor, *Food Texture and Viscosity (Second Edition)*, Food Science and Technology, pages 1 – 32. Academic Press, London, second edition edition.

Bourne, M. C. (2002b). Chapter 4 - principles of objective texture measurement. In Bourne, M. C., editor, *Food Texture and Viscosity (Second Edition)*, Food Science and Technology, pages 107 – 188. Academic Press, London, second edition edition.

Breward, C. J. W. (1999). *The Mathematics of Foam*. PhD thesis, St Anne's College, University of Oxford.

Briggs, H. B., Johnson, J. B., and Mason, W. P. (1947). Properties of liquids at high sound pressure. *The Journal of the Acoustical Society of America*, 19(4):664–677.

Brooker, B. E. (1993a). The stabilisation of air in cake batters - the role of fat. *Food Structure*, 12(3):285–296.

Brooker, B. E. (1993b). The stabilization of air in foods containing fat—a review. *Food Structure*, 12(2):115–122.

Canselier, J. P., Delmas, H., Wilhelm, A. M., and Abismaïl, B. (2002). Ultrasound emulsification—an overview. *Journal of Dispersion Science and Technology*, 23(1-3):333–349.

Cantat, I., Cohen-Addad, S., Elias, F., Graner, F., Höhler, R., and Pitois, O. (2013). *Foams: structure and dynamics*. Oxford University Press.

Cauvain, S. and Young, L. (2006a). *Baked Products: Science, Technology and Practice*. Wiley, High Wycombe, UK.

Cauvain, S. P. and Young, L. S. (2006b). *Baked Products: Science, Technology and Practice*, chapter 1: Current Approaches to the Classification of Bakey Products, pages 1–11. Blackwell Publishing.

Cauvain, S. P. and Young, L. S. (2006c). Key characteristics of existing bakery-product groups and typical variations within such groups. In Cauvain, S. P. and Young, L. S., editors, *Baked Products: Science, Technology and Practice*, chapter 2, pages 14–35. Blackwell Publishing, High Wycombe, UK.

Cauvain, S. P. and Young, L. S. (2009). *Bakery food manufacture and quality: water control and effects*. John Wiley & Sons.

Chanamai, R. and McClements, D. (2000). Creaming stability of flocculated monodisperse oil-in-water emulsions. *Journal of Colloid and Interface Science*, 225(1):214 – 218.

Chaudhary, V. K., Yamazaki, W. T., , and Gould, W. A. (1981). Relation of cultivar and flour particle size distribution to cake volume. *Cereal Chemistry*, 58(4):314–317.

Chorleywood-Food, C. . (2008). *Influence of glycerol monostearate (GMS) concentration on gas bubbles in sponge batters*. Campden BRI, Chipping Campden, UK.

Christensen, R. M. (1995). The hierarchy of microstructures for low density materials. In *Theoretical, Experimental, and Numerical Contributions to the Mechanics of Fluids and Solids*, pages 506–521. Springer.

Clark, D. C., Coke, M., Mackie, A. R., Pinder, A. C., and Wilson, D. R. (1990). Molecular diffusion and thickness measurements of protein-stabilized thin liquid films. *Journal of Colloid and Interface Science*, 138(1):207–219.

Clark, D. C., Mackie, A. R., Wilde, P. J., and Wilson, D. R. (1994). Differences in the structure and dynamics of the adsorbed layers in protein-stabilized model foams and emulsions. *Faraday Discussions*, 98:253–262.

Clements, R. L. and Donelson, R. (1982). Role of flour llipid in batter expansion in layer cakes .i. effect of aging. *Cereal Chemistry*, 59(2):121–124.

Cloke, J., Davis, E., and Gordon, J. (1984). Relationship of heat transfer and water-loss rates to crumb-structure development as influenced by monoglycerides [cake]. *Cereal Chemistry (USA)*.

Damodaran, S. (1997). Food proteins: An overview. In Damodaran, S. and Paraf, A., editors, *Food Proteins and their Applications*, pages 1–25. Mercel Dekker Inc, New York.

Derby, R. I., Miller, B. S., Miller, B. F., and Trimbo, H. B. (1975). Visual observation of wheat-starch gelatinization in limited water systems. *Cereal Chemistry*, 52:702–713.

Dickinson, E. (2010). Flocculation of protein-stabilized oil-in-water emulsions. *Colloids and Surfaces B: Biointerfaces*, 81(1):130 – 140.

Donelson, J. R., Gaines, C. S., and Finney, P. L. (2000). Baking formula innovation to eliminate chlorine treatment of cake flour. *Cereal Chemistry*, 77(1):53–57.

Foegeding, E. A., Luck, P., and Davis, J. (2006). Factors determining the physical properties of protein foams. *Food Hydrocolloids*, 20(2-3):284 – 292. 7th International Hydrocolloids Conference.

Frye, A. M. and Setser, C. S. (1992). Optimizing texture of reduced-calorie yellow layer cakes. *Cereal Chem*, 69:338–43.

Gaines, C. S. and Donelson, J. R. (1985). Effect of varying flour protein content on angel food and high-ratio white layer cake size and tenderness. *Cereal Chem*, 62:63–66.

Gaonkar, A. G. (1992). Effects of salt, temperature, and surfactants on the interfacial tension behavior of a vegetable oil/water system. *Journal of Colloid and Interface Science*, 149(1):256 – 260.

Gent, A. and Thomas, A. (1963). Mechanics of foamed elastic materials. *Rubber Chemistry and Technology*, 36(3):597–610.

Gibson, L., Ashby, M., Schajer, G., and Robertson, C. (1982). The mechanics of two-dimensional cellular materials. In *Proceedings of the Royal Society of London A: Mathematical, Physical and Engineering Sciences*, volume 382, pages 25–42. The Royal Society.

Gibson, L. J. and Ashby, M. F. (1999). *Cellular solids: structure and properties.* Cambridge university press.

Ginocchio, A., Fisher, N., Hutchinson, J., and Berry, R. (1983). Long-term toxicity and carcinogenicity studies of cake made from chlorinated flour: 2. studies in mice. *Food and Chemical Toxicology*, 21(4):435–439.

Grace, H. P. (1982). Dispersion phenomena in high viscosity immiscible fluid systems and application of static mixers as dispersion devices in such systems. *Chemical Engineering Communications*, 14(3-6):225–277.

Graham, D. E. and Phillips, M. C. (1976). The conformation of proteins at air-water interface and their role in stabilising foams. In Ackers, R. J., editor, *Foams*, pages 237–255. Academic Press, New York.

Greer, E. N. and Stewart, B. A. (1959). The water absorption of wheat flour: Relative effects of protein and starch. *Journal of the Science of Food and Agriculture*, 10(4):248–252.

Gujral, H. S., Rosell, C., Sharma, S., and Singh, S. (2003). Effect of sodium lauryl sulphate on the texture of sponge cake. *Food Science and Technology International*, 9(2):89–93.

Guy, R. C. and Sahi, S. S. (2006). Application of a lipase in cake manufacture. *Journal of the Science of Food and Agriculture*, 86(11):1679–1687.

Hailing, P. J. and Walstra, P. (1981). Protein-stabilized foams and emulsions. *C R C Critical Reviews in Food Science and Nutrition*, 15(2):155–203. PMID: 7023848.

Handleman, A. R., Conn, J. F., and Lyons, J. W. (1961). Bubble mechanics in thick foams and their effects on cake quality. *Cereal Chemistry*, 38(3):294.

Hareland, G. A. (1995). Evaluation of flour particle size distribution by laser diffraction, sieve analysis and near-infrared reflectance spectroscopy. *Journal of Cereal Science*, 20(2):183–190.

Heritage, L. (1894). *Cassell's New Universal Cookery Book*. Cassell.

Howard, N. B., Hughes, D. H., , and Strobel, R. G. K. (1968). Function of the starch granule in the formation of layer cake structure. *Cereal Chemistry*, 45:329–338.

Huang, G., Finn, J. W., and Varriano-Marston, E. (1982). Flour chlorination. ii. effects on water binding. *Cereal Chemistry*, 59(6):500–506.

Indrani, D. and Rao, G. V. (2008). Functions of ingredients in the baking of sweet goods. In Dumnu, S. G. and Sahin, S., editors, *Food Engineering Aspects of Baked Sweet Goods*, chapter 1, pages 31–48. CRC Press.

Johnson, A. and Hoseney, R. (1979). Chlorine treatment of cake flour. ii. effect of certain ingredients in the cake formula. *Cereal Chemistry*, 56(3):336–338.

Jyotsna, R., Prabhasankar, P., Indrani, D., and Rao, G. V. (2004). Improvement of rheological and baking properties of cake batters with emulsifier gels. *Journal of Food Science*, 69(1):SNQ16–SNQ19.

Kaldy, M. S. and Rubenthaler, G. L. (1987). Milling, baking, and physical-chemical properties of selected soft white winter and spring wheats. *Cereal Chem*, 64:302–307.

Kelly, I. (2009). *Cooking for Kings: The Life of Antonin Carême, the First Celebrity Chef*. Bloomsbury Publishing.

Kim, C. S. and Walker, C. E. (1992). Interactions between starches, sugars, and emulsifiers in high-ratio cake model systems. *Cereal Chemistry*, 69(2):206–212.

Kumagai, H., Torikata, Y., Yoshimura, H., Kato, M., and Yano, T. (1991). Estimation of the stability of foam containing hydrophobic particles by parameters in the capillary model. *Agricultural and Biological Chemistry*, 55(7):1823–1829.

Lakshminarayan, S. M., Rathinam, V., and KrishnaRau, L. (2006). Effect of maltodextrin and emulsifiers on the viscosity of cake batter and on the quality of cakes. *Journal of the Science of Food and Agriculture*, 86(5):706–712.

Lee, L. L., Niknafs, N., Hancocks, R. D., and Norton, I. T. (2013). Emulsification: Mechanistic understanding. *Trends in Food Science & Technology*, 31(1):72 – 78.

Lee, S., Inglett, G. E., and Carriere, C. J. (2004). Effect of nutrim oat bran and flaxseed on rheological properties of cakes. *Cereal Chemistry*, 81(5):637–642.

Li, M. and Fogler, H. (1978). Acoustic emulsification. part 1. the instability of the oil-water interface to form the initial droplets. *Journal of Fluid Mechanics*, 88(3):499–511.

Liu, Z. and Scanlon, M. G. (2003a). Modelling indentation of bread crumb by finite element analysis. *Biosystems engineering*, 85(4):477–484.

Liu, Z. and Scanlon, M. G. (2003b). Predicting mechanical properties of bread. *Food & Bioproduct Processing*, 81(3):224–238.

Manev, E., Sazdanova, S., and Wasan, D. (1984). Emulsion and foam stability—the effect of film size on film drainage. *Journal of Colloid and Interface Science*, 97(2):591 – 594.

Marmur, A. (1984). The effect of gravity on thin fluid films. *Journal of Colloid and Interface Science*, 100(2):407 – 413.

Mason, T. (1999). *Sonochemistry*. Oxford chemistry primers. Oxford University Press.

Mason, T., Chemat, F., and Ashokkumar, M. (2015). Power ultrasonics for food processing. In Gallego-Juárez, J. A., Graff, and F., K., editors, *Power Ultrasonics*, pages 815 – 843. Woodhead Publishing, Oxford.

Maziya-Dixon, B., Klopfenstein, C., and Walker, C. (1994). Freeze-dried wheat water solubles from a starch-gluten washing stream: functionally in angel food cakes and nutritional properties compared with oat bran. *Cereal chemistry (USA)*.

McClements, D. (2005). *Food Emulsions: Principles, Practice, and Techniques*. Contemporary Food Science. CRC Press.

Meza, B. E., Chesterton, A. K., Verdini, R. A., Rubiolo, A. C., Sadd, P. A., Moggridge, G. D., and Wilson, D. I. (2011). Rheological characterisation of cake batters generated by planetary mixing: Comparison between untreated and heat-treated wheat flours. *Journal of Food Engineering*, 104(4):592 – 602.

Miller, R. (2016). Cakes: Types of cakes. In Caballero, B., Finglas, P. M., and Toldrá, F., editors, *Encyclopedia of Food and Health*, pages 579 – 582. Academic Press, Oxford.

Mine, Y. (1995). Recent advances in the understanding of egg white protein functionality. *Trends in Food Science & Technology*, 6(7):225–232.

Monnier, H., Wilhelm, A.-M., and Delmas, H. (1999). Influence of ultrasound on mixing on the molecular scale for water and viscous liquids. *Ultrasonics Sonochemistry*, 6(1–2):67 – 74.

Morrison, I. D. and Ross, S. (2002). *Colloidal Dispersions: Suspensions, Emulsions, and Foams*. Wiley.

Morrison, W. R. (1978). Wheat lipid composition. *Cereal Chemistry*, 55(5):548–558.

Muller, H. (1969). Mechanical properties, rheology, and haptaesthesis of food. *Journal of Texture Studies*, 1(1):38–42.

Naofumi, K. and Etsushiro, D. (1988). Surface tension and foamability of protein and surfactant solutions. *Journal of Food Science*, 53(5):1542–1569.

Neduzhii, S. (1965). Nature of the disturbances giving rise to formation of the disperse phase of an emulsion in an acoustic field. *Sov. Phys. Acoust*, 10(4):390–397.

Nishioka, G. and Ross, S. (1981). A new method and apparatus for measuring foam stability. *Journal of Colloid and Interface Science*, 81(1):1 – 7.

Oliver, L. (2015). Cakes.

Pacek, A., Man, C., and Nienow, A. (1998). On the sauter mean diameter and size distributions in turbulent liquid/liquid dispersions in a stirred vessel. *Chemical Engineering Science*, 53(11):2005 – 2011.

Palav, T. (2016). Chemistry of cake manufacturing. In Wrigley, Colin, Corke, Harold, Seetharaman, Koushik, and Faubion, editors, *Encyclopedia of Food Grains (Second Edition)*, pages 367 – 374. Academic Press, Oxford, second edition edition.

Paton, D., Larocque, G. M., and Holme, J. (1981). Development of cake structure: Influence of ingredients on the measurement of cohesive force during baking. *Cereal Chemistry*, 58(6):527–529.

Pernell, C., Luck, P., AllenFoegeding, E., and Daubert, C. (2002). Heat-induced changes in angel food cakes containing egg-white protein or whey protein isolate. *Journal of Food Science*, 67(8):2945–2951.

Perry, P. and Donald, A. (2002). The effect of sugars on the gelatinisation of starch. *Carbohydrate Polymers*, 49(2):155 – 165.

Polyakov, V. I., Popello, I. A., Grinberg, V. Y., and Tolstoguzov, V. B. (1986). Thermodynamic compatibility of proteins in aqueous medium. *Food / Nahrung*, 30(3-4):365–368.

Povey, M. and Mason, T. (1998). *Ultrasound in Food Processing.* Springer.

Prins, A. (2005). Theory and practice of formation and stability of food foams. In Dickinson, E., editor, *Food Emulsions and Foams*, Woodhead Publishing Series in Food Science, Technology and Nutrition, pages 30 – 39. Woodhead Publishing.

Pyler, E. J. et al. (1973). Baking science & technology.

Raso, J., Mañas, P., Pagán, R., and Sala, F. J. (1999). Influence of different factors on the output power transferred into medium by ultrasound. *Ultrasonics Sonochemistry*, 5(4):157 – 162.

Sahi, S. S. (2008). Cake emulsions. In Dumnu, S. G. and Sahin, S., editors, *Food Engineering Aspects of Baked Sweet Goods*, chapter Cake Emulsions, pages 81–98. CRC Press.

Sahi, S. S. and Alava, J. M. (2003). Functionality of emulsifiers in sponge cake production. *Journal of the Science of Food and Agriculture*, 83(14):1419–1429.

Sahin, S. (2008). Cake batter rheology. In Dumnu, S. G. and Sahin, S., editors, *Food Engineering Aspects* *of Baked Sweet Goods*, chapter Cake Batter Rheology, pages 99–120. CRC Press.

Sahin, S. and Sumnu, S. (2007). *Physical Properties of Foods*. Food Science Text Series. Springer New York.

Sakiyan, O., Sumnu, G., Sahin, S., and Bayram, G. (2004). Influence of fat content and emulsifier type on the rheological properties of cake batter. *European Food Research and Technology*, 219(6):635–638.

Santos, H. M., Lodeiro, C., and Capelo-Martinez, J.-L. (2009). The power of ultrasound. In *Ultrasound in Chemistry*, pages 1–16. Wiley & Co.

Seguchi, M. (1990). Effect of heat-treatment of wheat flour on pancake springiness. *Journal of Food Science*, 55(3):784–785.

Seguchi, M. and Matsuki, J. (1977). Studies on pan-cake baking. i. effect of chlorination of flour on pan-cake qualities. *Cereal Chemistry*, 54(2):287–299.

Seguchi, M. and Yamada, Y. (1988). Hydrophobic character of heat-treated wheat starch. *Cereal Chemistry*, 4(65):375–376.

Shelke, K., Faubion, J., and Hoseney, R. (1990). The dynamics of cake baking as studied by a combination of viscometry and electrical resistance oven heating. *Cereal chemistry*, 67(6):575–580.

Shelke, K., Hoseney, R. C., Faubion, J. M., and Curran, S. P. (1992). Age-related changes in the properties of batters made from flour milled from freshly harvested soft wheat. *Cereal Chemistry*, 69(2):145–147.

Shepherd, I. and Yoell, R. (1976). Cake emulsions. In Freiberg, S., editor, *Food Emulsions*, pages 215–275. Mercel Dekker Inc, New York.

Shrestha, N., Vali, S., and Choudhary, P. (1990). Quality characteristics of cakes prepared from different fats and oil. *Journal of food science and technology*, 27(6):400–401.

Sinha, N., Yamamoto, H., and Ng, P. (1997). Effects of flour chlorination on soft wheat gliadins analyzed by reversed-phase high-performance liquid chromatography, differential scanning calorimetry and fluorescence spectroscopy. *Food Chemistry*, 59(3):387–393.

Sollars, W. F. and Rubenthaler, G. L. (1971). Performance of wheat and other starches in reconstituted flours. *Cereal Chem*, 48:397–410.

Spies, R. D. and Hoseney, R. C. (1982). Effect of sugars on starch gelatinization. *Cereal Chemistry*, 59(2):128–131.

Sugden, T. D. and Osborne, B. G. (2001). *Cereals and Cereal Products*, chapter Whear flour milling, pages 140–181. Aspen Publishers Inc.

Szczesniak, A. S. (1963). Objective measurements of food texturea. *Journal of Food Science*, 28(4):410–420.

Takeda, K. (1994). Effects of various lipid fractions of wheat flour on expansion of sponge cake. *Cereal Chemistry*, 71(1):6–9.

Tan, M., Chin, N., and Yusof, Y. (2011). Power ultrasound aided batter mixing for sponge cake batter. *Journal of Food Engineering*, 104(3):430 – 437.

Turabi, E., Sumnu, G., and Sahin, S. (2008). Rheological properties and quality of rice cakes formulated with different gums and an emulsifier blend. *Food Hydrocolloids*, 22(2):305 – 312.

und P. Walstra, H. M. (1975). The milk fat globule. emulsion science as applied to milk products and comparable foods. *Food / Nahrung*, 19(4):380–381.

Vankova, N., Tcholakova, S., Denkov, N. D., Ivanov, I. B., Vulchev, V. D., and Danner, T. (2007). Emulsification in turbulent flow: 1. mean and maximum drop diameters in inertial and viscous regimes. *Journal of Colloid and Interface Science*, 312(2):363 – 380.

Walstra, P. (1993). Principles of emulsion formation. *Chemical Engineering Science*, 48(2):333–349.

Walstra, P. and Smulders, P. (1998). Emulsion formation. In Binks, B. P., editor, *Modern Aspects of Emulsion Science*, pages 56–59. The Royal Society of Chemistry, Cambridge, UK, 1st edition edition.

Webb, C. and Owens, G. W. (2003). Milling and flour quality. In Cauvain, S. P., editor, *Breadmaking: Improving Quality*, pages 200–219. Woodhead Publishing.

Wilderjans, E., Luyts, A., Goesaert, H., Brijs, K., and Delcour, J. (2010). A model approach to starch and protein functionality in a pound cake system. *Food Chemistry*, 120(1):44 – 51.

Wootton, J. C., Howard, N. B., Martin, J. B., McOsker, D. E., and Holme, J. (1967). The role of emulsifiers in the incorporation of air into layer cake batter systems. *Cereal Chemistry*, 44:333–343.

Yamamoto, H., Worthington, S. T., Hou, G., and Ng, P. K. W. (1996). Rheological properties and baking qualities of selected soft wheats grown in the united states. *Cereal Chemistry*, 73(2):215–221.

Yang, X., Berry, T. K., and Foegeding, E. A. (2009). Foams prepared from whey protein isolate and egg white protein: 1. physical, microstructural, and interfacial properties. *Journal of food science*, 74(5):E259–E268.

Zghal, M., Scanlon, M., and Sapirstein, H. (1999). Prediction of bread crumb density by digital image analysis. *Cereal Chemistry*, 76(5):734–742.

3 Interfacial and Foaming Characterisation of Protein-Starch Mixtures

Abstract

The investigation in this chapter presents mixed protein-starch systems as effective foaming agents and stabilisers. The starch size and hydrophobicity played a dominant role in determining the nature of synergy between the protein and starch species. Egg White Protein (EWP) and Pea Protein Isolate (PPI) were selected at two concentrations (0.5, 1 wt. %) along with three starch species of concentrations 0.5 - 5wt. %. Two commercial OSA-modified starches were compared to a native granule and its heat-treated counter part. The systems' effectiveness to incorporate air (overrun) as well as their capacity to hold structure (half life) were evaluated. The effect of starch's physical character (contact angle and size) on the properties of the formed Air/Water (A/W) interface (interfacial dilatation rheology, surface tension) was also explored. The study demonstrated that addition of OSA treated starch to (EWP) foams could increase foam stability by up to 1200% without compromising the foaming capacity with observed increase in interfacial dilatational moduli. Where as the larger heat-treated starch granule was found to increase stability of wet foams by 800% through a combination of mechanisms.

Chapter published in *Food Hydrocolloids* ***Asghari, A. K.****, Norton, I., Mills, T., Sadd, P.,& Spyropoulos, F. (2016). Interfacial and foaming characterisation of mixed protein-starch particle systems for food-foam applications. Food Hydrocolloids, 53 , 311-319*

3.1 Introduction

The essential building component of many aerated structures found in products such as cakes, breads and ice creams are protein foams (Davis et al., 2004). The inherent instability of these systems means their use in industrial applications remains difficult. In products where the final quality is largely dependent on the original foam (*e.g.* high-ratio cake formulations) Egg white proteins (EWP) are most consistently used due to a combination of foaming capacity and stability. In such cases the foam is required to withstand considerable stresses during processing and remain aerated (Chesterton et al., 2015). However recent animal welfare and ecological concerns have driven a subsequent effort into finding alternative protein sources that can provide similar functionality in food systems (Damodaran, 1997).

Other species such as whey protein have been studied as potential replacers, however the lack of stability remains a major problem (Foegeding et al., 2002). More recently Pea protein isolate (PPI) has been the subject of some studies. Their non-allergic nature and high nutritional value, make them good candidates for food foaming applications (Gharsallaoui et al., 2009).

Within many food systems proteins are present along with other surface active species. pH and presence of co-solutes have been shown to influence the the nature of the adsorbed protein films at the Air/Water (A/W) interface (Gharsallaoui et al., 2009). Change of pH to the protein isoelectric point (PI), inhibits electro-static and intra-molecular interactions, thus increasing rate of diffusion and adsorption. However these fast forming films were shown to have much lower interfacial elasticities than those formed in an acid or alkaline condition (Gharsallaoui et al., 2009). Moreover the presence of surface active particles such as cellulose and OSA- starches has been shown to improve foam and emulsion stability (Murray et al., 2011). Several pertinent studies have demonstrated that use of particles as stabilisers within foams can yield strong interfacial layers that can retard rates of coalescence and disproportionation. Inorganic particles such as silica

(Binks and Horozov, 2005) and fat crystals (Murray et al., 2011) have been used for stabilisation of foams to good effect.

The stabilisation of food foams by particles has been reviewed by (Murray and Ettelaie, 2004) and more recently by (Dickinson, 2010). The major parameters of influence for the particles are contact angle and surface area, which determine the energy of desorption per particle (Hunter et al., 2008).

Starch in its native form is hydrophilic and is unlikely to adsorb at A/W interface. Hydrophobic modifications of starch is often induced through addition of Octenyl succinic anhydride (OSA) which is approved for food applications at an added amount of up to 3%.
A less process intensive and cost-effective method for increasing the hydrophobicity of starch granules is through heat-treatment (physical modification) (Seguchi, 2001). Dry-heating of starch changed it's surface character from hydrophilic to hydrophobic only by altering the nature of surface proteins (Rayner et al., 2012). Therefore no specific labeling is required for use in food applications.

The interactions of proteins and surface-active polysaccharides/particles within real food systems are of paramount importance as their control and manipulation (associative or dissociative) are key for formulation of novel food products (Damodaran, 1997). Functional properties of proteins such as surface activity, conformational stability, emulsifying and foaming capacity can all be manipulated with presence of polysaccharides, thus modifying the microstructure of the adsorbed layer (Damodaran, 1997; Schmidt et al., 2010). When the polysaccharide contains charged groups, these interactions can be electrostatic in origin (Patino and Pilosof, 2011). However shorter range interactions such as hydrogen bonding can also be present in some cases (Dickinson, 2010).

Studies into stabilisation of foams and emulsions by mixtures of proteins and particles have observed that proteins in the presence of surface-active particles formed more rigid interfacial layers, due to the facilitation of better packing at the interface. This augmentation of the interfacial layer was observed without any evidence of electrostatic interactions between the molecules (Murray et al., 2011). Even non-adsorbing particles can provide additional stability through the process of “Stratification” in the intervening film. As long as the particles are well below the initial radius of the thinning film they can be trapped and structure themselves into layers difficult to remove (Murray and Ettelaie, 2004).

The experiments presented within this chapter were undertaken based on two original hypotheses; (1) The existence of proteins along with surface active starch at pH7 should enhance the stability of wet foams comprising them. (2) The extent of any potential synergy between protein-starch particle systems should depend on protein type and starch physical and surface properties.

The focus of this study was to induce synergy at pH7 where electrostatic interactions between the molecules were restricted, thus seeking novel formulations for producing foams of higher stability but comparable foaming capacity to EWP based foams, which can (partially) replace them.

3.2 Materials and Methods

3.2.1 Materials

Egg white protein from chickens (EWP) and rice starch ($\leq$ 0.1wt. % Protein) were purchased from Sigma-Aldrich (UK). Pea protein isolate (PPI) was obtained from Kerry Ingredients (Listowel, Ireland). The compositions of the proteins used is shown in tab. 3.1. The two OSA-modified starches (commercially available food-grade) referred to as OSA1 & OSA2 were donated by an undisclosed source (for reasons of commercial confidentiality). Both starches were waxy maize derivatives with different levels of esterification (OSA substitution). The water used for all experiments was passed through a double distillation unit (A4000D, Aquatron, UK).

	EWP	PPI
Protein (wt.%)	85	86
Moisture (wt.%)	8.4	7.2
Fat (wt.%)	<0.1	0
Carbohydrate (-)	neg.	pos.
Ash (wt.%)	4.1	4.8
pH (-)	6.3	7.4

Table 3.1: Composition and pH (measured at concentration of 1wt. %) of EWP and PPI.

3.2.2 Starch treatment and characterisation

3.2.2.1 Heat-Treatment

Rice starch was spread out at a thickness of 1-2 *mm* in glass petri dishes and heated in an oven at 120 °C for 150 minutes (Seguchi, 2001; Rayner et al., 2012). Maximum stability of starch stabilised emulsions was reported when heat-treated starch at this optimum time-temperature combination was used by Rayner et al. (2012).

3.2.2.2 Particle size measurements

The sizes of OSA modified starches was measured by dynamic light scattering (DLS) using a Zetasizer Nano Series (Malvern Instruments, UK). The particle size distributions of the rice starch granules was determined by laser diffraction after dispersing the powders in water at a concentration of 1wt% and using a Malvern Mastersizer 2000 with a Hydro SM manual small volume dispersion unit attached (Malvern Instruments, UK). A refractive index (RI) of 1.53 (Bromley and Hopkinson, 2002) was used for the measurement.

3.2.2.3 Microstructure visualisation

Cryogenic scanning electron microscopy (Cryo-SEM; Philips XL30 FEG ESSEM) was used to visualise the microstructure of untreated and heat-treated rice starch in water. One drop of starch dispersion was frozen to approximately -180 °C in liquid nitrogen. Samples were then fractured and etched for 3 min at a temperature of -90 °C inside a preparation chamber. Afterwards samples were sputter coated with gold and scanned,

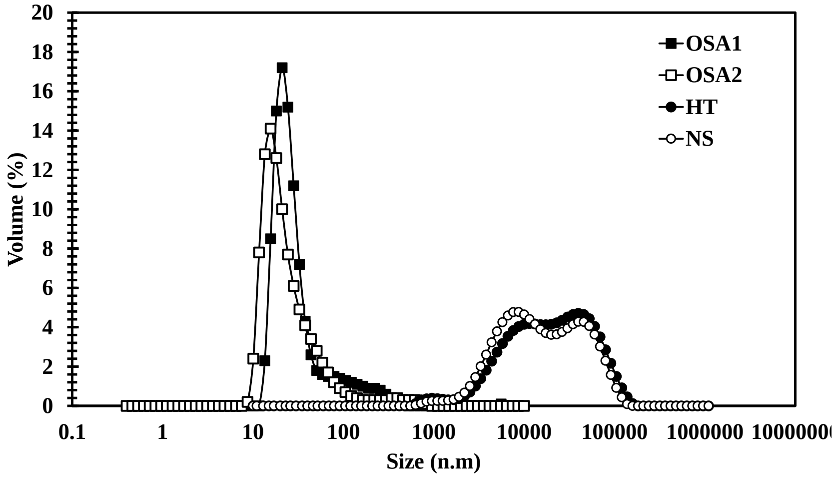


Figure 3.1: Particle size distributions of starch particles. OSA1, OSA2, Heat-treated rice starch (HT) and native rice starch (NS)

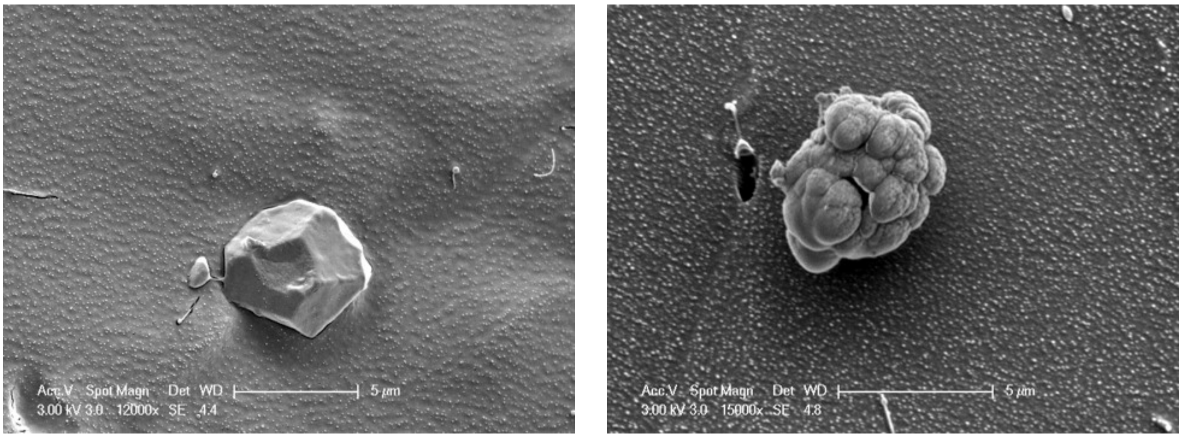
during which the temperature was kept below -160 °C by addition of liquid nitrogen to the system. Fig. 3.2 shows the explicit difference induced by HT on the surface character of the rice starch granule suspended in water.

3.2.2.4 Contact angle (θ) measurements

Contact angle measurements for the particles were undertaken after pelletisation of the starch powders (force$\sim$ 1000 N) so that a smooth surface was obtained for depositing of the water drop. A Goniometer (Krüss instruments, Germany) was used to measure θ by a dynamic sessile drop method. Tab. 3.2 shows the comparison of the contact angles for the three starch species used in this study.

Starch	Size (μm)	Contact Angle (θ)	ζ-Potential (mV)
OSA 1	0.15±0.03	$\sim$ 90±8	$\sim$ -20
OSA 2	0.09±0.01	$\sim$ 65±6	$\sim$ -30
HT	10.8±0.8	$\sim$ 38±7	-
N.S	9.6±0.1	$\sim$ 25±4	-

Table 3.2: Starch particle properties of OSA1, OSA2, Heat-treated rice starch (HT) and native rice starch (NS)



(a) Native (b) H.T

Figure 3.2: Cryo-SEM micrographs showing rice starch (a) untreated and (b) post heat-treatment.

3.2.2.5 ζ-potential measurements

The ζ-potential was determined using a Zetasizer (Malvern Instruments, UK). The ζ-potential of the two OSA-starches were measured after adjusting their pH to 7.0.

3.2.3 pH Adjustment

A SevenEasy pH meter (Mettler Toledo, UK) was used to adjust the dispersions' pH to 7.0 (using NaOH and HCL of 0.5M from Sigma Aldrich, UK) before foaming at a temperature of 20°C. The instrument was calibrated with standard buffer solutions of known pH.

3.2.4 Foam Stability

The biopolymer solutions were whipped using a Hobart mixing unit (Ohio, USA) at a shear rate of $\sim 123s^{-1}$ (maximum speed). The foam volume was then monitored over time using an automated web-cam (Logitech, Switzerland) taking pictures at predetermined 1 minute intervals. The time taken for the foam structure to collapse to half of its original height was derived from the recorded images.

3.2.5 Foaming Capacity

The capacity of the systems to incorporate air was characterised by overrun calculations. The foam generated in the mixing unit was weighing in a standard weight boat (100 ml). Weight measurements were used to calculate the overrun and air-phase fraction (φ_A) of the systems according to eqn.3.1 and eqn.3.2 respectively (Schmidt et al., 2010; Phillips et al., 1987).

$$\%Overrun = \frac{W_l - W_f}{W_f} \tag{3.1}$$

$$\varphi_A = \frac{Overrun}{(Overrun + 100)} \tag{3.2}$$

Where;

W_l is the weight of the pre-foamed solution for 100 ml volume

W_f is the weight of foam for 100 ml volume

3.2.6 Foam Physical Characterisation

Whipping profiles were obtained for all foamed systems prior to measuring their stability in order to ensure that maximum φ_A for each system was achieved and all systems were within the wet foam boundary. This was deemed necessary for an unbiased comparison of foam systems as foam structure is highly dependent on liquid fraction (ϕ_l). Only at ϕ_l>5%, the foam can be described as a wet foam. Above a critical liquid fraction, ϕ_l^* the bubbles no longer touch and the structure transitions into a suspension of bubbles. ϕ_l^*~0.26 for ordered 3D and ~0.36 for disordered 3D foams(Cantat et al., 2013).

3.2.7 Surface Tension

The surface tension between the aqueous phase and air was measured using a tensiometer K100 (Krűss, Germany) with a titanium Wilhelmy plate (length, width and thickness

.
Table 3.3: The maximum air-phase (φ_{max}) fractions achieved for all foaming systems and their respective minimum whipping times taken to achieve them (t_{min})

		EWP		PPI	
Protein Conc (wt%)	**Starch Conc (wt%)**	φ_{max}	T_{min}	φ_{max}	t_{min}
0.5[1]	0	0.82	3	0.84	3
	0.5	0.81	5	0.89	3
	1	0.79	5	0.86	5
	3	0.86	8	0.78	5
	5	0.85	8	0.77	5
1[1]	0	0.84	3	0.87	3
	0.5	0.80	3	0.86	3
	1	0.81	5	0.83	3
	3	0.88	8	0.76	3
	5	0.88	8	0.72	5
0.5[2]	0.5	0.81	3	0.88	3
	1	0.86	3	0.86	8
	3	0.93	5	0.86	8
	5	0.94	5	0.83	5
1[2]	0.5	0.80	3	0.84	5
	1	0.81	3	0.84	5
	3	0.88	3	0.75	8
	5	0.88	5	0.80	8
0.5[3]	1	0.85	5	-	-
	3	0.85	5	-	-
	5	0.84	5	-	-
1 [3]	1	0.82	3	0.8	5
	3	0.81	5	0.79	5
	5	0.79	5	0.81	5

[1] OSA1
[2] OSA2
[3] HT

of 19.9 *mm*, 10 *mm* and 0.2 *mm*, respectively). The plate was immersed in 20 *g* of aqueous phase to a depth of 3 *mm*. The test was conducted over 1000 *s* at 20 °C. The surface tension values and the error bars are reported as the mean and standard deviation, respectively of three replicate measurements.

3.2.8 Interfacial Dialational Rheology

The dynamic air/water (A/W) surface elasticity and viscosity were measured using a pendant-drop Sinterface PAT1 tensiometer (Sinterface, Berlin, Germany). A droplet of the liquid sample with an area of 20 mm^2 was formed automatically at the tip of a syringe driven by a motor plunger within a thermostatically controlled glass cuvette set to 20 °C.

The image of the drop was captured and digitised by a CCD camera. The interfacial tension (A/W) was calculated by analysing the profile of the drop and fitting it to the Laplace equation. After allowing 1000 s for equilibrium to be reached, sinusoidal oscillations of the interface were induced by injecting and extracting a set volume into and from the drop while the response in surface tension was recorded. The relative amplitude (dA/A) of the oscillations was set to 5% in order to stay within the linear viscoelastic region and the frequency ranged from 0.01 to 0.2 Hz while 0.01 Hz was the frequency chosen as the one relevant to foams (Schmitt et al., 2014). The dilatational parameters were calculated through a Fourier transformation algorithm implemented in the software package. The dilatational elasticity and viscosity were calculated from eqn. 3.3 and eqn. 3.4.

$$|E| = A.\frac{\Delta\sigma_{A/W}}{(\Delta A)} = E' + iE'' \tag{3.3}$$

$$\eta_d.\frac{E''}{\omega} \tag{3.4}$$

where A is the area of the drop (mm^2), $\sigma_{A/W}$ the Air/Water interfacial tension (mNm^{-1}), E' the dilatational elasticity (mNm^{-1}), E'' the loss dilatational modulus (mNm^{-1}), η_d the dilatational viscosity ($mNsm^{-1}$) and ω the frequency (Hz).

3.2.9 Statistical Analysis

All measurements are reported as means with one standard deviation derived from three replicates, analysed using MS Excel (Microsoft, Redmond, WA, USA).

3.3 Results and Discussion

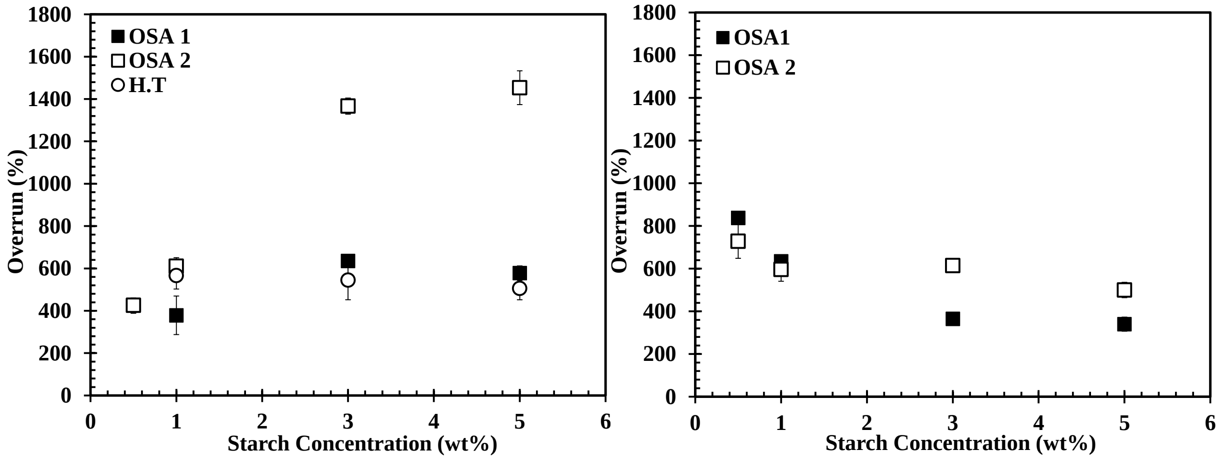
3.3.1 Foaming Capacity of Mixed Protein-Starch Systems

3.3.1.1 OSA Starch Systems

It is observable from tab. 3.3 that increased addition of starch species resulted in longer times taken for φ_{max} to be reached regardless of the protein type used. This was expected for rice starch systems, as associative interactions could reduce firstly the mobility of the proteins and secondly their effective concentration due to potential hydrophobic protein-protein interactions (with de-natured surface proteins of the starch).

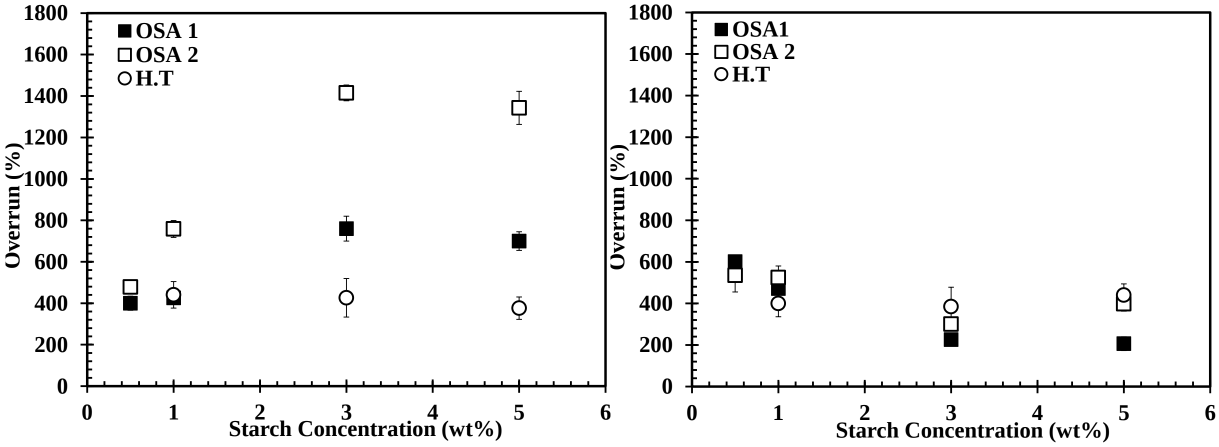
Fig. 3.3 shows the foaming capacity of the systems at different starch-protein ratios. The PPI systems showed little tolerance to the presence of starch and the overruns were compromised compared to the original protein foams (∼650%). EWP foams on the other hand showed no compromise and when combined with OSA2, they observed a three-fold increase compared to their original overruns. The diversity of the proteins that form EWP and the fact that they all serve different functions, means EWP could entrap high volumes of air (overrun from ∼ 550%). Since the quality of the foam is mainly dependent on the protein conformation at the interface, EWP is very effective in partially unfolding its flexible macromolecule, thus observing better amphiphilic properties (Zmudzinski et al., 2014). This property will facilitate easier formation of foams and thus the property that makes EWP such good foaming agents (Yang and Foegeding, 2010; Murray and Ettelaie, 2004). However the pea globulins are more sensitive to beating times, conditions and the presence of co-solutes (this was underlined by the φ_A profiles in table. 3.3. At pH 7.0, PPI was shown to have higher molecular association (*i.e.* the molecular structure is not dissociated) thus significantly reducing its amphiphilic nature (Gharsallaoui et al., 2009).

When the interfacial forces (resistive to bubble break-up) are lowered, the foaming capacity should increase accordingly. Therefore changes in surface tension(σ) induced by the addition of starch at different concentrations are presented in fig. 3.4.



(a) EWP-0.5wt%

(b) PPI-0.5wt%



(c) EWP-1wt%

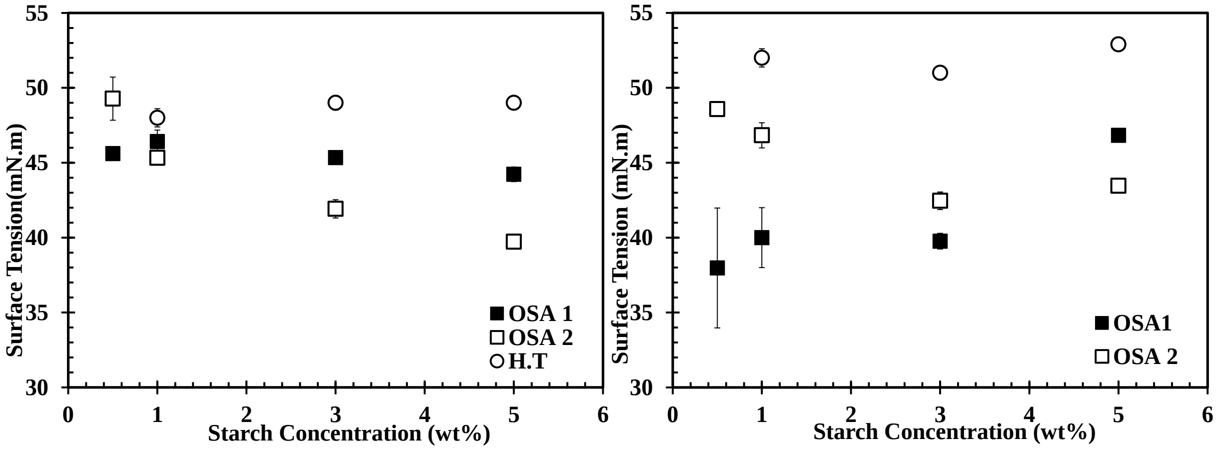
(d) PPI-1wt%

Figure 3.3: The effect of starch concentration on the foaming capacity of protein solutions. a)EWP-0.5wt%, b)PPI-0.5wt%, c)EWP-1wt% & d)PPI-1wt%. Error bars represent one standard deviation derived from three replicates.

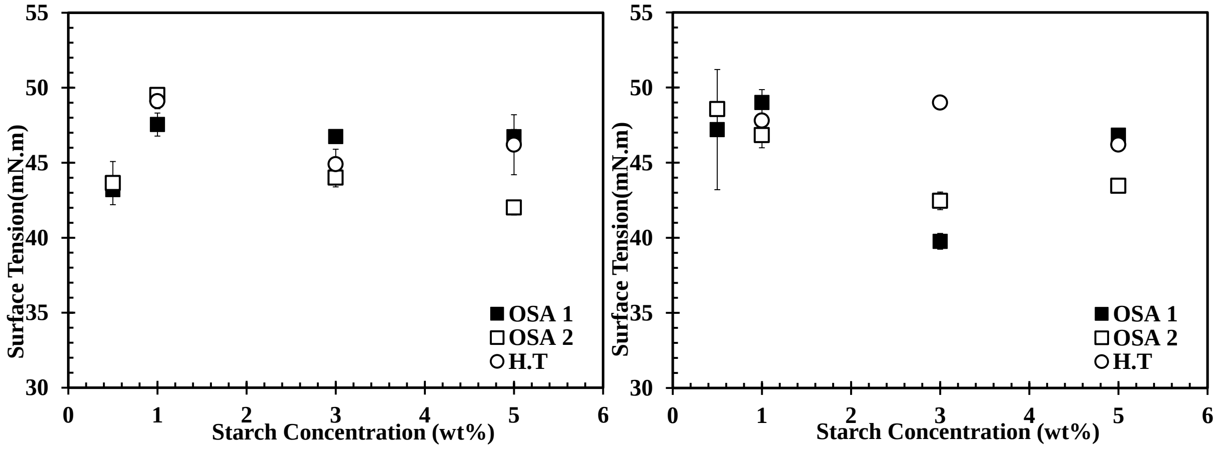
The surface tensions of the original protein solutions were ∼45 & ∼42 mN/m for 0.5 & 1wt.% EWP respectively and ∼47 & ∼46 mN/m for 0.5 & 1wt.% PPI respectively. Fig.3.4 highlights that for both protein systems at 1 % the σ values remained largely unchanged regardless of the starch concentration when combined with OSA1 and HT starches and a minor decrease was observed for OSA2 systems. Indicating that at higher protein concentrations, the starch had little influence on σ. However at 0.5 % EWP there was a marked decrease in σ (*i.e.* increase in OSA2 concentration yielded a reduction in the surface tensions observed), which was in agreement with the overruns observed in Figure.3.3a, where reductions of ∼ 8 mN/m and ∼3 mN/m for 5wt% OSA2 and OSA1 respectively translated to higher overruns displayed by these systems.

It was apparent that the more hydrophobic OSA1 did not increase the overruns for either protein system, whereas OSA2 observed synergy with EWP. The better performance of OSA2 in terms of overrun with EWP systems could be attributed to its level of hydrophobicity. It has been shown that $\theta_{optimum}$ for foams is ∼ 60-70° and above these values the hydrophobicity of the particle could be detrimental to foam formation and stabilisation (Hunter et al., 2008). Tab. 3.2 shows the contact angle of the three starches used within this study. The more suitable contact angle of OSA2, meant that it had the potential to compete with the protein for the interface. Therefore OSA2 could preferentially adsorb at the interface which could explain the results obtained at 1 % EWP where little reduction in σ was observed. According to Naofumi and Etsushiro (1988) the rate of change in σ was more significant than its final value for facilitation of foam formation for studied $\beta-casein$ systems (as presented in Fig 2.12). Thus the smaller starch species with lower diffusion co-efficients could induce quicker reductions in σ resulting in the higher overruns.

For PPI systems both OSA starches reduced the overrun dramatically (by up to ∼ 50%) when present at concentrations of ≥3wt%. The surface tensions corresponding to the PPI systems showed less of a correlation (Fig. 3.4b and 3.4d), although OSA1 and OSA2 systems demonstrated an overall increase in σ with increasing concentration, which



(a) EWP-0.5wt%   (b) PPI-0.5wt%



(c) EWP-1wt%   (d) PPI-1wt%

Figure 3.4: The equilibrium surface tensions of the mixed protein-starch particle systems. (a) EWP-0.5wt%, (b) PPI-0.5wt%, (c) EWP-1wt% & (d) PPI-1wt%.Error bars represent one standard deviation derived from three replicates.

was reflected in the lower overruns for PPI systems (Fig. 3.4b). The poor performance of these systems could be at least in part attributed to the fact that at higher pH above their iso-electric point, pea globulins are not completely dissociated(Gharsallaoui et al., 2009), so that less surface-active groups are less available for adsorption.

3.3.1.2 HT Starch Systems

The presence of HT starch had little effect on the surface tension of the systems at 1 % protein, but a slight increase is observed for 0.5 % protein systems (Fig. 3.4), which was reflected in an overrun compromise of $\sim 20\%$ for EWP systems. Moreover its antagonistic influence was underlined when used in PPI systems which resulted in the production of only a bubbly suspension (below the wet-foam threshold). The reduction in foaming capacity could be due to a number of factors, however it has been reported that polydispersity of particles in wet foam systems can lead to dramatic reductions in foaming capacity (Dickinson, 2015). It was observed with Silica particles, that as the film thickness approaches the size of larger particles, they become pinned, constraining their diffusivity leading to a subsequent osmotic pressure gradient. This gradient will draw water from regions rich in larger bubbles thus thinning the film around the bubbles leading to rupture (Dickinson, 2015). Moreover the non-uniformity of particles surface, has been shown to cause piercing of the air-bubbles thus acting as an anti-foaming agent (Hunter et al., 2008) as indicated by the overrun reductions shown in fig. 3.3. However it was interesting that the EWP systems were less susceptible to these effects and their overruns and T_{min} values remained largely unaffected. The HT starch will form a different mixture with the protein. It's larger surface area and highly branched structure meant that coacervation, phase segregation and even complexation were possible.

3.3.2 Foam Stability of Mixed Protein-Starch Particle Systems (OSA1 and OSA2)

The stability of a given wet foam system is dependent on its air-phase fraction, with more dense foams staying stable for longer. However fig. 3.5 showed that the foam systems analysed throughout this study were all within the wet-foam boundary thus reported stabilities were largely independent of φ_A.

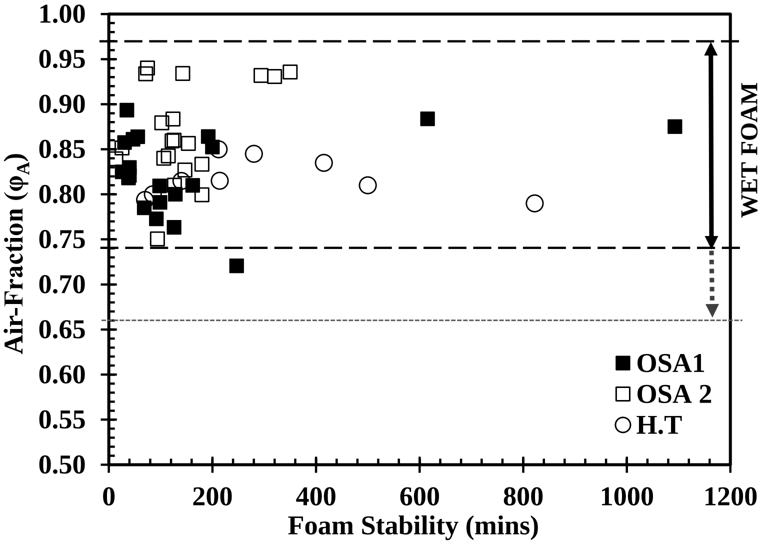
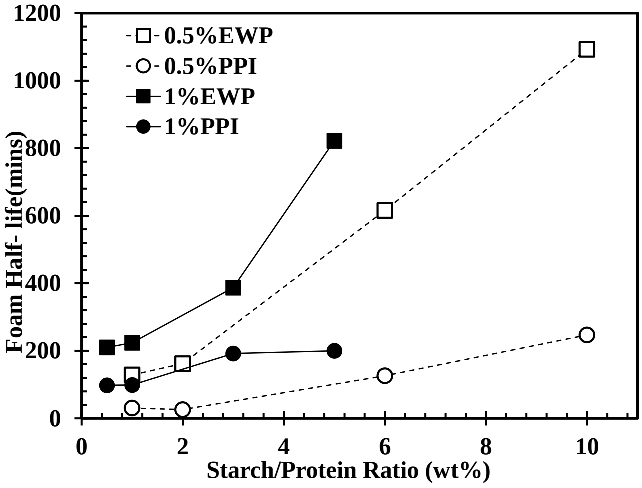
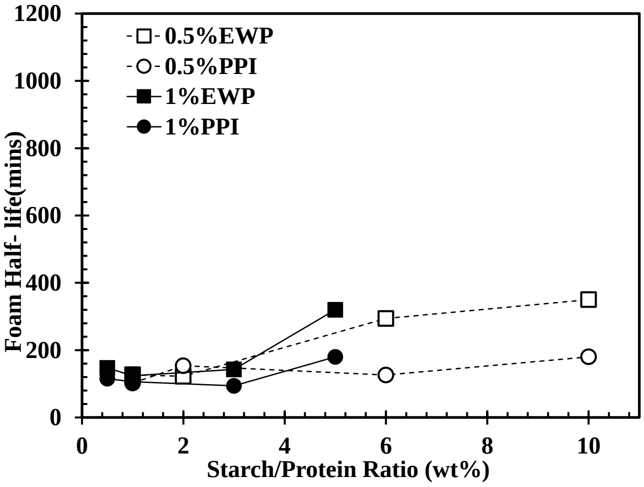


Figure 3.5: The Air-phase fraction of all foamed systems as a function of their stability. All air-phase fractions were within the wet foam boundary.

The capacity of the foamed systems to hold structure and remain stable was characterised by measuring their half-lives, represented in fig. 3.6. It was clear that the stabilities of the systems were highly dependent on the protein type. Without the presence of starch, EWP foams had half-life stabilities of $\sim$ 108 mins and $\sim$ 106 mins for 1wt.% and 0.5wt.% respectively, where as PPI foams were less stable at $\sim$ 75mins and $\sim$ 48mins. As alluded to earlier, the hydrophobic groups of pea globulins at pH $\approx$ 7.0 are not sufficiently exposed thus surface-active groups are less available for adsorption. This potentially implied easier exclusion of PPI from the inetrfacial layers, when combined with surface-active OSA starches. Whereas the major constituent ovalbumin in EWP has been observed to readily



(a) OSA1



(b) OSA2

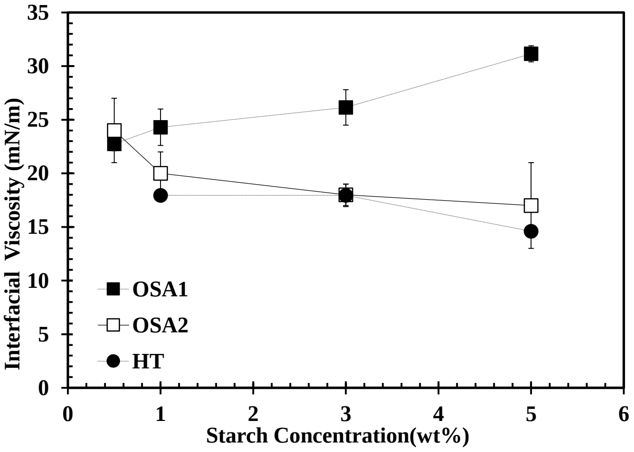
Figure 3.6: The half-life stability of OSA-Protein stabilised foam systems. (a) stability of OSA1-Protein stabilised foam systems & (b) OSA2-Protein stabilised foam systems

coagulate at the interface forming viscoelastic interfacial layers when present on its own (Zmudzinski et al., 2014).

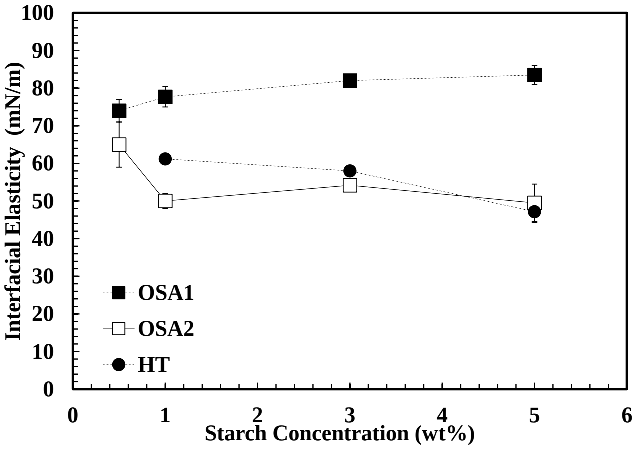
EWP foam stability dramatically increased (twelve fold increase when combined with OSA1) at starch/protein ratios ≥ 3, despite little change in overruns compared to the original EWP foam. However OSA2 starch only enhanced the stability of EWP foams three folds at the same ratios (Fig. 3.6b); a far less significant synergy than the twelve fold increase observed for the more hydrophobic OSA1 (Fig. 3.6a). Since associative interactions between the protein and the starch were minimal at pH 7.0 (Tab. 3.2), the additional increase in stability for OSA1 systems could be attributed to a number of mechanisms.

Firstly as both molecules possess a negative ζ-potential, it could be that the net repulsion at a molecular level causes thermodynamic incompatibility (the system spontaneously separates into two distinct phases), which has been cited as causing synergistic effects (Patino and Pilosof, 2011). This effect would transition from low starch concentrations (*i.e.* where both are intimately mixed and form one-phase solution) to high starch concentrations, where phase separation occurs and a two-phase solution is formed. This would correspond to the trends observed in the half-life data (Fig 3.6a), where at low starch/protein ratios (*i.e.* > 3), the protein and starch co-exist in a single phase and mutually exclude one another. At concentrations below the critical starch/protein ratio, little or no enhancement compared to the original protein system is observed. Therefore within these systems the critical concentration seems to be $\sim$3, above which due to limited thermodynamic compatibility the starch could increase the effective concentration of EWP protein at the interfacial layer.

Moreover the increased stability of OSA1-EWP systems could be due to an induced augmentation of the EWP interfacial layer because of the interactions of the starch with the EWP interfacial layer. Volume exclusion effects have been shown to modify the thermodynamic activity of the protein at the interface (Patino and Pilosof, 2011). Therefore the adsorbed protein film at the interface would become more concentrated, leading to an increase in surface pressure. It is the strength of the interfacial film which provides an energy barrier that prevents the diffusion of gas between different sized bubbles (disproportionation). During disproportionation the bubbles tend to shrink and in order for that to happen the bubbles have to work against the interfacial elasticity and viscosity which can suppress shrinkage (Murray et al., 2011).Therefore if the additional stability was due to an augmented interfacial layer, a corresponding enhancement in the interfacial rheological properties of the formed interfacial layer could be expected. Dilatation moduli of EWP systems (Fig. 3.7), observed an increase in both the elastic modulus (of $\sim$15 mN/m) viscous modulus (of $\sim$10 mNs/m), which at least in part supported the augmentation hypothesis, where molecular crowding could be increasing the effective concentration of



(a) Interfacial Viscosity



(b) Interfacial Elasticity

Figure 3.7: The effect of starch addition on the interfacial dilatational properties; comparison between OSA1 and H.T starch at frequency of 0.1 Hz @ 1% EWP (most stable formulations)

EWP at the interface resulting in a more viscoelastic interfacial layer.

OSA2 systems on the other hand observed lower levels of synergy. Due to an optimum contact angle, it was hypothesised that OSA2 could be competing with the protein for interfacial space. The fact that little change in the foam half-life was observed above ratios of 3 could be an indication that OSA2 was initially dominating at the interface (due to smaller size and optimum contact angle) thus facilitating higher overruns and it is the protein that is excluded. The protein then displaced the OSA2, at which point the excluded particles structured in the lamella and contributed to drainage stability. The decrease in the dilational moduli (Fig. 3.7) was indicative of competitive adsorption between the protein and the starch. However increasing the starch/protein ratio from 1-6 marked an increases of 20 % in half-life stabilities. This could be due to the non-adsorbing OSA2 species structuring in the films (stratification), hence increasing systems stability through retardation of rates of drainage, which was underlined by their drainage performance (discussed later). The mechanism for enhanced stability was unlikely to be reduced rates of disproportionation/coalescence as there was no increase observed in the dilational moduli of the adsorbed films (Fig. 3.7). Therefore the increased stability was likely to be mainly due to stratification. Similar results have been observed for for micellar surfactants (Nikolov and Wasan, 1997), solid silica particles (Binks and Horozov, 2005) and sodium caseinate systems (Dickinson, 2015) where formation of a particle network within the intervening films resulted in notable increases in stability of the foams.

3.3.3 Stability of Heat-Treated Starch Systems

The stabilities of rice starch systems showed better correlation to starch concentration than starch/protein ratio as shown in fig. 3.8. The heat-treated starch due to its much larger size and more hydrophillic nature, was less likely to adsorb at the A/W interface, even though heat has been cited to expose the hydrophobic groups of starch's surface proteins and increase its adsorption capacity (Timgren et al., 2013).

The nature of synergy observed with heat-treated starch was significantly different. HT starch showed no increase in the interfacial dilational moduli. In fact the presence of starch was detrimental to the dilational moduli, indicating that the enhancement in stability was not down to an interfacial effect. Although the size distribution of the HT starch was polydisperse, there was no evidence of any interfacial contribution by its smaller fractions (Fig. 3.7). As the bulk phase viscosity decreases the mobility of the continuous phase around the air bubbles hence reducing the rate of liquid drainage (Yang and Foegeding, 2010), it was deemed necessary for shear viscosities of the pre-foamed solutions to be determined (Fig. 3.9). Half-life stability of the foams showed a weak correlation with shear viscosities for all foam systems studied, however the increase in

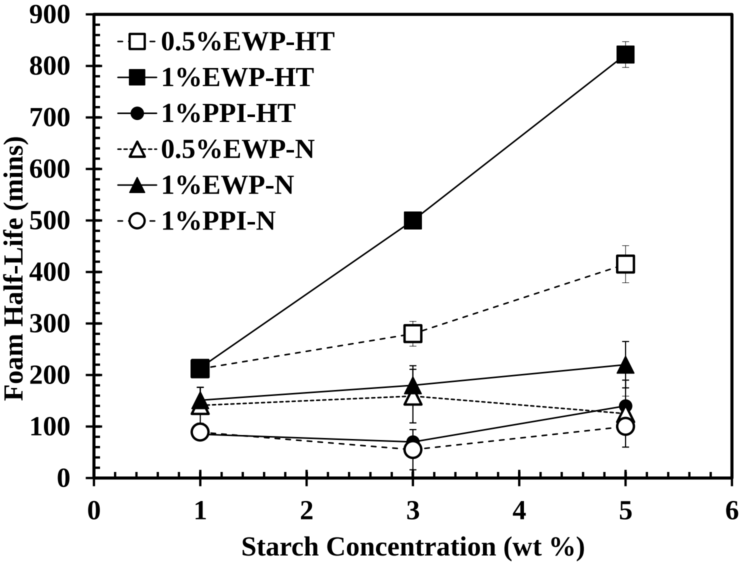


Figure 3.8: Half-life stability of protein-HT starch systems shown as a function of starch concentration.

viscosity was small (up to ~0.6 $Pa.s$) indicating that decrease in the rates of drainage due to bulk phase viscosity effects were not significant enough to describe the dramatic increase in foam stabilities.

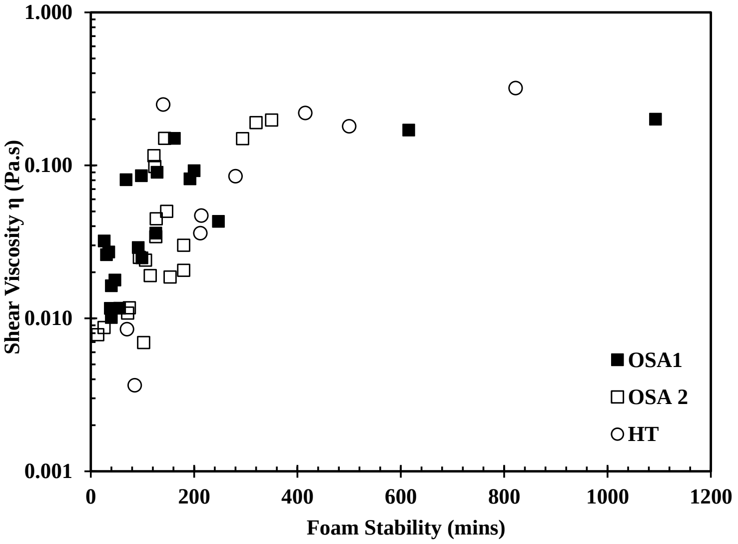
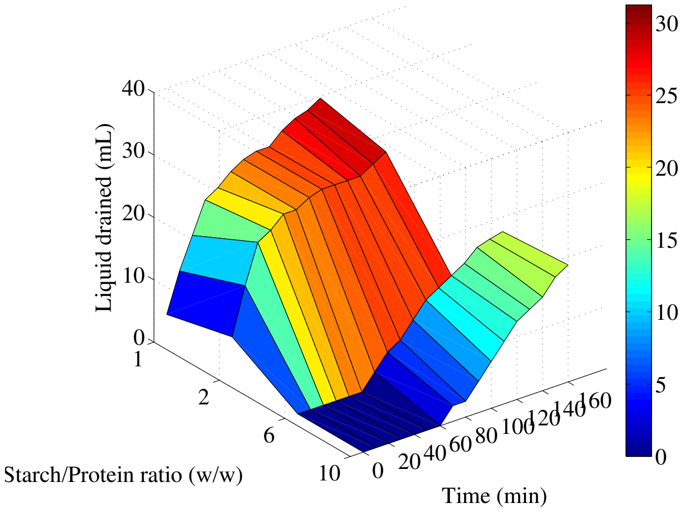


Figure 3.9: Average shear viscosities (at $0.1s^{-1}$) of all foaming systems shown as a function of foam half-life stability

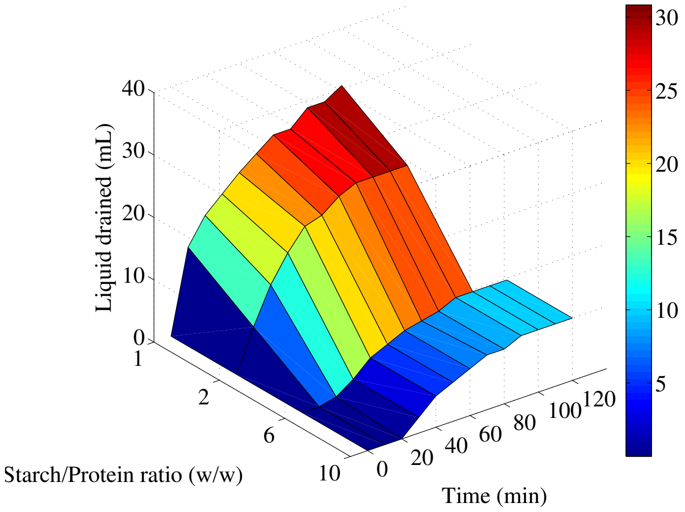
The HT systems had lower overall volumes of liquid drained compared to OSA systems, despite greater initial rate of drainage. This along with no evidence of interfacial participation suggested the HT starch only contributed to the overall structural stability, where HT starch-protein networks induced through protein-protein interactions reduced the overall volume of drained liquid (Fig. 3.10c). Similar results have been observed with large micro-gel particulates formed from whey protein by (Lazidis et al., 2016). They concluded that these large protein particles (up to 150 μm) reduced the rate of local liquid drainage by acting like mobile 'corks' anchored to the A/W interface.

The acquisition of the rate at which liquid drained from the structures was visualised as a function of foam systems' compositions (Fig. 3.10). The drainage behaviour for the two OSA starches (Fig. 3.10a & 3.10b) showed comparable trends. The initial rates and the final volume of liquid drained were significantly reduced as the starch/protein ratio increased. This was reflective of a step-wise thinning of the liquid films ascribed to stratification. It has been observed that the total number of stepwise transitions increase with particle concentration and decrease with increasing particle size (Hunter et al., 2008; Dickinson, 2010; Binks and Horozov, 2005). This was particularly highlighted for the rates of drainage for OSA2 systems (Fig. 3.10b). At ratios $\geq$6, the volume of drained liquid is reduced by $\sim$ 60 %. Although these systems showed higher overruns (*i.e.* lower liquid fractions), the reduction remains a good indication of the dominant stabilising mechanism. As the starch/protein ratio was increased to ratios of $\geq$6, the accumulation and structuring of the non-adsorbing starch could be the responsible mechanism for increased half-life stabilities in EWP-OSA2 systems.

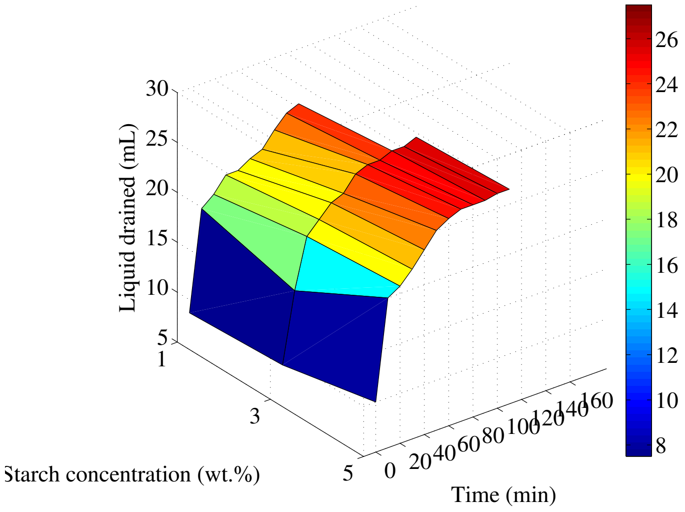
OSA1 systems exhibited similar effects with a reductions of $\sim$ 50 % in drained liquid volume as the starch/protein ratio approached 6. Structural stabilisation by non-adsorbing particles is most effective when the structured particles are close to being mono dispersed (Dickinson, 2015). Thus highlighting the mechanistic difference between the different drainage behaviours of OSA and H.T systems (Fig. 3.1).



(a) OSA1



(b) OSA2



(c) HT

Figure 3.10: The effect of starch concentration on the rates of drainage of (a) OSA1, (b) OSA2 and (c) HT systems.

3.4 Conclusions

It was demonstrated that EWP-starch systems can act as effective stabilisers for wet foam systems. The size and extent of starch's hydrophobicity played an important role in the level and the mechanism of synergy between the protein and the starch. More hydrophobic OSA modified starch, showed significantly higher foam stabilities (up to 12 fold) when above the critical starch/protein ratios (*i.e.* ≥ 3). Increase of interfacial moduli were observed and ascribed to interactions of the starch with the protein interfacial layer. Moreover inhibition of initial rates of drainage were observed, eluding to the fact that more than one mechanism could be responsible for the increased stability.

Less hydrophobic and smaller OSA2, did not exhibit the same levels of synergy with either protein (only increase of $\sim$3-folds with EWP). It was hypothesised that protein eventually displaces OSA2 at the A/W interface, thus excluding it to the foam lamella, where at high concentrations stratification lowered levels of liquid drainage.

Heat-treated rice starch granules which were much larger in size, also showed synergy with EWP for stabilising wet-foams. The nature of synergy was different compared to OSA starches; lower drained liquid volumes were observed, indicating that the dominant stabilising mechanism was likely to be due to film viscosity effects forming a barrier to drainage.

Acquisition of more information on drainage as well as detailed analysis of the thinning and rupture of the aqueous films would be useful for better understanding of the mechanistic factors determining the stability enhancements observed for EWP-starch particle wet foams.

3.5 References

List of References

Binks, B. P. and Horozov, T. S. (2005). Aqueous foams stabilized solely by silica nanoparticles. *Angewandte Chemie*, 117(24):3788–3791.

Bromley, E. and Hopkinson, I. (2002). Confocal microscopy of a dense particle system. *Journal of Colloid and Interface Science*, 245(1):75 – 80.

Cantat, I., Cohen-Addad, S., Elias, F., Graner, F., Höhler, R., and Pitois, O. (2013). *Foams: structure and dynamics*. Oxford University Press.

Chesterton, A., Wilson, D., Sadd, P., and Moggridge, G. (2015). A novel laboratory scale method for studying heat treatment of cake flour. *Journal of Food Engineering*, 144(0):36 – 44.

Damodaran, S. (1997). *Food Proteins and Their Applications*. Food Science and Technology. Taylor & Francis.

Davis, J., Foegeding, E., and Hansen, F. (2004). Electrostatic effects on the yield stress of whey protein isolate foams. *Colloids and Surfaces B: Biointerfaces*, 34(1):13 – 23.

Dickinson, E. (2010). Food emulsions and foams: Stabilization by particles. *Current Opinion in Colloid & Interface Science*, 15(1-2):40 – 49.

Dickinson, E. (2015). Structuring of colloidal particles at interfaces and the relationship to food emulsion and foam stability. *Journal of Colloid and Interface Science*, 449:38 – 45. Liquid Films, Interfaces and Colloidal DispersionsHonoring Professor Darsh T. Wasan.

Foegeding, E., Davis, J. P., Doucet, D., and McGuffey, M. K. (2002). Advances in modifying and understanding whey protein functionality. *Trends in Food Science & Technology*, 13(5):151 – 159.

Gharsallaoui, A., Cases, E., Chambin, O., and Saurel, R. (2009). Interfacial and emulsifying characteristics of acid-treated pea protein. *Food Biophysics*, 4(4):273–280.

Hunter, T. N., Pugh, R. J., Franks, G. V., and Jameson, G. J. (2008). The role of particles in stabilising foams and emulsions. *Advances in Colloid and Interface Science*, 137(2):57 – 81.

Lazidis, A., Hancocks, R., Spyropoulos, F., Kreuß, M., Berrocal, R., and Norton, I. (2016). Whey protein fluid gels for the stabilisation of foams. *Food Hydrocolloids*, 53:209 – 217. Functional Hydrocolloids: A Key to Human Health.

Murray, B. S., Durga, K., Yusoff, A., and Stoyanov, S. D. (2011). Stabilization of foams and emulsions by mixtures of surface active food-grade particles and proteins. *Food Hydrocolloids*, 25(4):627 – 638. Food Colloids 2010: On the Road from Interfaces to Consumers.

Murray, B. S. and Ettelaie, R. (2004). Foam stability: proteins and nanoparticles. *Current Opinion in Colloid & Interface Science*, 9(5):314 – 320.

Naofumi, K. and Etsushiro, D. (1988). Surface tension and foamability of protein and surfactant solutions. *Journal of Food Science*, 53(5):1542–1569.

Nikolov, A. and Wasan, D. (1997). Effects of film size and micellar polydispersity on film stratification. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 128(1-3):243 – 253. A collection of papers presented at the 11th International Symposium on Suryactants in Solution.

Patino, J. M. R. and Pilosof, A. M. (2011). Protein-polysaccharide interactions at fluid interfaces. *Food Hydrocolloids*, 25(8):1925 – 1937. 25 years of Advances in Food Hydrocolloid Research.

Phillips, L. G., Haque, Z., and Kinsella, J. E. (1987). A method for the measurement of foam formation and stability. *Journal of Food Science*, 52(4):1074–1077.

Rayner, M., Sjöö, M., Timgren, A., and Dejmek, P. (2012). Quinoa starch granules as stabilizing particles for production of pickering emulsions. *Faraday discussions*, 158(1):139–155.

Schmidt, I., Novales, B., Boue, F., and Axelos, M. (2010). Foaming properties of protein/pectin electrostatic complexes and foam structure at nanoscale. *Journal of Colloid and Interface Science*, 345(2):316 – 324.

Schmitt, C., Bovay, C., and Rouvet, M. (2014). Bulk self-aggregation drives foam stabilization properties of whey protein microgels. *Food Hydrocolloids*, 42, Part 1(0):139 – 148.

Seguchi, M. (2001). Oil binding ability of chlorinated and heated wheat starch granules and their use in breadmaking and pancake baking. *Starch*, 53(9):408–413.

Timgren, A., Rayner, M., Dejmek, P., Marku, D., and Sjöö, M. (2013). Emulsion stabilizing capacity of intact starch granules modified by heat treatment or octenyl succinic anhydride. *Food Science & Nutrition*, 1(2):157–171.

Yang, X. and Foegeding, E. A. (2010). Effects of sucrose on egg white protein and whey protein isolate foams: Factors determining properties of wet and dry foams (cakes). *Food Hydrocolloids*, 24(2-3):227 – 238.

Zmudzinski, D., Ptaszek, P., Kruk, J., Kaczmarczyk, K., Roznowski, W., Berski, W., Ptaszek, A., and Grzesik, M. (2014). The role of hydrocolloids in mechanical properties of fresh foams based on egg white proteins. *Journal of Food Engineering*, 121:128 – 134.

4 Influence of Heat-treatment on Performance of Wheat Flour in a Model Foam Cake Formulation

Abstract

Two flours, one that was subjected to Heat-Treatment (HT) and one without treatment (WF), were compared in an angel cake formulation in order to evaluate the influence of heat-treatment on batter and final cake structure. The concentration of the egg white protein (EWP) as the emulsifying/foaming component was incrementally decreased and replaced with water in order to observe the effect of HT firstly on the aeration capacity of the batter and secondly on the final cake structure formed. The rheology of angel cake batters all showed $G' > G$" regardless of EWP concentrations with HT flours (HTF) consistently showing lower specific gravities. Higher G' and lower δ values were obtained for HTF compared to the untreated flour. The results supported the hypothesis that heat-treated flours can enhance air entrapment at the mixing stage. The final cake's Young's modulus E increased with reduction in EWP whilst the bulk modulus values observed a marked increase for samples WF. In both cases the values obtained for the HT flours varied considerably less with EWP reduction, compared to the untreated WF samples.

4.1 Introduction

The criteria for cake quality evaluation is generally in terms of a high volume and a low density crumb structure. The occurrence of this is highly dependent on the dynamic events occurring in the batter during mixing and baking to yield a cake with fine, relatively uniform crumb structure (Pernell et al., 2002). Mixing increases the air/water interfacial area which requires stabilisation against mechanical and gravitational stresses (Sumnu and Sahin, 2008). Similarly, during baking the constructive events constituting bubble expansion should dominate over destructive ones that contribute to coalescence and disproportionation. This is mainly achieved by the EWP forming highly elastic interfacial films that allow expansion whilst maintaining stability (Foegeding et al., 2006; Yang and Foegeding, 2010). However it is also in part achieved by the water binding capacity of the flour (mainly starch's absorbing capacity and glutens water-binding role) to yield a more viscous batter restricting bubble movement. Moreover the interspersed starch serves a very important function during baking (Wilderjans et al., 2010). At this stage the starch acts to adsorb the excess water and thus contributes to the viscosity and strength of the baking gel matrix facilitating air-bubble entrapment. Once a maximum cake height is reached, the cake can partially collapse or maintain volume by formation of a network that "sets" the cake (Yang and Foegeding, 2010; Foegeding et al., 2006; Pernell et al., 2002). The individual EWP components such as globulin, ovomucin and ovalbumin fractions all contribute significantly towards cake volume (MacDonnell et al., 1955). For example Johnson and Zabik (1981b) observed that lysozyme contributed to cake volume by limiting the extent of collapse at the latter stages of baking, due to formation of a ovomucin-lysozyme complex thus altering the heat-induced denaturation and aggregation of EWP (Johnson and Zabik, 1981a).

The use of heat-treated flour within high ratio cake production is predominant for improved baking performance. The amount of water absorbed is increased heat treatment of the cake flour (Sumnu and Sahin, 2008), which increases viscosity and helps to trap and subdivide air during baking. Additionally heat-treatment has an explicit denaturing effect on starch surface proteins (Seguchi and Yamada, 1988) facilitating formation of more aerated batters. Therefore the aim of this study is to compare the rheological properties of batters composed of Heat-treated flours (HTF) to batters of same composition made from native wheat flours and understand how the rheology informs the final cake structures after baking.

4.2 Materials and Methods

4.2.1 Materials

Two flours were analysed as part of the study, a commercial non-treated cake flour (8.81-10.87 % moisture, 0.76-0.79 % Ash and 7.95 - 8.81 % protein) and an Industrial Heat-treated Flour followed by partial re-humidification (6.10 - 8.14 % moisture, 0.76-0.79 % Ash and 7.95 - 8.81 % protein) (Premier Foods, UK). Egg white protein (EWP) from chickens was purchased from Sigma Aldrich UK. Sugar was commercially sourced. The water used for all experiments was passed through a double distillation unit (A4000D, Aquatron, UK).

4.2.2 Batter and Cake Preparation

A control sample with 10wt% Egg White Protein (EWP) was used as the reference and the concentration incrementally lowered to 5 % EWP. To produce the cake batter, the ingredients were mixed in a Hobart N50-110 (Hobart UK, London) planetary mixer. The protein solutions were whipped along with 100 *g* of sucrose using the Hobart DWHIP-SST005 Stainless Steel Wire Whip attachment at 259 RPM for 5 minutes. 50 *g* and 2 *g* of flour and baking powder respectively were simultaneously added into the wet foam before another mixing stage at speed 61 RPM, folding the flour into the mixture for 2 minutes until a homogeneous batter was obtained. 90 *g* of cake batter was consequently weighed and baked for 30 minutes at a constant temperature of 180 °C. A trial study was conducted prior to determine the optimum time-baking temperature combination to yield maximum final cake volume.

4.2.3 Flour Particle size

Particle size measurements were made using a Sympatec HELOS/RODOS (Sympatec, Germany) which employs particle laser diffraction. Each flour sample underwent two measurements, successively using an R5 (0.5-875 μm) and R1 (0.18-35 μm) lenses.

4.2.4 Specific Gravity (SG)

The SG of the raw batter was measured with a small cup of known volume. It was determined gravimetrically by dividing the weight of this known volume of batter by the weight of an equal volume of water (Baixauli et al., 2008) as follows;

$$SG = \frac{W_{bat}}{W_{water}} \tag{4.1}$$

4.2.5 Rheological Methods

4.2.5.1 Oscillatory

All rheological measurements were performed using a Kinexus Rheometer (Malvern, UK) with parallel plate geometry. The foam batter was placed onto the lower plate before the upper plate was lowered to give a gap of 2 mm. To determine the rheology of the batter, a frequency sweep (0.1-10 Hz) was performed at a temperature of 25 °C. For comparison of the numerous samples, G' (Storage/elastic Modulus) and δ (phase angle) values were extracted at a standard frequency of 1 Hz.

4.2.6 Cake Macrostructure

The cake structures maintained a uniform shape down to 5 % EWP concentrations. So the height of the cakes was measured as the parameter determining the cake volume. Cake heights were measured after 1 hour at 20 °C. The presented data are mean and standard deviation of two repeats.

4.2.7 Cake Mechanics

Mechanical properties were evaluated through uniaxial compression cycles, (Stable Microsystems, London UK). The tests were performed by using a 40 mm cylindrical aluminium probe, a force trigger of 0.05 N and a test-speed of 1 mm/s to a maximum strain of 50 %. Two main parameters were extracted from the resultant data; (1) The Young's modulus (E) and (2) Bulk Modulus (K). Compression force and change in sample height (strain) were then used to determine the stress (eqn. 4.2) and strain (eqn. 4.3), true stress (eqn. 4.4) and true strain (eqn. 4.5), subsequently the Young's modulus (E) was taken as the gradient of the initial linear region of the true stress strain curve to represent the load range over which the cake may be elastically deformed (*i.e.* return to prior equilibrium position after unloading). The bulk Modulus (K) represented the gradient of the true stress strain curve up to 50 % deformation of the first cycle of the TPA.

$$\delta_E = \frac{F}{A_0} \tag{4.2}$$

$$\epsilon_E = \frac{H_0 - h}{H_0} \tag{4.3}$$

$$\delta_T = \delta_E(1 - \epsilon_E) \tag{4.4}$$

$$\epsilon_H = -ln(1 - \epsilon_E) \tag{4.5}$$

4.3 Results and Discussion

4.3.1 Flour particle Size

The size distribution of the flour greatly influences the volume of the cake, with direct correlations between finer particle sizes and higher final cake volumes reported (Sahi and Alava, 2003).

Fig. 4.1 represents that the particle size distribution (as determined on volume basis) which observes a marked difference between HTF and WF samples. There's an indication of a trimodal distribution which would be in accordance to Meza et al. (2011)'s observation for a similarly sourced flour. There is a shoulder at 3-4 μm, a peak at 20-25 μm and a second peak at around 70-90 μm. It is noticeable that the HT flour has a higher proportion

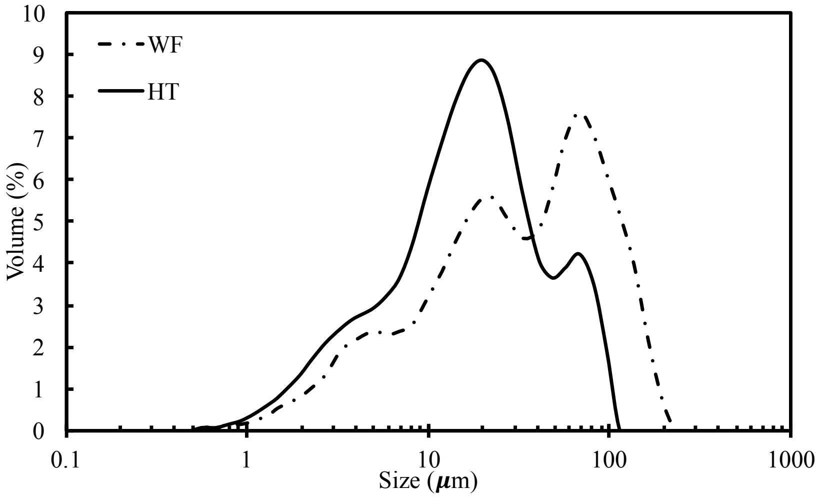


Figure 4.1: The particle size distributions of HTF and WF samples

of finer particles and a lower population of larger particles. This has been attributed to the additional processing HT flour is subjected to as previously reported by Meza et al. (2011). The discrepancy in the results observed here could be down to the method of particle sizing as Meza et al. (2011) used a solvent as a dispersing medium for their laser diffraction technique.

4.3.2 Batter Aeration

The storage modulus G' is a measure of the energy stored in a material subjected to deformation or elasticity of the material. As air incorporation during mixing reduces the specific gravity (SG) of the batter (Sumnu and Sahin, 2008), the results obtained showed good correlation between G' and SG of the batter which was in accordance with Mleko et al. (2007) who observed that G' of EWP foams was linked to the amount of aeration of these systems. The oscillatory behaviour of the batters is shown in fig. 4.2. All batters tested showed similar gel-like bahaviour thus mechanical parameters were taken at 1 Hz for comparison of other systems.

Regardless of flour type, there is a direct correlation between EWP concentration and G' of the batter (Fig. 4.3), contributing to the notion that reducing the amount of EWP is

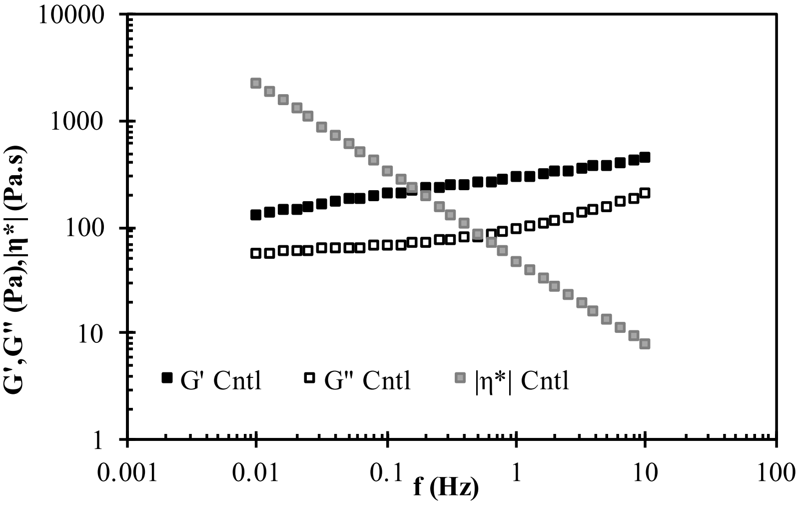
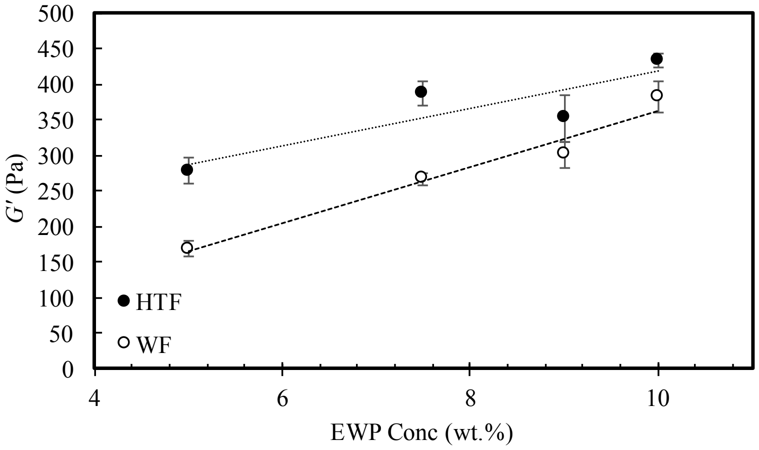


Figure 4.2: Mechanical spectra for the control sample at 10 % EWP concentration.

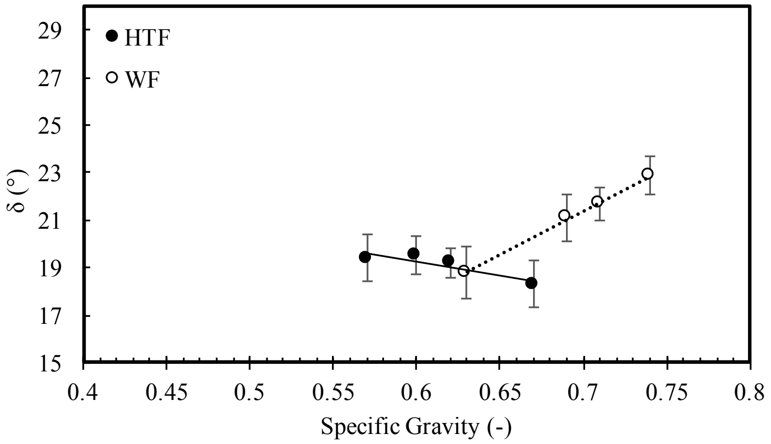
accompanied by a reduced ability of the mixture to incorporate and maintain air (Miller, 2016).

The G' of the HTF batters is shown to be consistently higher at all EWP concentrations. This is shown in fig. 4.3a which highlights the higher capacity of HTF networks to withstand the EWP reduction compared to WF samples which display a more significant drop in the extent of network formation. The results obtained when using HTF thus indicated that the consistently higher G' was suggestive of production of a more aerated, pre-bake structure (Sahi and Alava, 2003; Sakiyan et al., 2004). This is synonymous with the SG measurements depicted in fig. 4.3b. In accordance with previous observations, the SG of the batter gives a strong indication of the amount of air trapped in the pre-bake batter network, thus higher G' values signify the presence of a stronger network hence higher levels of air incorporation (Chaiya and Pongsawatmanit, 2011). The SG of the batters made using the HTF were consistently lower than those of the commercial flour; however lower particle size has also been reported to be inversely proportional to batter aeration capacity (Moiraghi et al., 2013).

Fig. 4.3b reiterates the correlation between viscoelastic behaviour of the batters formed and the specific gravity of the batters (*i.e.* the aeration of the batters). A phase angle above 45° indicates the sample is behaving more like a viscous liquid; while a phase angle below 45° indicates behaviour more like an elastic solid. As the phase angle approaches 0, the sample is exhibiting a stronger elastic behaviour (Pernell et al., 2002). Fig. 4.3b shows that there was little correlation between the phase angle for HTF, however in the case of the WF systems the phase angle is directly proportional to the specific gravity of the batters. The reduction in EWP in systems composed of the WF were accompanied by a transition to more viscous behaviours, whereas batters composed of HTF displayed similar viscoelastic behaviour regardless of the EWP concentrations. Although the G' values decreased with reduction of EWP in batters composed of HT flours, the decrease was proportional between G' and G'', thus not affecting the δ values. Fig. 4.3b highlights that use of HTF can facilitate higher tolerances of the cakes for EWP reduction without compromising the extent of vicoelasticity of the batter.



(a) G' as a function of EWP conc

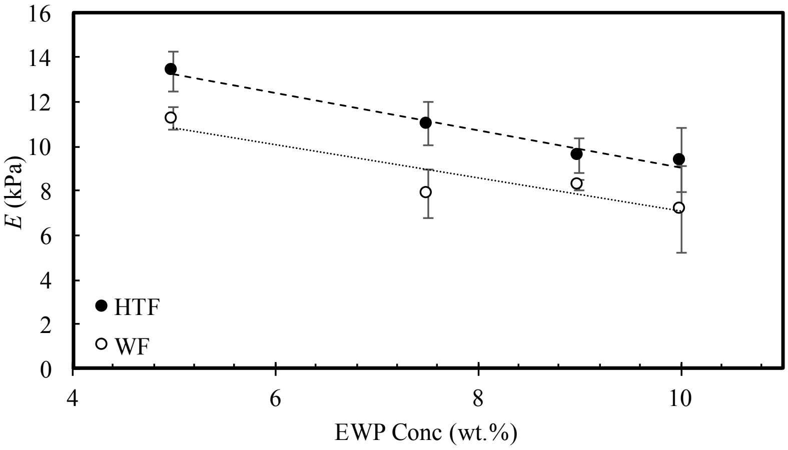


(b) Phase angle δ vs. SG

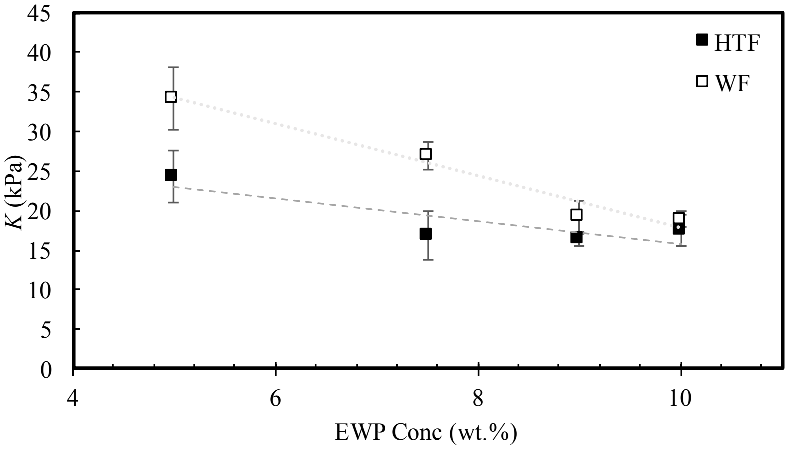
Figure 4.3: The influence of EWP concentration on batter aeration levels and consequent effect on the viscoelastic behaviour of the batters.

4.3.3 Final Cake Texture

Finally, it was found that reducing EWP increased the bulk moduli of the cakes (Fig 4.4). The moduli for WF samples increased sharply, at least in part due to the larger particle size of the flour reducing the cake volumes obtained. By comparison, the HTF produced cakes more capable of retaining air leading to cakes of lower E values. The higher K values for the WF were as a consequence more dense, less aerated structures providing greater resistance to compression. This is in good accordance with the data obtained in tab.4.1. The Young's moduli of the cakes increased at a similar rate with EWP reduction and in all cases EWP concentration was proportional to E. From the data in tab. 4.1 it is apparent that increase in cake height was directly responsible for the marked differences observed in the E and K values. This is in agreement to the model proposed by Gibson and Ashby (1987) for cellular solids, which has been used for breads by Zghal et al. (2002).



(a) Young's Modulus (E)



(b) Bulk Modulus (K)

Figure 4.4: The Mechanical properties of the final cakes at different EWP concentrations for HTF and WF flours. (a) The Young's Moduli (b) Bulk Moduli of final cakes.

The effect of flour particle size on cake structure has been shown to negatively influence the final volume of cakes (Yamamoto et al., 1996; Gaines and Donelson, 1985; Chaudhary et al., 1981). However, the amount of starch damage due to additional processing also influences the final cakes volumes and textures obtained (Sahi and Alava, 2003). Moreover, it has been demonstrated that the additional post-milling processes (*i.e.* pin-milling) can

Table 4.1: The heights of the cakes as a function of the EWP concentration and the flour type used.

EWP Conc (wt.%)	Cake Height (mm)	
	WF	HTF
10	24±0.5	27±1.0
9	21.5±2.0	26±1.5
7.5	20.5±0.5	22.5±1.0
5	16.5±0.5	19.5±0.5

also contribute to better final cake volume and texture (Chaudhary et al., 1981).

4.4 Conclusions

It was shown that batters made with HTF all generated higher level of network formation which were less dependent on EWP concentrations. G' values for batters correlated well to the SG's calculated. The elastic moduli of the WF batters was highly dependent on the EWP content which also had a direct influence on the δ values. This signified a more liquid-like response for WF batters, while HTF showed little dependency in their phase behaviour at any EWP concentration. These effects were attributed to a combination of more aerated batters during mixing which translated into final cake structures with more consistent E and K values at all EWP concentrations. Moreover, the cakes made from WF, all exhibited a significant increase in K values with EWP reduction. The absorption capacity of starch subjected to HT is reported to increase (Sumnu and Sahin, 2008). The final baked cake quality is highly dependent on baking viscosity and thus capacity of the batter to avoid water vapour escape and phase separation due to buoyancy effects. Therefore the effect of HT on the water absorption capacity of the system as well as the specific influence of HT on individual flour components namely the starch requires a more systematic investigation.

4.5 References

List of References

Baixauli, R., Sanz, T., Salvador, A., and Fiszman, S. (2008). Muffins with resistant starch: Baking performance in relation to the rheological properties of the batter. *Journal of Cereal Science*, 47(3):502 – 509.

Chaiya, B. and Pongsawatmanit, R. (2011). Quality of batter and sponge cake prepared from wheat-tapioca flour blends. *Kasetsart J (Nat Sci)*, 45(45):305–313.

Chaudhary, V. K., Yamazaki, W. T., , and Gould, W. A. (1981). Relation of cultivar and flour particle size distribution to cake volume. *Cereal Chemistry*, 58(4):314–317.

Foegeding, E. A., Luck, P., and Davis, J. (2006). Factors determining the physical properties of protein foams. *Food Hydrocolloids*, 20(2-3):284 – 292. 7th International Hydrocolloids Conference.

Gaines, C. S. and Donelson, J. R. (1985). Effect of varying flour protein content on angel food and high-ratio white layer cake size and tenderness. *Cereal Chem*, 62:63–66.

Gibson, L. J. and Ashby, M. F. (1987). *Cellular Solids; Structure and properties.* Pergamon Press.

Johnson, T. M. and Zabik, M. E. (1981a). Egg albumen proteins interactions in an angel food cake system. *Journal of Food Science*, 46(4):1231–1236.

Johnson, T. M. and Zabik, M. E. (1981b). Response surface methodology for analysis of protein interactions in angel food cakes. *Journal of Food Science*, 46(4):1226–1230.

MacDonnell, L., Feeney, R., Hanson, H., Campbell, A., and Sugihara, T. (1955). The functional properties of the egg white proteins. *Food Technology*, 9:49–53.

Meza, B. E., Chesterton, A. K., Verdini, R. A., Rubiolo, A. C., Sadd, P. A., Moggridge, G. D., and Wilson, D. I. (2011). Rheological characterisation of cake batters generated by planetary mixing: Comparison between untreated and heat-treated wheat flours. *Journal of Food Engineering*, 104(4):592 – 602.

Miller, R. (2016). Cakes: Types of cakes. In Caballero, B., Finglas, P. M., and Toldrá, F., editors, *Encyclopedia of Food and Health*, pages 579 – 582. Academic Press, Oxford.

Mleko, S., Kristinsson, H., Liang, Y., and Gustaw, W. (2007). Rheological properties of foams generated from egg albumin after ph treatment. *LWT-Food Science and Technology*, 40(5):908–914.

Moiraghi, M., de la Hera, E., Pérez, G. T., and Gómez, M. (2013). Effect of wheat flour characteristics on sponge cake quality. *Journal of the Science of Food and Agriculture*, 93(3):542–549.

Pernell, C., Luck, P., AllenFoegeding, E., and Daubert, C. (2002). Heat-induced changes in angel food cakes containing egg-white protein or whey protein isolate. *Journal of Food Science*, 67(8):2945–2951.

Sahi, S. and Alava, J. M. (2003). *Sponge Cake batters*, chapter 5, Cake Batter Rheology. CRC Press.

Sakiyan, O., Sumnu, G., Sahin, S., and Bayram, G. (2004). Influence of fat content and emulsifier type on the rheological properties of cake batter. *European Food Research and Technology*, 219(6):635–638.

Seguchi, M. and Yamada, Y. (1988). Hydrophobic character of heat-treated wheat starch. *Cereal Chemistry*, 4(65):375–376.

Sumnu, S. and Sahin, S. (2008). *Food Engineering Aspects of Baking Sweet Goods*. Contemporary Food Engineering. CRC Press.

Wilderjans, E., Luyts, A., Goesaert, H., Brijs, K., and Delcour, J. (2010). A model approach to starch and protein functionality in a pound cake system. *Food Chemistry*, 120(1):44 – 51.

Yamamoto, H., Worthington, S. T., Hou, G., and Ng, P. K. W. (1996). Rheological properties and baking qualities of selected soft wheats grown in the united states. *Cereal Chemistry*, 73(2):215–221.

Yang, X. and Foegeding, E. A. (2010). Effects of sucrose on egg white protein and whey protein isolate foams: Factors determining properties of wet and dry foams (cakes). *Food Hydrocolloids*, 24(2-3):227 – 238.

Zghal, M., Scanlon, M., and Sapirstein, H. (2002). Cellular structure of bread crumb and its influence on mechanical properties. *Journal of Cereal Science*, 36(2):167 – 176.

5 Evaluation of Quinoa Starch Performance in a Model Foam Cake Formulation

Abstract

The performance of quinoa starch was compared to wheat starch in a model angel cake system. The effect of dry heat-treatment on the performance of the starch during batter formation and on the final cake structure and mechanics was evaluated. Quinoa and wheat were compared through progressive substitution with a commercial heat-treated (HT) flour (starch:flour ratios, 0.4, 0.6 & 1). Two different egg white protein (EWP) concentrations (5 & 10 wt.%) were used to study the influence of free water content on starch performance. The rheological properties of the batters studied at ambient temperature showed significant differences between HT and native fractions (at 100wt.% starch substitution levels). The strength of starch-EWP gels formed at 90 and 100°C was higher for wheat, and stronger gels were formed upon heat-treatment of wheat, while quinoa performance remained unaffected by heat treatment. The final cake volumes increased when substituting the flour with up to 60 wt. % quinoa or Wheat starch. It was possible to achieve ~30 % increase in porosity of cake formulations through flour dilution with starch, which could facilitate reduction of EWP by 50wt.% whilst maintaining comparable cake volume and quality. Quinoa starch showed comparable performance to wheat only at higher EWP concentrations.

5.1 Introduction

Cakes comprise a significant portion of the snack market, with their sales in the UK increasing by over 2.5 % annually. Snack consumption behaviour is changing and demands for healthier formulations with nutritious ingredients -including ancient grains, demonstrate opportunities for innovation (Mintel, 2016). However cake research remains empirical and in many cases lacks basic insight (Cauvain and Young, 2006). Cakes are macro-porous systems with great diversity and complexity in their formulations. Examples of simple cake systems are Angel cakes which are without egg yolks and oil, making them low fat, low calorie dessert options (Sumnu and Sahin, 2008).

Angel cakes mainly rely on egg white protein (EWP) for their structure and texture. Thus the flour used in angel food cakes is usually weak wheat flour (*i.e.* flour with low protein content of ∼4 % compared to commercial cake flours of ∼8-11 %) and often wheat starch is added to dilute the flour. This simplicity in the formulation makes them ideal systems for studying starch and protein functionality. The function of flour in angel food cake is completely different than in almost all other bakery goods; in general, the gluten fraction is of great importance for the final structure and quality of baked products (Cauvain and Young, 2006), while the starch is the most significant fraction of the flour with regards to angel cakes (Miller, 2016). The starch gelatinisation dries the foam by absorbing the available water, causing the structure to set during baking (Palav, 2016; Delcour et al., 2009; Sumnu and Sahin, 2008). Furthermore during the baking stage, the air bubbles expand and EWP coagulate around them, giving permanence to the foam structure. If EWP are under-foamed the volume and texture of the finished product will be negatively affected. On the other hand, over-foamed EWP lack elasticity and cannot expand properly when heated. Due to the production routes of these cakes, they are often referred to as foam type cakes and are made in two general steps; 1. the entrapment of the air into the EWP and sugar by mechanical agitation, 2. the folding of the flour into the mixture (Palav, 2016). Furthermore the high sugar incorporation usually required increases the stress put on the structure building components (EWP and starch) (Miller, 2016).

Rheology is the prevalent characterisation tool for the batter stage. Several works have also reported improved cake volume with reduction of flour particle size (Moiraghi et al., 2013; Sumnu and Sahin, 2008). Viscoelastic properties of the batters can also elucidate the extent of network formation and is generally a function of formulation and temperature (Sanz et al., 2005; Xue and Ngadi, 2007). Starch gelatinisation is a fundamental phenomena during baking and it informs the final textural and structural characteristics (Delcour et al., 2009). According to Donovan (1977), starch granules serve two major simultaneous functions during angel cake baking: they swell to form the 'building bricks' of the final crumb and in doing so bind to the excess water. Starch hence acts as a temperature-triggered water-sink in cake and is responsible for the transformation of an aqueous fluid batter into a solid porous cake structure, as a consequence, the degree of cross-linking of the amylopectin, the amount of damaged starch, amylose and amylopectin present are also considered important parameters for determining starch performance (Delcour et al., 2009).

The gelatinisation temperature depends upon the botanical origin of the starch, the amount of water present, pH and concentration of sugar (Delcour et al., 2009). Starch gelatinisation is also influenced by the presence of EWP as they compete with starch for the available water. Mohamed and Rayas-Duarte (2003) proved that protein extract and gluten interact differently with starch and influence its gelatinisation parameters and water evaporation. Recently, Wilderjans et al. (2010) used a model approach to explore starch and protein functionality in a pound cake system. They concluded that the combination of a protein network formed during baking, and the starch gel formed during cooling, make up the crumb cell walls and determine cake quality.

Heat-treatment is seen as an effective substitute for chlorination, whereby the performance of the flour is enhanced. Seguchi and Yamada (1988) have hypothesised about exposure and denaturation of surface proteins and their contribution at the air interfaces created during mixing. Meza et al. (2011) investigated the effect of heat-treatment of flours by recreating the conditions for commercial heat-treatment and reported a difference in rheological development between base and heat-treated flours which they related to baking performance. Ozawa et al. (2009) have reported a decrease in the onset gelatinisation temperature of heat-treated flours as well as higher peak viscosities. However the changes observed have been attributed to the gluten fraction. They have also observed that at temperatures close to the gelatinisation point of the starch, significant differences are observed. However aside from the aforementioned cake research, (Rayner et al., 2012) have observed a significant difference in adsorption capacity at the air/water (A/W) interface, both upon heat-treatment of starch and also in reducing its size. In agreement with these authors Asghari et al. (2016) also observed a significant increase in foam stability upon heat-treatment of the starch particles used in conjunction with proteins.

Quinoa presents a highly nutritious and versatile alternative to wheat. Its gelatinisation point is similar to that of wheat's, whilst its small and mono-dispersed granules can aid foam formation and air-retention during processing. Additionally quinoa has been reported to have high water binding capacity and better swelling performance than wheat (Lorenz, 1990).

As a consequence quinoa can enhance air incorporation and retention due to its small and mono-dispersed granules, while heat-treatment could facilitate improved air-retention and strengthen the gel structures formed during baking. Therefore the aims of this investigation are to study Quinoa starch's potential as an alternative to wheat, and the effect of dry heat-treatment on its performance.

5.2 Materials and Methods

5.2.1 Materials

Quinoa grain was purchased from Whole Foods Online Ltd (UK) and the starch was extracted according to the method proposed by Rayner et al. (2012) (refer to Appendix A for details of procedure). A white soft flour with typical $\sim$ 9.4 % protein produced from milling a blend of wheats, followed by heat treatment and partial re-humidification (6.10 - 8.14 % moisture, 0.76-0.79% Ash and 7.95 - 8.81 % protein) was provided by Premier Foods (UK). Egg white protein (EWP) from chickens and wheat starch ($\leq$ 0.1

wt. % protein) were supplied by Sigma-Aldrich (UK). (Tab.5.1). The water used for all experiments was passed through a double distillation unit (A4000D, Aquatron, UK).

Table 5.1: Composition and pH (measured at concentration of 1wt.%) of EWP

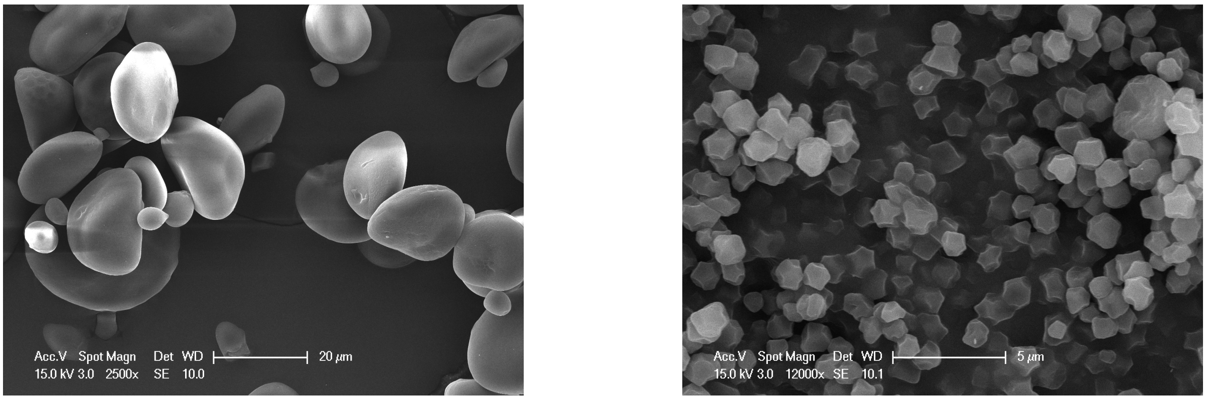
Content	Amount (wt.%)
Protein	85
Moisture	8.4
Fat	<0.1
Carbohydrate	neg.
Ash	4.1
pH	6.3

5.2.1.1 Starch Heat-treatment

Wheat and Quinoa starch were spread out at a thickness of 1-2 mm in glass petri dishes and heated in an oven at 120 °C for 150 minutes (Seguchi and Yamada, 1988; Rayner et al., 2012).

5.2.1.2 Particle Visualisation - Scnning Electron Microscopy (SEM)

For SEM imaging, starch samples in their native and heat-treated forms were sprinkled on double-sided adhesive tape mounted on an aluminium stub. Then they were coated with a thin gold film using sputter coater Emitech K550X (Quorum Technologies Ltd. UK) and observed in a Vega2 microscope (Tescan, Czech Republic) at an accelerating potential of 20 kV.



(a) Wheat starch (b) Quinoa starch

Figure 5.1: Topography of (a) wheat starch and (b) quinoa starch topography observed through SEM imaging.

Table 5.2: Starch properties : granule size, peak gelatinisation temperature (G.T) and amylose (AM) content.

Source	Size (μm)	G.T (°C)	Amylose(%)
Wheat	1-10 & 15-40	58-62	17-29
Quinoa	0.6-2	59-64	9-11

5.2.2 Batter and Cake Preparation

The ingredients for the model angel cake formulation are listed in tab. 5.3. Through progressive dilution/replacement of wheat flour with different amounts of quinoa and wheat starch (40, 60 & 100 wt. %), a comparison between these two granules was highlighted in order to evaluate the overall performance. To ensure that the final formulations had the same moisture content, the amount water was varied according to the moisture of the starch component used. The EWP was beaten in the first stage for 5 minutes, then the sucrose was added and beaten for a further 2 minutes and lastly the dry ingredients (i.e.flour-starch blend & baking powder) were mixed in using low shear. Subsequently 90 grams of the batter was transfered to a baking tin and baked for 30 minutes at 180 °C.

Table 5.3: Model Angel cake formulation for batter preparation

Ingredient	Conc (Weight %)	Weight (g)
Flour	20	50
Sugar	40	100
EWP	4	10
Baking Powder	0.4	1
Water	35-38	82-95

5.2.3 Specific Gravity (SG) Measurements

The SG of the raw batter was measured with a small cup of known volume. It was determined gravimetrically by dividing the weight of this known volume of batter by the weight of an equal volume of water (Baixauli et al., 2008) as follows;

$$SG = \frac{W_{bat}}{W_{water}} \tag{5.1}$$

All measurements were made in duplicate.

5.2.4 Rheological Measurements

Rheological measurements were performed on a Kinexus controlled stress rheometer (Malvern Instruments, London, UK) using parallel plates (40 mm diameter and 1.5 mm gap). A thin film of silicone oil (1 Pa s) was applied to the exposed sample edges to prevent water loss. The temperature was controlled by a peltier system with a sensitivity of ± 0.2 °C. After loading, each sample was held for 3 minutes before testing to allow stress relaxation and temperature equilibration. All measurements were made in duplicate at 25 °C.

5.2.4.1 Steady Shear Rheology

The steady shear behaviour of the batters was studied at 25 °C. The apparent viscosity, η_{app}, was determined as function of shear rate, $\dot{\gamma}$, over the range of 0.01 to 10 s^{-1}. In order to obtain steady-state conditions, samples were sheared for 5 seconds at each value of $\dot{\gamma}$. The shear rate dependency with η_{app} was fitted to the power-law relationship;

$$\eta_{app} = K\dot{\gamma}^{(1-n)} \tag{5.2}$$

where η_{app} is the apparent viscosity, k is the consistency index, $\dot{\gamma}$ is the shear rate and n is the flow index.

5.2.4.2 Dynamic Oscillatory Rheometry

The viscoelastic behaviour was studied for batters at 25 °C within the linear viscoelastic region. The elastic modulus G', viscous modulus G'', complex modulus, G* and complex viscosity, η^*, were determined in the linear viscoelastic region at a fixed stress amplitude of 0.05 Pa at the highest frequency of 10 Hz.

5.2.4.3 Gel Strength

The frequency dependency of cake batters at 90 °C and 100 °C were fitted to the weak gel model for foods proposed by Gabriele et al. (2001). The model describes the structure of the food material as a cooperative arrangement of flow units, with interacting strands. Thus the complex modulus is expressed as;

$$\mid G^* \mid = \sqrt{(G'^2) + (G''^2)} = A_F \omega^{1/z} \tag{5.3}$$

where ω is the angular frequency, z is the interaction factor describing the number of flow units interacting with each other within the three-dimensional network, and A_F is gel strength, and therefore can be interpreted as the strength of the interaction between the flow units.

5.2.5 Final Cake Characterisation

The cake macrostructure was analysed at room temperature (after cooling at 20 °C) using a digital camera (Canon, Tokyo, Japan). Subsequently squares of 3x3 cm were cut from the middle of the cakes using an electrical carver (Cookworks, Swansea, UK).

5.2.5.1 Texture Analysis

The cake's mechanical properties were evaluated using a double compression cycle, commonly referred to as Texture profile analysis (TPA) (Stable Micro Systems London UK). The tests were performed by using a 40 mm cylindrical aluminium probe, a force trigger of 0.05 N and a test-speed of 1 mm/s. Two main parameters were extracted from the resultant data; 1. The Young's modulus (E), 2. Hardness (defined as the maximum force required to compress the cake slices to 50 % strain). Compression force (F), change in sample height ($H_0 - h$), and sample area in contact with the probe (A_0) were then used to determine the stress (eqn.5.4) and strain (eqn.5.5), true stress (eqn.5.6) and true strain (eqn.5.7) and subsequently the Young's modulus (eqn.5.8).

$$\delta_E = \frac{F}{A_0} \tag{5.4}$$

$$\epsilon_E = \frac{H_0 - h}{H_0} \tag{5.5}$$

$$\delta_T = \delta_E(1 - \epsilon_E) \tag{5.6}$$

$$\epsilon_H = -ln(1 - \epsilon_E) \tag{5.7}$$

$$E = \frac{\delta_T}{\epsilon_H} \tag{5.8}$$

5.2.5.2 Cake Sample Preparation

Cylinders of 2 cm in diameter were drilled out from the middle of the cake using a hole-saw (Bosch cordless drill, Germany). The structural parameters of the cake samples measured were average of 3D structural analysis from two separate batches. The principles and procedures for image acquisition and processing for porosity analysis using X-ray micro-CT by image reconstruction softwares were adapted from Mousavi et al. (2007) and Tan et al. (2016).

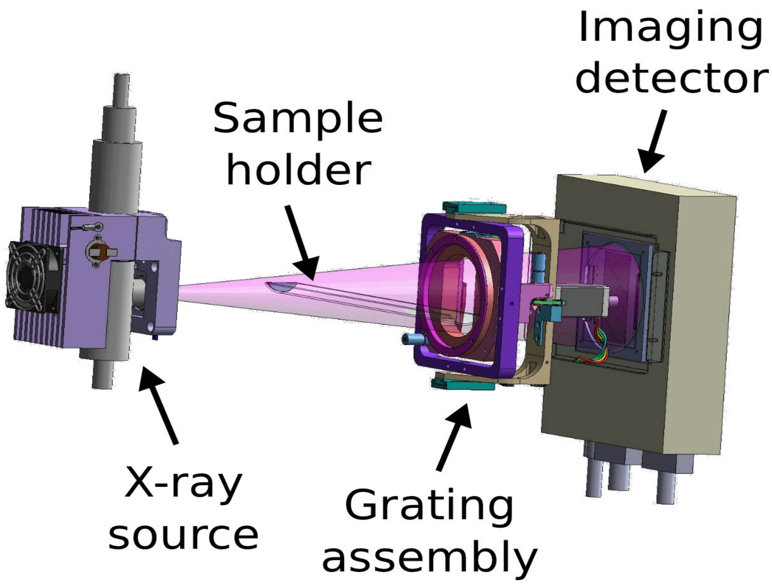


Figure 5.2: The basic principles of operation for Micro-CT scanning. Adapted from (MedPhysics, 2012).

5.2.5.3 X-ray Micro-CT Principles

Micro-CT is a combination of X-ray microscopy and tomographical algorithms. Micro-CT consists of passing X-rays through an object, and the object under study is then rotated inside the X-ray chamber to be viewed from different angles (Fig. 5.2 shows the basic operation). A computer records each of the projected images and measures the intensity of the X-ray beam after it passes through the material (*i.e.* phase contrast imaging due to varying attenuation of x-rays). Once the initial data (shadow images) are obtained, special image reconstruction is accomplished via a filtered back-projection algorithm utilising cone-beam reconstruction.

The reconstruction results in a set of 2D cross-sectional images, or slices, for the entire sample, which thereby reveals the internal microstructure of the sample. These slices are separated by a distance equal to the pixel size of the system (in this case 20 μm). A 3D model of the object can be then obtained by layering all the horizontal 2D slices one on top of another. The cake samples were scanned using a high resolution Skyscan 1172 desktop microCT system, which consisted of a microfocus sealed X-ray tube with a spot size of 10 μm capable of operating at a voltage of 100 kV and current of 200 μA. The X-ray detector consisted of a 1024 x 1024 pixel 12-bit digital cooled CCD camera connected to a computer. For microtomographical reconstruction, X-ray images were acquired from 200 rotational views through 180 degrees of rotation. The scanning process was controlled by a Skyscan software package via a computer, which also allowed reconstruction. The software package also included image processing and analysis facilities, stereo-visualisation and 3D visualisation with a facility to rotate and cut the image of the object on-screen.

5.2.5.4 X-ray Image Analysis For Obtaining Structural Parameters

For image analysis after the reconstruction of the 2D cross-sectional images by in built software N-Recon, c-Tan software was used to first find the threshold for binerisation of the 2D images (here is the limitations of the technique and why it can only be used for relative comparison rather than finding of absolute structural values, however for consistency, the binerisation process was automated (using Otsu algorithm as seen in fig. 5.3)).

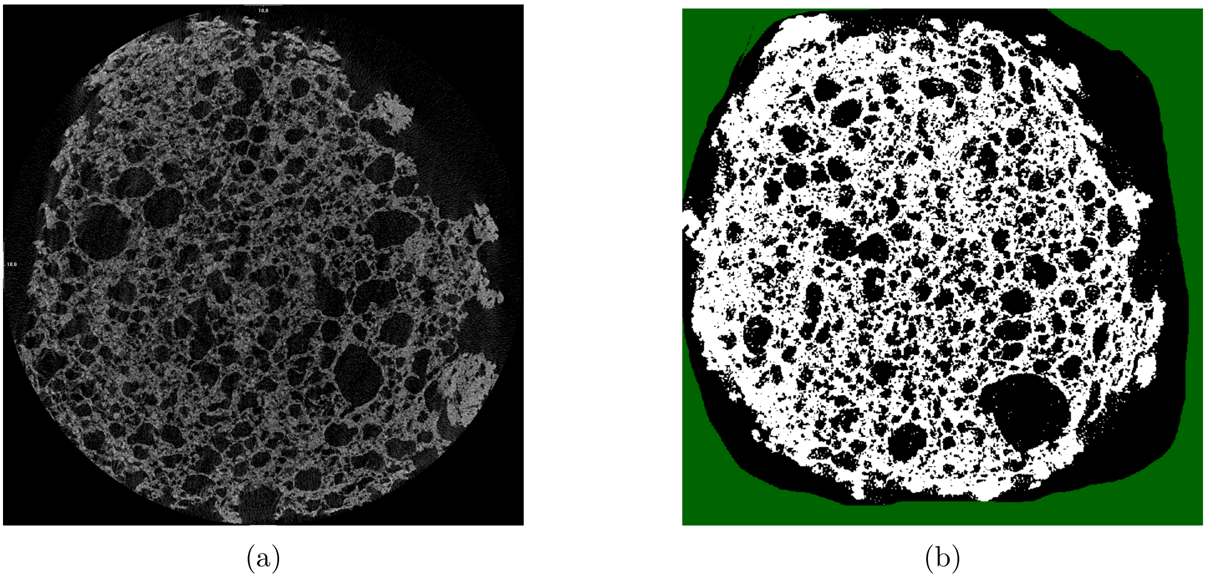

(a) (b)

Figure 5.3: Cross sectional 2D images obtained from micro-CT (a) Cross-sectional image and (b) image after binerisation before de-speckling function.

After binerisation a de-speckling function was used for the removal of noise pixels from the binerised image. The region of interest was then shrink-wrapped and the original image once again projected on the shrink-warped area of interest. A morphological operations step was performed to close the open boundaries of the cells at the outer edges

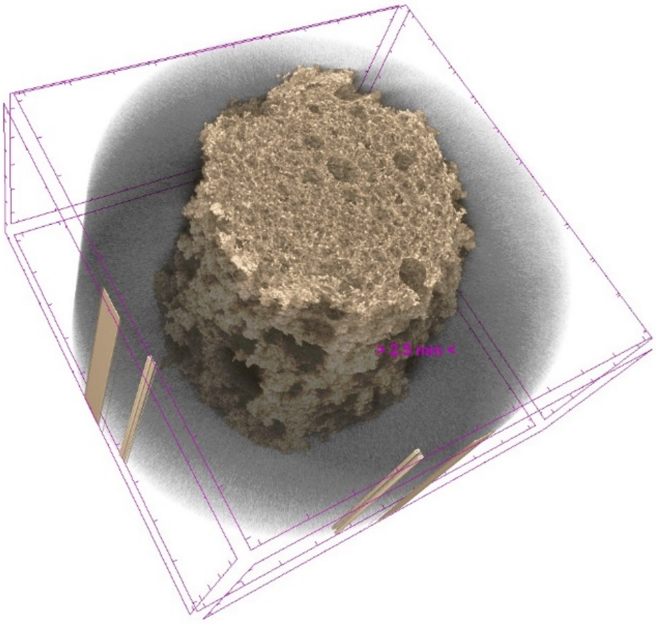


Figure 5.4: 3D volume rendered image built from 2D slices of the scanned cake sample.

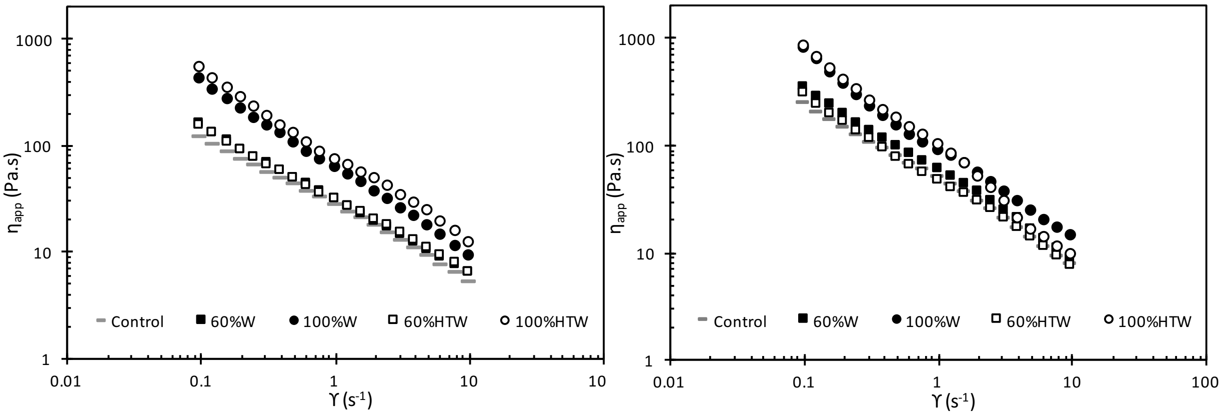
of the image. Finally, 3D analysis of the entire volume of interest was performed for extraction of structural parameters. The cross-sectional images included were taken at 2 *mm* from the top and bottom of the cakes.

5.2.6 Statistical Analysis

Analysis of variance (ANOVA) was applied and, when the effect of the factors was significant ($P < 0.05$), the multiple rank HSD Tukey test used (95% confidence level). Statistical analysis was performed using Minitab 15.0 (Minitab Inc., State College, Pennsylvania, USA).

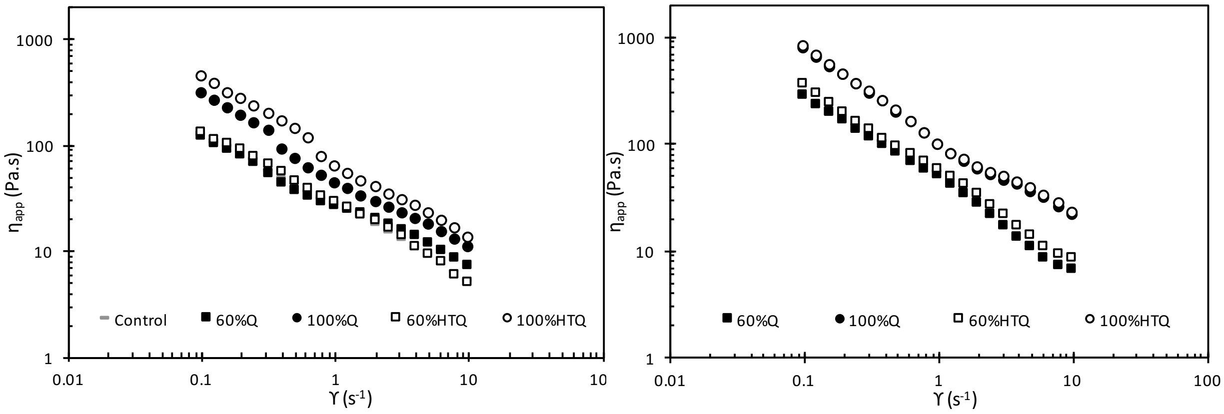
5.3 Results and Discussion

The power law model from eqn. 5.2 adequately described the experimental data for flow curves of all samples ($R^2\sim$ 0.95 to 0.99). The consistency and flow behaviour indices allowed a comparison of the viscosity and the rate of shear-thinning of the batters. Steady shear flow curves of batters with different starch:flour ratios and EWP concentrations are shown in Fig. 5.5. Cake batters comprised of different formulations have all been reported as observing shear-thinning behaviour. Meza et al. (2011) reported shear thinning behaviour for batters both at the foam-stage and upon the addition of oil. Baixauli et al. (2008) observed similar behaviour for muffin batters with higher specific gravities. However in all cases the values of the η_{app} were considerably lower than those reported here at starch:flour ratios greater than 0.6. Flow behaviour and consistency indices reported by Xue and Ngadi (2007) for cake batters containing different combinations of flour and hydrocolloids were comparable to the control samples within this study ($n \sim 0.46$ and $k \sim 69.2\ Pas^n$). In any case shear-thinning behaviour was ascribed to a combination of effects; firstly the spatial distribution of the particles will be altered within the shear-field and they will align themselves with the shear-field, secondly the disruption or even destruction of the structure (in this case air-bubbles) due to the shear-field (McClements, 1998). The rheological data suggests two distinct ranges of values for n and k, which are dependent on the degree of flour dilution. Control samples and those up to and including starch:flour ratio of 0.6 observed no significant statistical differences, however at ratio a of 1, n values were significantly lower and k values significantly higher.



(a) Wheat-5wt.% EWP

(b) Wheat-10wt.% EWP

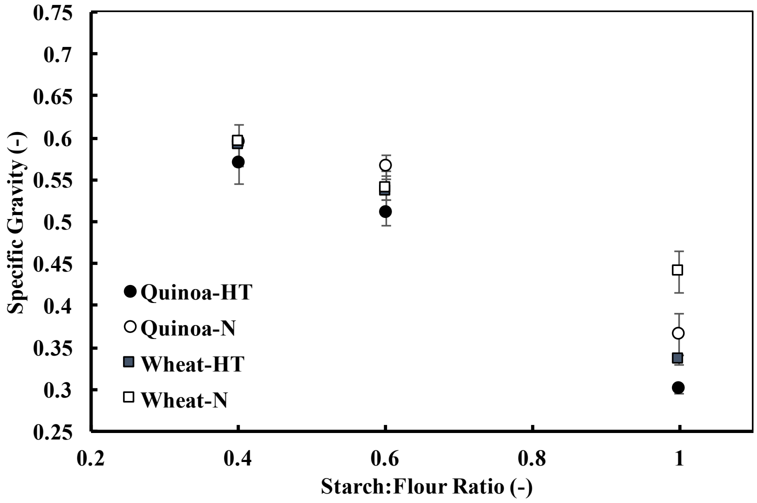


(c) Quinoa-5wt.% EWP

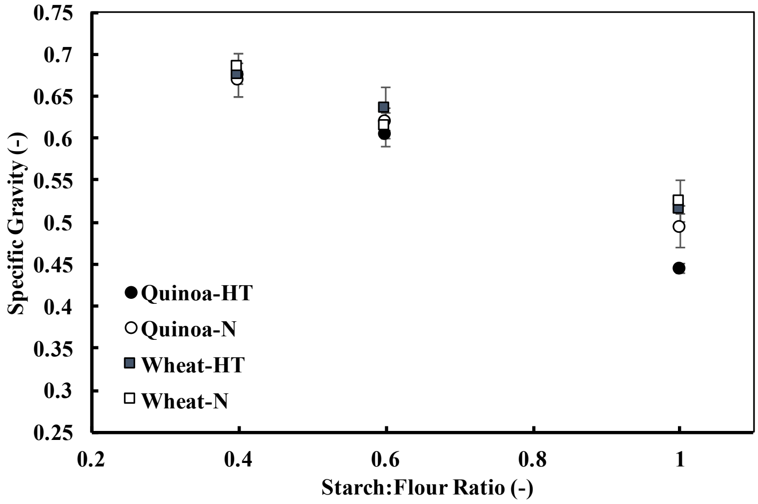
(d) Quinoa-10wt.% EWP

Figure 5.5: Flow curves of heat-treated and untreated starch at 0.6 and 1.0 starch:flour ratio and their comparison to the control samples

Fig. 5.6 suggests that this change in η_{app} was correlated to the specific gravity of the batters and thus the air-phase fraction. Both EWP and starch concentrations enhanced air-entrapment and thus produced more viscous batters. At higher starch:protein ratios and hence less gluten and other flour components, flow indices decreased, imparting a higher degree of pseudo-plasticity to the batter samples regardless of the starch species and treatment. The reduction in the amount of air and thus η_{app} in the system due to halving of the EWP concentration could be offset through addition of starch (Fig. 5.6) suggesting that the smaller particle sizes associated with starch granules as compared to other flour components minimised the de-aeration of batter. At starch:flour ratios of 1, the consistency index (*i.e* viscosity at $\dot{\gamma}$=1) of the batters was higher for quinoa systems with 10 % EWP, however there were no statistical differences observed with 5 % EWP.



(a) 5wt.% EWP



(b) 10wt.% EWP

Figure 5.6: The specific gravities of the batters at at different starch:flour ratios for Quinoa and Wheat starches at (a) 5 % EWP and (b) 10 % EWP.

This effect can be due to possibility of formation of smaller bubbles with 10 wt. % EWP (*i.e.* higher interfacial areas). This would imply that quinoa can provide better packing at the interface or at least within the foam lamella due to its smaller granule size. Moreover, heat-treated (HT) and native quinoa presented the same behaviour in terms of flow and consistency indices at all levels of starch substitution. This is in accordance with previous work done on the effect of heat-treatment on batter rheology by Meza et al. (2011), who did not observe any difference between HT and native fractions at ambient temperature. However the results in Fig. 5.6 shows higher SG values for heat-treated than native fractions at a starch:flour ratio of 1, indicating better air-entrapment capacity for the systems made with HT starch. This is in good agreement with Seguchi and Yamada (1988) who demonstrated that heat-treated starch granules in flours can bind with air-cells and provide additional stability. This hypothesis was further confirmed by studies of Rayner et al. (2012) and Asghari et al. (2016), who observed evidence of better binding capacity at oil and air interfaces for heat-treated starch granules compared to their native counter-parts. Thus higher consistency and lower flow behaviour indices with HT fractions can be attributed to the the additional binding capacity of these starch fractions to protect the air-cells against collapse within the shear field, and so can explain why this effect is only apparent at 10 wt. % EWP.

Table 5.4: Power law parameters of the batters extracted from steady shear tests, for 5 and 10 % EWP

Sample	5%EWP			10%EWP		
	$n^a(-)$	$k^a(Pa.s^{1-n})$	R^2	$n^a(-)$	$k^a(Pa.s^{1-n})$	R^2
Control	0.34±0.02	26.9±2.7	0.99	0.26±0.03	48.0±2.2	0.98
0.4W[1]	0.27±0.04	25.2±3.4	0.99	0.19±0.05	45.0±1.9	0.95
0.6W	0.32±0.02	31.7±1.8	0.99	0.22±0.02	58.9±3.6	0.98
1.0W	0.21±0.01	63.8±0.8	0.99	0.15±0.01	98.1±4.2	0.90
0.4HTW[2]	0.35±0.02	27.5±2.5	0.99	0.16±0.04	40.6±3.2	0.97
0.6HTW	0.33±0.03	31.8±2.7	0.99	0.23±0.01	48.9±4.4	0.98
1.0HTW	0.22±0.02	80.5±4.8	0.97	0.18±0.02	92.9±2.2	0.90
0.4Q [3]	0.40±0.02	22.2±2.1	0.99	0.36±0.03	38.6±5.2	0.99
0.6Q	0.42±0.04	28.9±2.5	0.98	0.21±0.04	44.4±3.5	0.91
1.0Q	0.27±0.04	51.9±6.5	0.92	0.16±0.03	115±6.2	0.89
0.4HTQ [4]	0.36±0.01	30.0±1.7	0.99	0.30±0.04	44.9±1.2	0.98
0.6HTQ	0.30±0.03	25.6±3.5	0.98	0.23±0.03	53.7±4.2	0.96
1.0HTQ	0.22±0.02	74.4±5.3	0.93	0.18±0.01	119±3.9	0.90

[1] Wheat

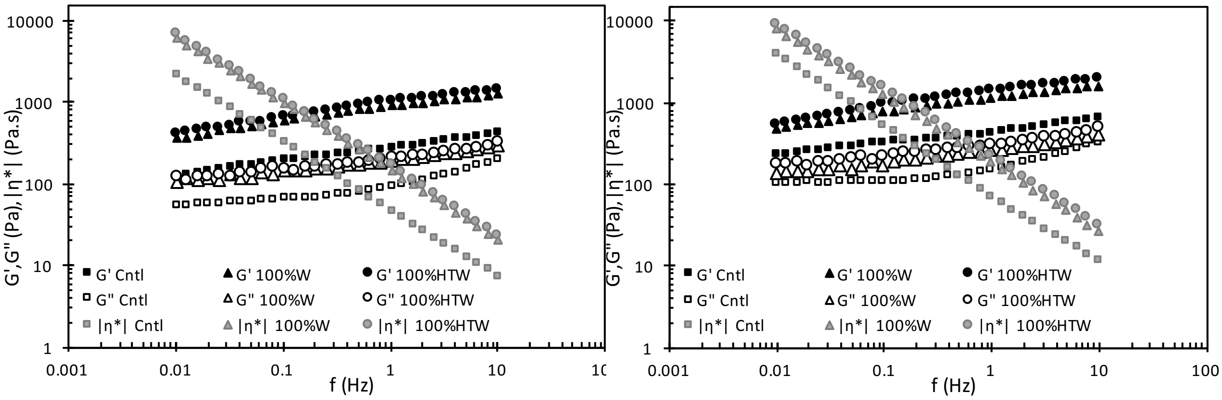
[2] Heat-treated wheat

[3] Quinoa

[4] Heat-treated quinoa

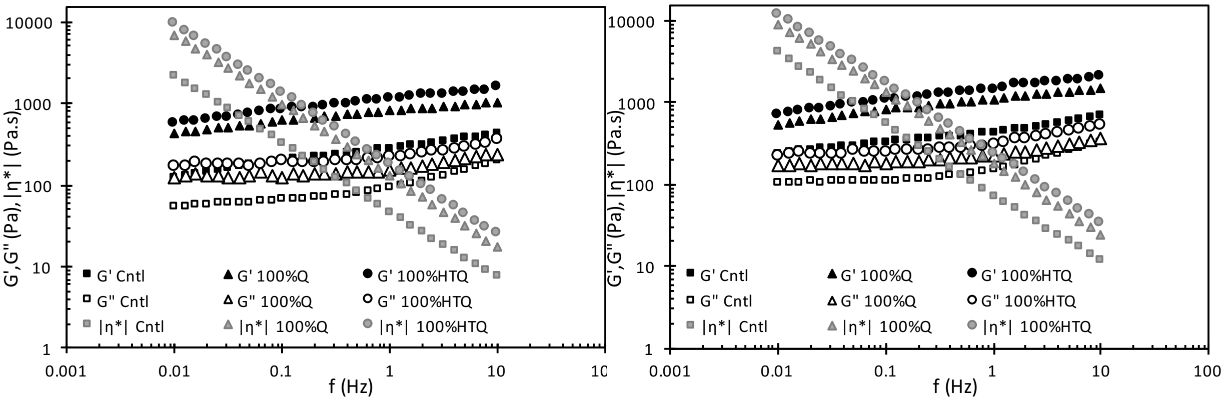
5.3.1 Dynamic Oscillatory Rheology

Mechanical spectra of batters can be used to determine the phase behaviour of the batters. Their acquisition provided information on the extent of network formation formed after mixing. Therefore the influence of different starch:flour ratio, starch treatment and EWP concentration was studied using oscillatory rheology. The mechanical spectra for the control and 100 wt.% starch are presented for both EWP concentrations in fig.5.7. For all samples including the control, G' was higher than G''. The viscoelsticity of cake batters largely depends on the formulation and on processing conditions due to the variability of EWP foaming (Foegeding et al., 2006). However here the EWP was adjusted in pH and temperature and was allowed to equilibrate for 24 hours before use to avoid this type of variability. It is clear that the level of structuring attributed to the incorporation of air into the system dominated the extent of the system's elasticity. Sahi and Alava (2003) have observed a solid-like behaviour in sponge cake formulations with the addition of emulsifiers (glyceryl monostearate and polyglycerol ester). They reported a $G' \sim$ 60-1050 Pa and $G'' \sim$ 80-900 Pa, demonstrating a more viscous behaviour than the systems presented in tab. 5.5. The oscillatory behaviour is highly dependent on formulation and thus the use of oil in the vast majority of studies tends to make pahse behaviour of the batters more viscous (Sumnu and Sahin, 2008). However Sanz et al. (2005) have examined oil-free batters in the presence of methyl cellulose and reported $G' \sim$ 30-1000 Pa and $G'' \sim$ 10-500 Pa (frequency range 0.002-15.9 Hz), but no air volume fractions were reported. Tab. 5.5 shows significantly higher G' compared to G" (stronger gel-like behaviour) with increasing starch:flour ratios. At lower starch:flour ratios, the increase in EWP concentration doubled the G' of the batters, however the effect on G' is dominated by starch concentration over EWP at starch:flour ratios above 0.6. This effect was independent of starch type suggesting enhanced air incorporation at higher starch concentrations (shown in fig. 5.6). This is due to the processing method used for angel food cakes. The folding of the flour into the already foamed meringue structure inevitably disrupted the structure and removed some of the air entrapped within the system which was minimised at higher starch:flour ratios. The smaller size and lower density of the starch fractions compared to other components in the commercial flour lead to a reduction in air bubble disruption during batter mixing.



(a) Wheat-5wt%EWP

(b) Wheat-10wt%EWP



(c) Quinoa-5wt%EWP

(d) Quinoa-10wt%EWP

Figure 5.7: Mechanical spectra of heat-treated and untreated starch at starch:flour ratio of 1 and their comparison to the control

Furthermore tab. 5.5 shows a comparison of the loss and storage moduli for batters at 1 Hz. In general the use of HT starch resulted in a more elastic phase behaviour and this effect was more profound at 5 wt.% EWP. This could be explained by the incorporation of higher air-phase fractions facilitated at 10wt.%EWP. Whereas at lower EWP concentrations the contribution of starch in allowing more air-capture was better observed. Higher G' and G'' values indicated higher levels of interaction within the network. Although the phase angle values for HT starch remained constant, higher moduli values indicated

Table 5.5: The mechanical properties of the cake batters at frequency of 1 Hz, expressed in terms of elastic modulus (G'), viscous modulus (G'') and the phase angle (δ)

Sample	5%EWP			10%EWP		
	$G'(Pa.s)$	$G''(Pa.s)$	$\delta(^\circ)$	$G'(Pa.s)$	$G''(Pa.s)$	$\delta(^\circ)$
Control	278±8	92±5	18.3±1.1	434±10	153±9	19.4±1.0
0.4W	261±11	82±9	17.5±1.1	570±29	163±11	15.9±2.0
0.6W	361±39	101±13	15.6±1.2	744±53	202±16	15.2±1.1
1.0W	889±41	188±23	11.9±1.4	1160 ±106	260±32	12.7±1.1
0.4HTW	268±12	83±11	17.3±2.0	542±29	151±8	15.6±1.4
0.6HTW	403±18	109±16	15.2±1.0	664±48	176±21	14.9±2.0
1.0HTW	1040±54	211±20	11.4±1.4	1400±111	311±25	12.5±1.1
0.4Q	297±19	86±18	16.2±2.2	523±19	148±9	15.8±1.0
0.6Q	340±24	103±10	16.9±1.2	570±24	163±21	15.9±1
1.0Q	790±111	154±60	11.0±1.3	1100±77	228±29	12.8±0.3
0.4HTQ	347±34	105±18	17.7±2.0	588±28	171±11	16.3±2.1
0.6HTQ	410±59	113 ±26	15.5±2.1	587±14	173±7	16.4±1.0
1.0HTQ	1100±87	218±38	11.2±1	1440±71	308±17	12.1±0.4

similar phase behaviour with a more extensive network formation.

This discrepancy between HT and native starch could be explained in terms of the denaturation of the granule's surface proteins as first hypothesised by Seguchi (2001). These proteins were rendered more hydrophobic upon dry heat treatment and therefore induced further interaction with the available air-interfaces but also with the EWP within the continuous phase. This effect was apparent regardless of the starch species used and no significant differences could be observed between wheat and quinoa. It has been shown by Asghari et al. (2016) that heat-treatment of starch dramatically inhibited the volumes of liquid drained within wet-foams when present with EWP. This was ascribed to the interaction of the hydrophobic surface proteins with the interfacial protein films, trapping starch granules within the foam lamella and inhibiting the drainage phenomena. This hypothesis is consistent with the present results, as the packing/structuring of the granules within the foam structure could facilitate the formation of more extensive, stronger netwroks within the batter.

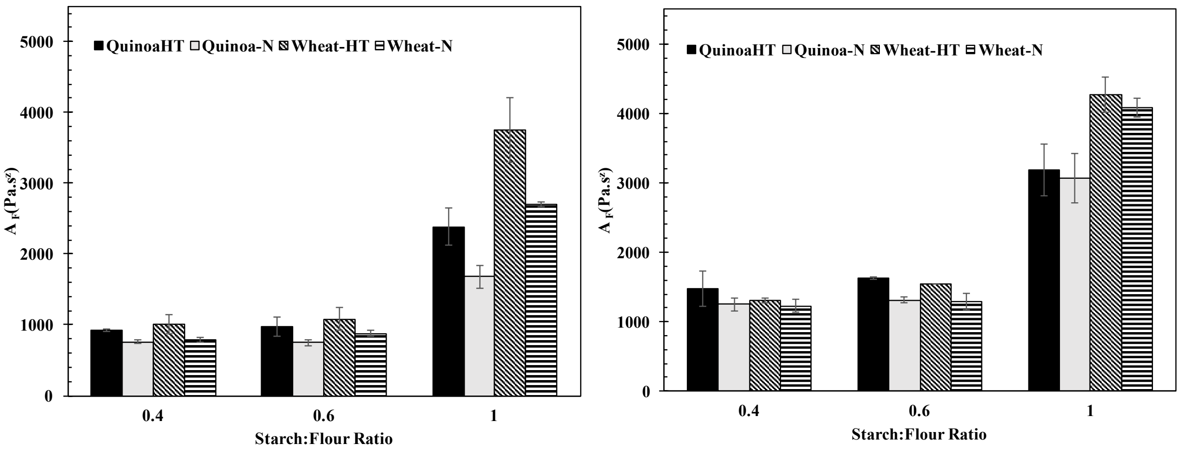
5.3.2 Influence of Starch at 90 and 100 °C

Due to the high amounts of sugar, starch gelatinisation and thus the setting of the structure occured at higher temperatures, and was dependent on starch type. The formation of a gel network is due to the EWP denaturation and aggregation (Pernell et al., 2002; Foegeding et al., 2006). The inter-dispersed starch granules swell and absorb the available water thus augmenting and reinforcing the structural rigidity of the network. Therefore within this model the uptake of water by the granule determines the strength of the gel network formed.

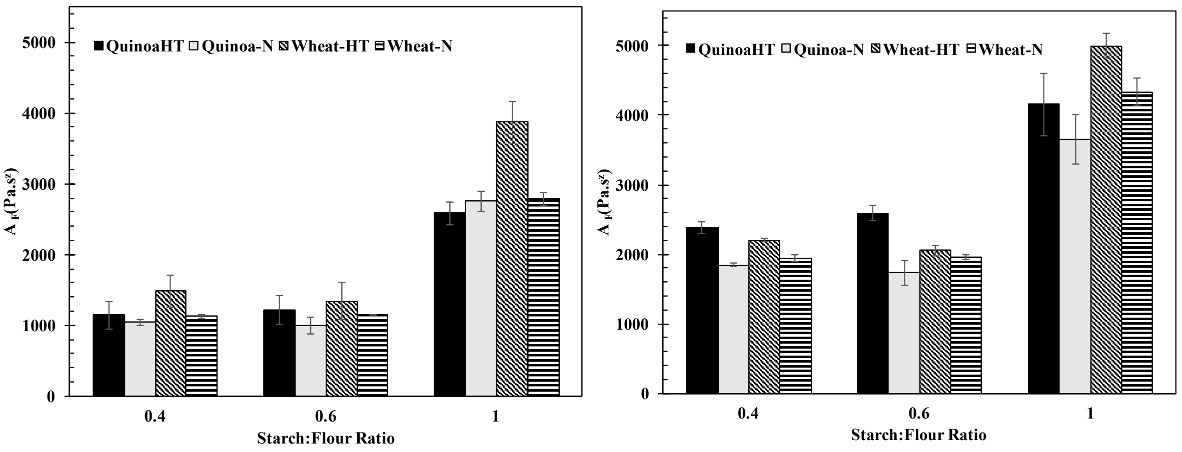
The small amplitude deformations at temperatures of 90 and 100 °C, where the combined effect of starch gelatinisation and EWP coagulation were suspected to give rise to a gel, were analysed using the weak gel model. The A_F values were extracted for the batters and are shown in fig. 5.8. A good correlation was obtained for all samples tested ($R^2 > 0.91$).

As it is highlighted in fig. 5.8, the A_F values were largely determined by the starch:flour ratio in the system. As the ratio increased, the water absorbing capacity of structure also increased yielding stronger gel networks. However this effect was only significant at starch:flour ratio of 1. Below this ratio all systems for a given temperature and EWP concentration were similar to the control samples (960 - 1350 $Pa.s^z$ for 90 °C, and 1500 - 2100 $Pa.s^z$ at 100 °C). Moreover the strength of the gel (A_F) was also dependent on the EWP concentration at both 90 and 100 °C, suggesting that EWP denaturation and coagulation co-determined the final gel-structure's strength.

Meza et al. (2011) have suggested that the interaction of the proteins at air-bubble interface and those present in the continuous phase increased the gel strength for their high ratio cake batter. In addition it was found that the gels formed with 100 % wheat were stronger than those formed by quinoa at the same concentration. This can be due to the better capacity of wheat to absorb water. Therefore increasing the EWP concentration would decrease the available water for the starch, thus enhancing the strength of the gels formed with quinoa.



(a) 90°C-5wt%EWP (b) 90°C-10wt%EWP



(c) 100°C-5wt%EWP (d) 100°C-10wt%EWP

Figure 5.8: Gel strength (A_F) in the weak gel model for foods at (a) 90 °C-5 % EWP, (b) 90 °C-10 % EWP, (c) 100 °C-5 % EWP and (d) 100 °C-10 % EWP. Bars represent standard deviation of two replicates.

The use of a gel model shown in eqn. 5.3 for cake batters has been reported by Meza et al. (2011). In their systems with considerably lower air-phase fractions (*i.e.* 0.12-0.48), A_F values of 20-70 $Pa.s^{1/z}$ and z values of 2-4 were obtained. Meza et al. (2011) used 3wt.% fat (which acts a tenderiser) accounting for the lower gel strengths they observed. The contrast of their system to the one presented here (Figure.**??**) is vast. Gabriele et al. (2001) reported A_F value of 262,170 $Pa.s^{1/z}$ and z value of 8.5 for bread dough at 70 °C.

5.3.2.1 Effect of Heat-treatment at High Temperatures

The effect of heat-treatment was also analysed using low deformation rheology at elevated temperatures in order to obtain more information on the effect of HT on the strength of the gels formed. Heat-treatment of wheat increased the strength of the gels formed at both 90 and 100 °C only at 5 wt.% EWP. At lower EWP concentrations the A_F values were more influenced by the type of starch and it's pre-treatment due to the higher concentration of the unbound water.

No significant difference was observed between the z values for the two EWP concentrations used (30>z>11). This result implied that the number of interactions remained constant despite the additional EWP. The only significant difference for z values was observed when starch:flour ratios of 1 were used. At 100 wt. % starch the values were 30>z>20, where as at all lower ratios the values were 15>z>11, regardless of starch type. This implied that moving to a more pure flour system with less components (100wt% starch) did not yield less interaction. However this could be due to the fact that as the starch concentration increased so did the number of air-cells present in the system (*i.e.* a higher air-phase fraction) (Tab. 5.4), therefore accounting for the higher z values observed.

Sun et al. (2014) reported that dry heat-treatment of proso-millet flour and starch independently, increased the pasting viscosity of the flour gel, while it only increased the final viscosity of the starch gel. They observed that the gel structure became more compact and the starch particles were plumper. However Ozawa et al. (2009) in their study of dry heat-treatment of flour components, reported no observable difference in the onset gelatinisation temperature or peak viscosities of the starch gels. They concluded that the aforementioned changes in the flour were due to the effect of heat-treatment on the gluten fraction and not the starch. However the results observed here contradict the results obtained by Ozawa et al. (2009) and in pure starch systems, considerable rheological differences were observed at higher temperatures. Ozawa and Seguchi (2006) suggested the higher hydrophobicity upon dry heating improves the interactions between the different starch fractions, when the temperature increases, thus yielding a stronger structure during the baking process.

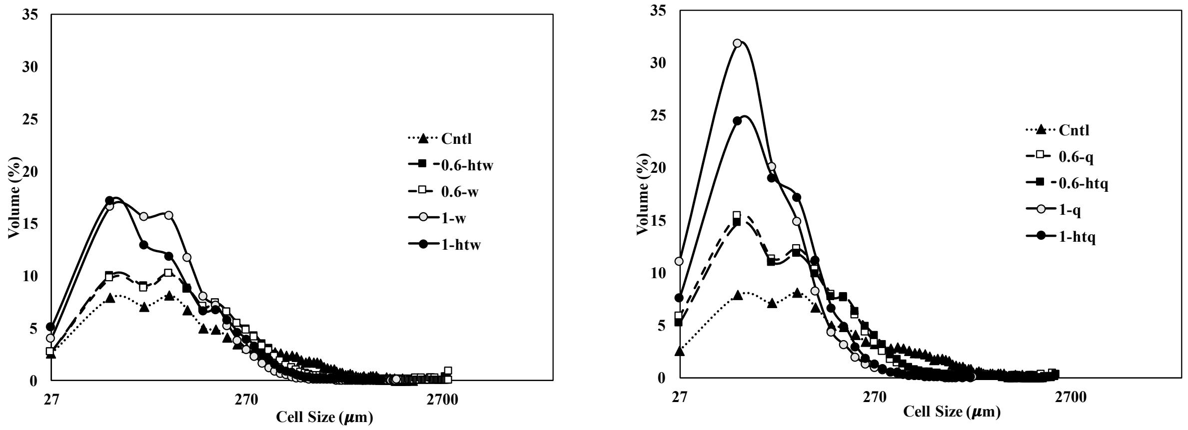
5.3.3 Cake Microstructure

5.3.3.1 Pore Size and Porosity

The porosity and pore size distributions were investigated using an x-ray CT in order to compare the final microstructure of the baked cakes. The pore size distributions (Fig. 5.9) were inversely proportional to the starch: flour ratio, independent of the starch type used. At all substitution levels of starch, wheat starch systems were more porous structures compared to the quinoa and control systems (0.59 & 0.62 for 5 & 10 wt.% EWP respectively). The highest population of smaller air cells corresponded to the highest A_F values observed (Fig. 5.8). There are likely to be a couple of mechanisms responsible for this change in cell size distribution; firstly smaller starch granules are more likely to participate at the air cell interfaces during the batter formation stage. Secondly during baking, the higher strength of the gels formed by starch and EWP, retard the extent of air-cell disproportionation and coalescence.

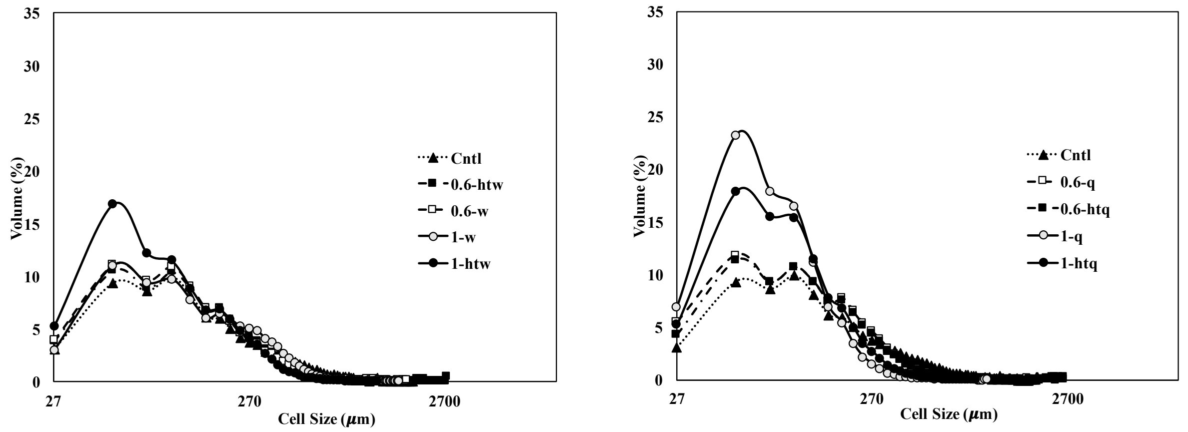
The porosity of the cakes was studied as a function of starch:flour ratio and EWP concentration (Fig. 5.10). The EWP concentration determined the quinoa starch performance. When increasing the EWP concentration, the porosity values obtained for quinoa systems sharply increased (from $0.54<\varphi<0.62$ to $0.64<\varphi<0.73$), and thus the difference between wheat and quinoa systems became less apparent (Fig. 5.10).

Aside from the effect of EWP in increasing initial air-incorporation during mixing of the batter, Donovan (1977) has also showed using differential scanning calorimetry that smaller fractions of EWP *i.e.* lysozyme and conalbumin denature at temperatures below the maximum temperature attained during baking ($\sim$ 100 °C). Protein denaturation in this temperature range (from 70 to 90 °C), increased the batter viscosity thus preventing movement of air cells and contributing to baking stability.



(a) Wheat - 10wt.%EWP

(b) Quinoa - 10wt.%EWP

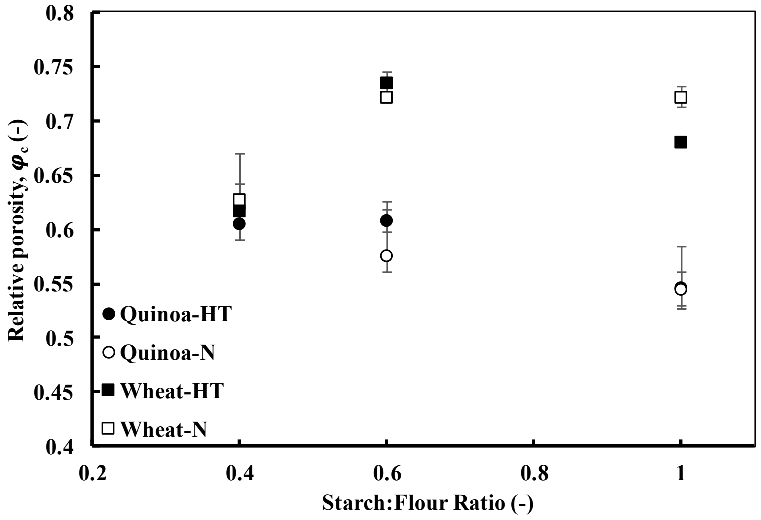


(c) Wheat - 5wt.%EWP

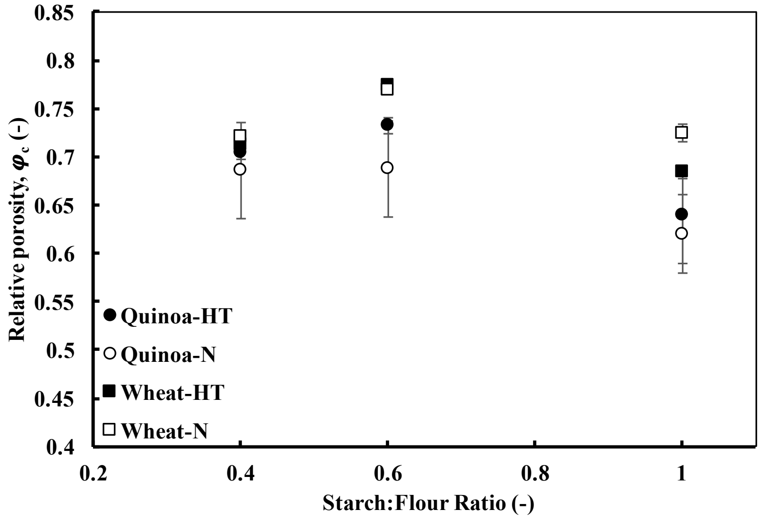
(d) Quinoa - 5wt.%EWP

Figure 5.9: The air-cell size distributions within cake structure. (a) size distribution for wheat starch and (b) size distribution for quinoa starch systems

All cakes observed bi-modal size distributions with EWP concentration and starch size both inversely proportional to pore size. Quinoa cakes had a larger population of smaller cells (Fig. 5.9) as analysed by x-ray CT at all starch:flour ratios compared to wheat systems. This could be explained by the lower porosity yielding thicker structures, as evident from the structure distributions (*i.e.* cell wall thickness distributions) shown in fig. 5.11. This is due to a combination of effects. Firstly quinoa cakes demonstrated higher weight loss during baking (tab. 5.6) compared to wheat. This might be evidence of higher water absorbing capacity of wheat starch (less unbound water evaporating during baking). The higher weight loss could also have been responsible for the lower cake volume in these cases. It was also observed that with 5 wt.% EWP, HT wheat showed a lower weight loss. Derby et al. (1975) have demonstrated that when wheat starch was within its protein matrix, its contact and reaction with water is limited and less swelling was observed. Thus heat-treatment can potentially disrupt this matrix and increase the swelling capacity of wheat. However this effect reduced quinoa's absorption capacity, probably due to size effects and too much molecular disruption.



(a) 5wt.% EWP



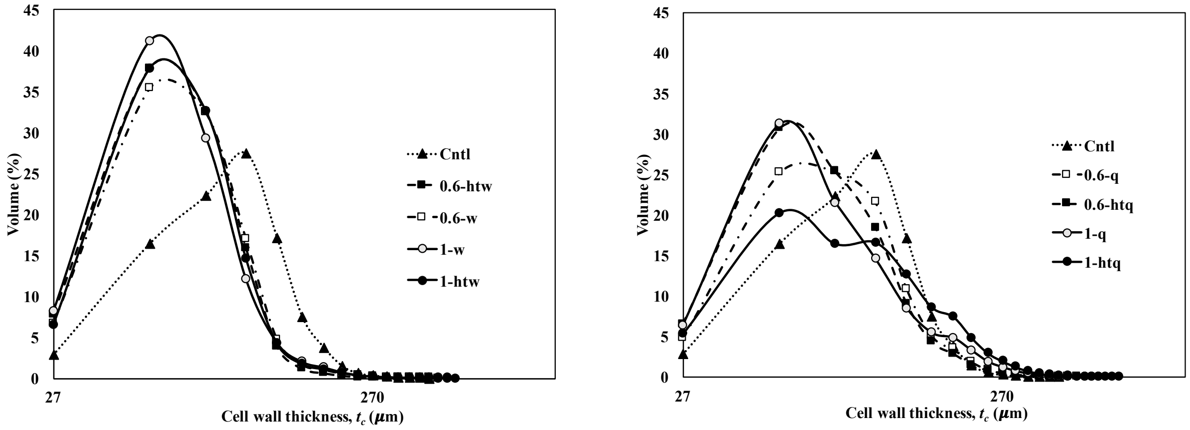
(b) 10wt.% EWP

Figure 5.10: The relative porosity of the cakes as measured by x-ray CT; a comparison between Quinoa and Wheat at (a) 5 %EWP and (b) 10 % EWP. The error bars represent standard deviation from two replicate cakes.

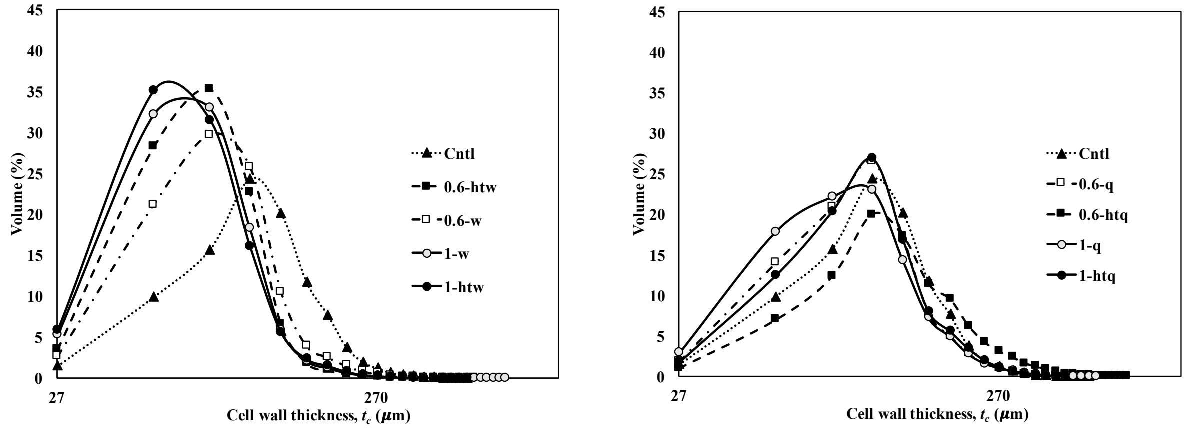
Table 5.6: Weight Loss at starch:flour ratio of 1; shown for Quinoa and Wheat starches. Contrast of performance at two different water contents

Sample	Weight Loss (g)	
	5wt.%EWP	10wt.%EWP
Quinoa	24.3±0.7	22.4±0.8
HT Quinoa	27.1±1.8	19±1.0
Wheat	21.7±1.0	19.5±0.7
HT Wheat	19.7±0.5	19±0.4

Moreover the combined effect of the swollen starch granules, and the continuous EWP gel surrounding them, provides the firm structure of the cell wall material (Wilderjans et al., 2010). According to Donovan (1977) the angel cake structure *i.e.* the cake composite, permeated by gas bubbles is similar to bricks and mortar. The starch grains form the bricks and the protein, the mortar. Based on this proposed model the structure of the systems were analysed according to cell wall thickness, t_c. The t_c was found to be inversely proportional to porosity (Fig. 5.10). The final cell wall structures are determined by removal of water by starch granule and thus properly baked cakes should contain sufficiently swollen starch granules that make mutual contact (Delcour et al., 2009). Hence the removal of excess water from the crumb by the starch granule determines the physical characteristic of the cell walls. Quinoa systems showed a sharp increase in t_c with reduction of EWP (Fig .5.11). In addition cake structure is also dependent on starch granules maintaining a recognisable granular shape after swelling (Howard et al., 1968). Therefore the increase in the t_c can be related to complete disintegration of quinoa in a system where high amounts of water was available.



(a) Wheat - 10wt.%EWP (b) Quinoa - 10wt.%EWP



(c) Wheat - 5wt.%EWP (d) Quinoa - 5wt.%EWP

Figure 5.11: The cell wall thickness distributions within cake structures. (a) and (c) size distribution for wheat starch (b) and (d) size distribution for quinoa starch systems

The strength and character of the micellar network within the granule is the major factor controlling the swelling behavior of starch. Thus, highly associated starches with an extensive and strongly bonded micellar structure should be more resistant to swelling (Lorenz, 1990). Higher extent of swelling in starches with low amylose content (quinoa, wild rice and amaranth) was observed by Lorenz (1990) who concluded that amylose reinforces the internal network of the granule and restricts swelling. Therefore, the structural bonding is weak. Although, the starch granules swell during baking, the cake structure cannot stand the stress involved during baking or the subsequent cooling.

It was observed that all cakes had a reduction in cake height during the cooling, however this was higher (by 7-13 %) in quinoa systems. According to Wilderjans et al. (2010) the starch gel forms too late during cooling to prevent collapse, but can contribute significantly to cell wall texture and thus initial firmness of the cake. Upon cooling the starch will re-associate to a more ordered state and amylose forms a continuous crystalline network (Delcour et al., 2009). Thus the strength of the starch gel and amylose content of the starch play an important role in preventing post-bake collapse and the initial crumb firmness. This means that the cake structure cannot stand the stress involved in subsequent cooling and a more significant collapse was observed for system with 100 % quinoa starch.

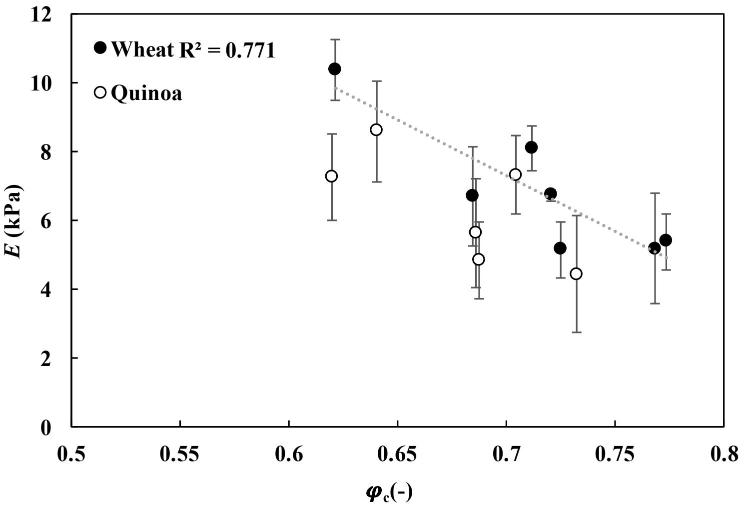
5.3.3.2 Mechanical Properties of Cakes

According to Gibson and Ashby (1987) the most important parameter for characterising the mechanical properties of cellular solids is the relative density of the system. The theory of cellular solid mechanics has successfully been applied to bread crumb and successful correlations of porosity to both Young's modulus and critical stress have been derived (Zghal et al., 2002). For simplified starch bread formulations it was found that the relative Young's modulus E/E_s (where E is the Young's modulus of the overall bread structure and E_s is the Young's modulus of the solid material comprising the cell walls) had the following power-law relationship with relative density of the bread (Zghal et al., 2002);

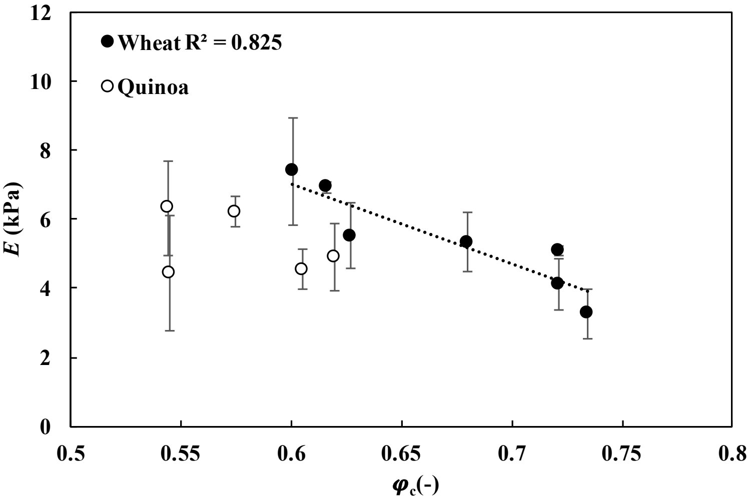
$$\frac{E}{E_s} = C[\frac{\rho}{\rho_s}]^n \tag{5.9}$$

where ρ is the density of the bread crumb, ρ_s is the solid material's density and C and n are empirical constant that are dependent on whether cells are open or closed and the relative thickness of the cell walls. Thus in fig. 5.12 the effect of relative density was normalised and the Young's modulus as a function of starch species and EWP concentration is displayed. This was achievable as porosity can be simply expressed as;

$$\varphi_c = 1 - \frac{\rho}{\rho_s} \tag{5.10}$$



(a) 10 % EWP



(b) 5 % EWP

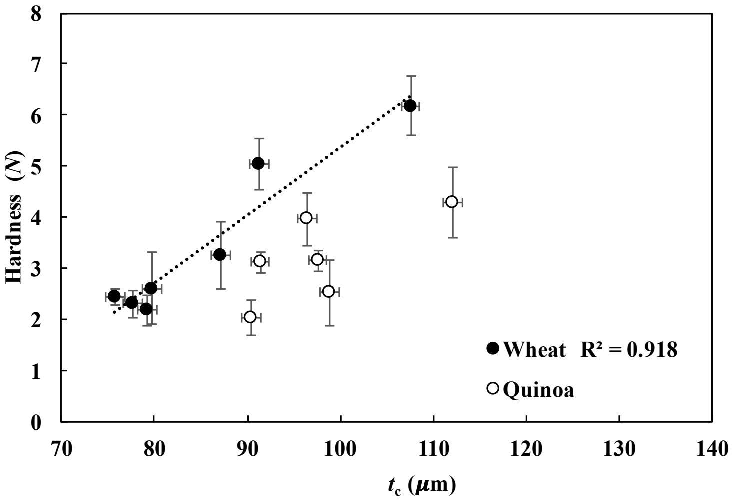
Figure 5.12: The correlation of Young's modulus (E) of cakes to cake porosity (φ_c) for wheat and quinoa starch systems at (a) 10 % EWP and (b) 5 % EWP

With regards to wheat systems, E evolved linearly as a function of porosity (Fig. 5.12), whilst this relationship was only observed with 10 % EWP for quinoa systems. Wilderjans et al. (2010) showed that formation of a protein network and starch recrystallisation during cooling, determined the stability of the final structure. They concluded in their study of a pound cake system that springiness is in fact determined by the amount of extractable protein within the system and that the initial cake hardness is only determined by the starch. Hence it is reasonable to assume that protein concentration will influence the recovery of the structure from any compressive deformation. Moreover Attenburrow et al. (1989) observed a significant decrease in E and the critical stress when increasing the water activity of sponge cakes. They reported that as water activity increased, both E and the critical stress fell by an order of magnitude. Therefore the lower E values for quinoa systems could be attributed to the higher water activity as a consequence of lower water binding/absorbing capacity compared to wheat however as water activity was not measured, this hypothesis requires validation. In any case even the use of 10 % EWP with quinoa presented weak trends between E and porosity, but here the E and φ_c values were comparable to those of wheat systems unlike those reported at 5 % EWP, where much lower φ_c values were observed.

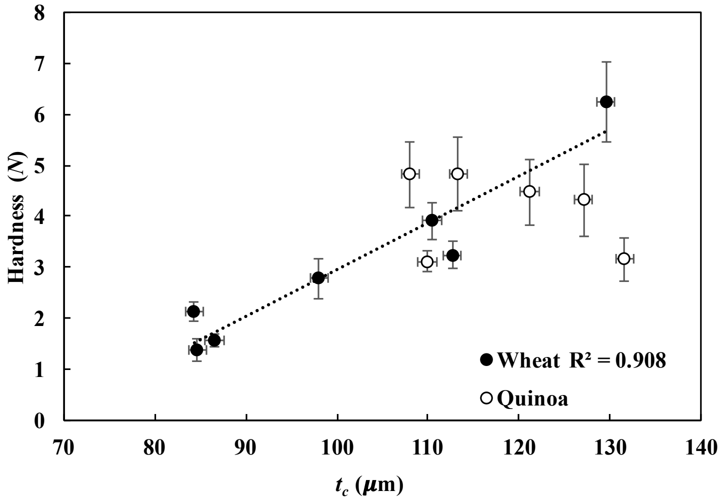
Another important characterisation parameter in cakes is the initial firmness of its structure. This is determined by porosity and physical and mechanical properties of the formed cell walls. Zghal et al. (2002) reported a linear correlation between the yield stress of 'starch bread' and its microstructural parameters. The critical stress at which the cellular structure yields or buckles has been related theoretically to the relative density of the cellular structure (Gibson and Ashby, 1987). Thus the relative density term is related to microstructural dimensions t_c and l for a three-dimensional open cell model by;

$$\frac{\rho}{\rho_s} \propto \frac{t_c}{l} \tag{5.11}$$

where t_c is the thickness of cell walls and l is the cell-edge length of cells. Within the systems of this study porosity could be correlated to the cell wall thickness, t_c ($R^2 = 0.82$). Thus the mechanical properties should exhibit changes according to micro-structural parameters within these cellular structures.



(a) 10 %EWP



(b) 5 % EWP

Figure 5.13: The correlation of hardness of cakes to structural parameter cell wall thickness (t_c) for wheat and quinoa starch systems at (a) 10 % EWP and (b) 5 % EWP

Fig. 5.13 presents hardness of wheat and quinoa systems as a function of the cell-wall thickness. Wheat systems were characterised by a linear relationship while quinoa starch cake parameters showed no correlation. At both 10wt.% EWP and 5 wt. % EWP a constant hardness independent of t_c was observed (R^2 = 0.4 - 0.5). Accordingly, hardness did not show a significant correlation to the t_c with quinoa at any EWP concentration, but EWP content impacted the t_c of quinoa systems.

5.3.4 Cake Macrostructure

Using high EWP concentrations (10 %), quality of the cakes comprised of wheat starch up to and including 100 % starch were good with a homogeneous crumb. However at 5 % EWP, the cakes with the highest level of substitution lost volume around their sides despite good cake height (a mushroom effect), fig. 5.14. What is detrimental to the crumb quality seems to be availability of water. Howard et al. (1968) who reported a similar effect, concluded that this can be attributed to the non-gelling tendency of amylopectin/ amylose mixture in the absence of granular starch (*i.e* the folding of granule structure and its disintegration in excess water which leads to undesirable cake quality (Derby et al., 1975; Howard et al., 1968)).

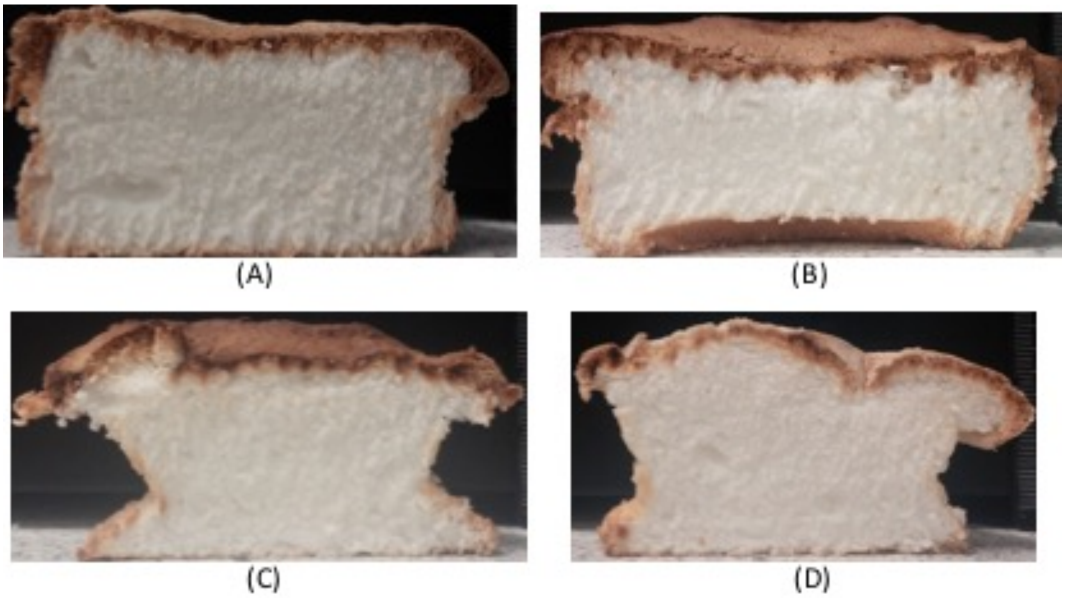
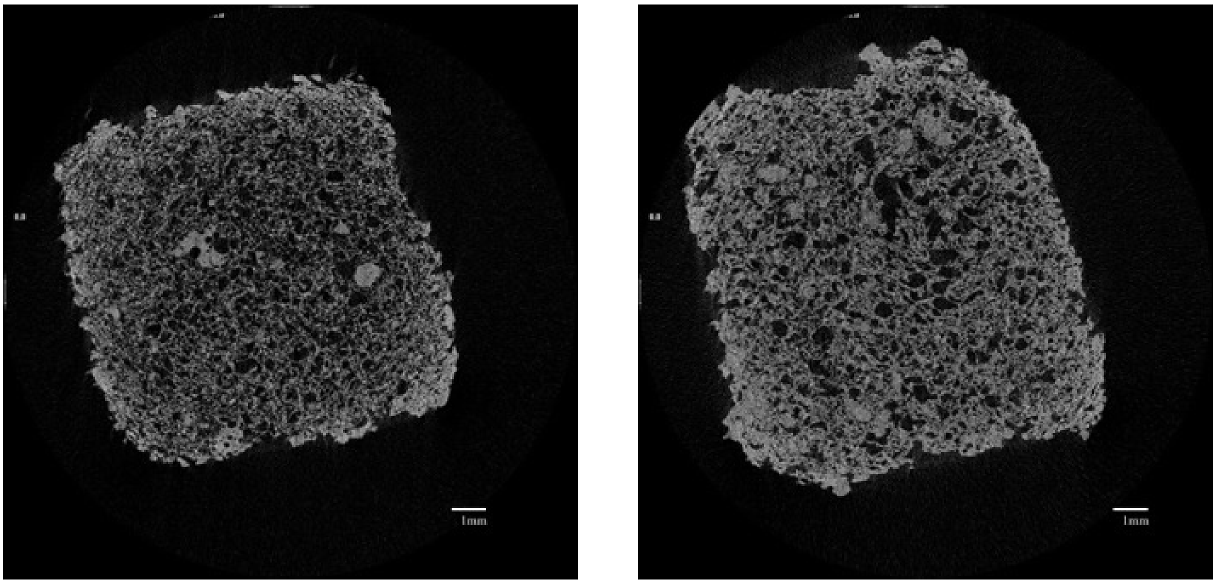


Figure 5.14: The effect of EWP and heat-treatment for 100 % wheat starch systems. (A) 10 % EWP-HT, (B) 10 %EWP-native, (C) 5 %EWP-HT and (D) 5 %EWP-native. The bases of all cakes are 7 cm in diameter.

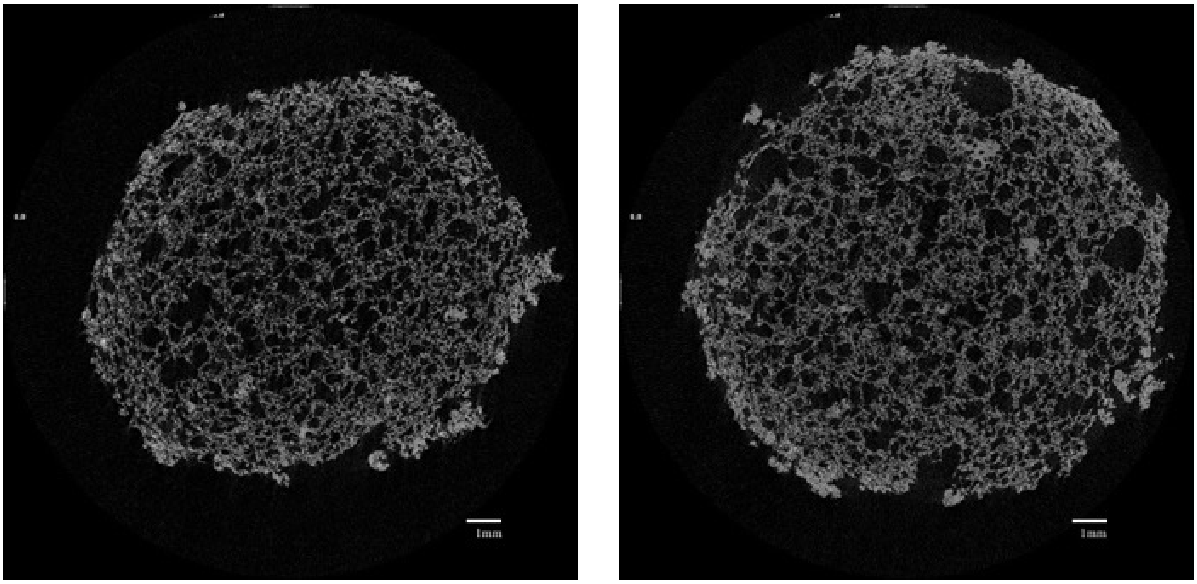
Fig. 5.15 shows with quinoa starch:flour ratios > 0.6, the structure of the cakes was no longer homogeneous at 5 % EWP. There was a dense bottom layer formed. The density gradient observed in the cross-sectional images (Fig. 5.15) was further confirmed by x-ray cross-sectional images (Fig. 5.16). This densification near the bottom of the cake was not observed at 10 % EWP. This was attributed to a baking fault well known in bread baking, known as 'bone' as these dense patches represented areas of collapse. This phenomena is caused by the starch matrix surrounding the bubbles not setting rigidly enough by the end of baking. Consequently they collapse during cooling as the steam in them condenses (Cauvain, 2001). It is likely that starch granules are not disintegrating in this case, but are just not producing gels of sufficient strength at a low enough temperatures. With bubble buoyancy effects the dense layer would most likely be at the very bottom of the cake. Once again the reason is down to available water, where lower quantities of available water at 10 % EWP meant quinoa starch granules could produce a more rigid matrix at lower temperatures as represented by the A_F values (Fig. 5.8). The higher cell wall thicknesses at the bottom of the cake and this density gradient meant that the fit of the structural parameters were not as coherent either. Therefore a hypothesis could be that at higher concentrations of free water (5 %EWP), quinoa systems with smaller size and lower amylose content yielded less viscous batters at higher temperatures, where the weaker quinoa starch gels, couldn't cause baking collapse of the gas cells and hence the inhomogeneity could be due to post-bake condensation of the entrapped water vapour.

Figure 5.15: The effect of EWP and heat-treatment for 100 % quinoa starch systems. (A) 10 %EWP-HT, (B) 10 % EWP-native, (C) 5 %EWP-HT and (D) 5 % EWP-native. The bases of all cakes are 7 cm in diameter.

These results were in concordance with those by Sanz et al. (2005) who have suggested that the batter viscosity at high temperatures had an important effect on the bubble movement. This was concluded as an important parameter in determining final cake volume.



(a) Quinoa - Top (b) Quinoa - Bottom



(c) Wheat - Top (d) Wheat - Bottom

Figure 5.16: Microstructural cross-sectional images (x-rayCT scans) from Quinoa and Wheat cakes at starch:flour ratio 1 and EWP concentration of 5 %

Moreover the HT seemed to influence the quinoa systems negatively. Whereas within wheat systems the final quality of the cake with lower EWP concentration did not show a significant difference, but the microstructural properties did show the formation of higher number of smaller pores. Lorenz and Coulter (1991) reported that chlorinated wheat flour improved both the colour and properties of high ratio cakes (those with high proportions of sugar and liquid in the recipe), but chlorination of the quinoa flour did not substantially improve cake characteristics.

5.4 Conclusions

Substitution of starch for flour yielded more viscous systems with higher levels of pseudo-plasticity. The size of starch was also inversely proportional to the specific gravities of the batters formed. The higher levels of starch substitution translated to better air incorporation producing more elastic gel networks with higher shear viscosities during batter formation. Heat treatment of wheat and quinoa presented batters with stronger networks at ambient temperature, attributed to surface denaturation effects facilitating interfacial contribution of the starch granules. At high temperatures, it was observed that HT wheat starch formed stronger gels than its native counterpart.

Quinoa starch showed good potential and comparable mechanical and physical properties at high EWP concentrations, where the lower concentrations of free water did not expose it's inferior water absorbing capacity compared to wheat. However at lower EWP concentrations the quinoa cakes showed inhomogeneity in their structures, ascribed to formation of weaker gels. This along with lower amylose content and molecular association resulted in weak setting of the starch gel matrix *i.e.* bone effect. The weaker gel strengths observed at 90 and 100 °C were a testament to this phenomena. Moreover, the substitution of 60 % of flour content for either starch granule could facilitate up to 50 % EWP reduction whilst maintaining similar batter and cake properties. Finally the gel-strength was seen as a good indicator of the final cake structure within wheat systems. This parameter determined the extent of disproportionation and air-cell movement and so the final pore sizes. The final porosity was co-dependent on the cooling process and whether starch fractions could maintain an intact granular structure during baking. X-ray CT analysis was shown to be an effective tool for comparison of cake microstructures, where marked differences were observed between; firstly the different starch:flour ratio systems and secondly systems comprising of different starch species. Good correlations for the mechanical properties and the structural parameters (obtained from image-analysis of x-ray scans) were observed for wheat systems. Whereas quinoa systems mainly depended on EWP for their final structures. Finally quinoa was found to be an effective substitute for wheat, when used either in combination with a minimum amount (40wt.%) of wheat flour or in systems with lhigh concentrations of EWP.

5.5 References

List of References

Asghari, A. K., Norton, I., Mills, T., Sadd, P., and Spyropoulos, F. (2016). Interfacial and foaming characterisation of mixed protein-starch particle systems for food-foam applications. *Food Hydrocolloids*, 53:311 – 319. Functional Hydrocolloids: A Key to Human Health.

Attenburrow, G., Goodband, R., Taylor, L., and Lillford, P. (1989). Structure, mechanics and texture of a food sponge. *Journal of Cereal Science*, 9(1):61 – IN1.

Baixauli, R., Sanz, T., Salvador, A., and Fiszman, S. (2008). Muffins with resistant starch: Baking performance in relation to the rheological properties of the batter. *Journal of Cereal Science*, 47(3):502 – 509.

Cauvain, S. (2001). Improvers. In S P Cauvain, L. S. Y., editor, *Baking Problems Solved*, chapter 4, pages 52–62. Woodhead Publishing Limited.

Cauvain, S. and Young, L. (2006). *Baked Products: Science, Technology and Practice.* Wiley.

Delcour, J. A., Bruneel-Liesbeth, C., J. Derde, S. G., Putseys, B. A., Wilderjans, E., and Lamberts, L. (2009). Fate of starch in food processing: From raw materials to final food products. *FOOD SCIENCE AND TECHNOLOGY*, 1:87–111.

Derby, R. I., Miller, B. S., Miller, B. F., and Trimbo, H. B. (1975). Visual observation of wheat-starch gelatinization in limited water systems. 52:702–713.

Donovan, J. W. (1977). A study of the baking process by differential scanning calorimetry. *Journal of the Science of Food and Agriculture*, 28(6):571–578.

Foegeding, E. A., Luck, P., and Davis, J. (2006). Factors determining the physical properties of protein foams. *Food Hydrocolloids*, 20(2–3):284 – 292. 7th International Hydrocolloids Conference.

Gabriele, D., de Cindio, B., and D'Antona, P. (2001). A weak gel model for foods. *Rheologica Acta*, 40(2):120–127.

Gibson, L. J. and Ashby, M. F. (1987). *Cellular Solids; Structure and properties.* Pergamon Press.

Howard, N. B., Hughes, D. H., and Strobel, R. G. K. (1968). Function of the starch granule in the formation of layer cake structure. 45:329–338.

Lorenz, K. (1990). Quinoa (chenopodium quinoa) starch: Physico-chemical properties and functional characteristics. *Starch*, 42(3):81–86.

Lorenz, K. and Coulter, L. (1991). Quinoa flour in baked products. *Plant Foods for Human Nutrition*, 41(3):213–223.

McClements, D. (1998). *Food Emulsions: Principles, Practice, and Techniques.* Contemporary Food Science. Taylor & Francis.

MedPhysics (2012). Micro-ct scanner with rotating gantry.

Meza, B. E., Chesterton, A. K., Verdini, R. A., Rubiolo, A. C., Sadd, P. A., Moggridge, G. D., and Wilson, D. I. (2011). Rheological characterisation of cake batters generated by planetary mixing: Comparison between untreated and heat-treated wheat flours. *Journal of Food Engineering*, 104(4):592 – 602.

Miller, R. (2016). Cakes: Types of cakes. In Caballero, B., Finglas, P. M., and Toldrá, F., editors, *Encyclopedia of Food and Health*, pages 579 – 582. Academic Press, Oxford.

Mintel (2016). Cakes and cake bars - uk - march 2016.

Mohamed, A. A. and Rayas-Duarte, P. (2003). The effect of mixing and wheat protein/gluten on the gelatinization of wheat starch. *Food Chemistry*, 81(4):533 – 545.

Moiraghi, M., de la Hera, E., Pérez, G. T., and Gómez, M. (2013). Effect of wheat flour characteristics on sponge cake quality. *Journal of the Science of Food and Agriculture*, 93(3):542–549.

Mousavi, R., Miri, T., Cox, P. W., and Fryer, P. J. (2007). Imaging food freezing using x-ray microtomography. *International Journal of Food Science & Technology*, 42(6):714–727.

Ozawa, M., Kato, Y., and Seguchi, M. (2009). Investigation of dry-heated hard and soft wheat flour. *Starch - Stärke*, 61(7):398–406.

Ozawa, M. and Seguchi, M. (2006). Relationship between pancake springiness and interaction of wheat flour components caused by dry heating. pages 167–172.

Palav, T. (2016). Chemistry of cake manufacturing. In Wrigley, Colin, Corke, Harold, Seetharaman, Koushik, and Faubion, editors, *Encyclopedia of Food Grains (Second Edition)*, pages 367 – 374. Academic Press, Oxford, second edition edition.

Pernell, C., Luck, P., AllenFoegeding, E., and Daubert, C. (2002). Heat-induced changes in angel food cakes containing egg-white protein or whey protein isolate. *Journal of Food Science*, 67(8):2945–2951.

Rayner, M., Sjöö, M., Timgren, A., and Dejmek, P. (2012). Quinoa starch granules as stabilizing particles for production of pickering emulsions. *Faraday discussions*, 158(1):139–155.

Sahi, S. and Alava, J. M. (2003). *Sponge Cake batters*, chapter 5, Cake Batter Rheology. CRC Press.

Sanz, T., Salvador, A., Vélez, G., Munoz, J., and Fiszman, S. (2005). Influence of ingredients on the thermo-rheological behaviour of batters containing methylcellulose. *Food Hydrocolloids*, 19(5):869 – 877.

Seguchi, M. (2001). Oil binding ability of chlorinated and heated wheat starch granules and their use in breadmaking and pancake baking. *Starch - Stärke*, 53(9):408–413.

Seguchi, M. and Yamada, Y. (1988). Hydrophobic character of heat-treated wheat starch. *Cereal Chemistry*, 4(65):375–376.

Sumnu, S. and Sahin, S. (2008). *Food Engineering Aspects of Baking Sweet Goods*. Contemporary Food Engineering. CRC Press.

Sun, Q., Gong, M., Li, Y., and Xiong, L. (2014). Effect of dry heat treatment on the physicochemical properties and structure of proso millet flour and starch. *Carbohydrate Polymers*, 110:128 – 134.

Tan, M., Chin, N., Yusof, Y., and Abdullah, J. (2016). Novel 2d and 3d imaging of internal aerated structure of ultrasonically treated foams and cakes using x-ray tomography and x-ray microtomography. *Journal of Food Engineering*, 183:9 – 15.

Wilderjans, E., Luyts, A., Goesaert, H., Brijs, K., and Delcour, J. (2010). A model approach to starch and protein functionality in a pound cake system. *Food Chemistry*, 120(1):44 – 51.

Xue, J. and Ngadi, M. (2007). Rheological properties of batter systems containing different combinations of flours and hydrocolloids. *Journal of the Science of Food and Agriculture*, 87(7):1292–1300.

Zghal, M., Scanlon, M., and Sapirstein, H. (2002). Cellular structure of bread crumb and its influence on mechanical properties. *Journal of Cereal Science*, 36(2):167 – 176.

6 | Emulsification by Ultrasound at Higher Continuous Phase Viscosities: Comparison to Mechanical Agitation

Abstract

Viscosity influenced the emulsification process differently depending on the mechanism by which droplets were broken. For power Ultrasound (US) and high-shear mechanical mixing (HS) the influence of continuous phase viscosity in an oil/water system was compared. High shear emulsification by mechanical agitation was affected differently compared to break-up due to cavitations generated by power ultrasound. Using the same model system (Sucrose/water/rapeseed oil and Polyoxyethylene sorbitol monoestearate), the influence of continuous phase viscosity on final droplet size was analysed. Its influence on droplet diameter as a function of the processing time (20-120 seconds), surfactant concentration (0.1-3 wt. %), oil-phase volume fractions (0.1, 0.2 & 0.4) and mean energy dissipation was investigated. It was found that at lower viscosities power ultrasound was more efficient with regards to processing time, emulsifier concentration and power dissipated, consistently yielding sub-micron emulsions. However at high viscosities ($\sim 160\ mPa.s$), the difference in droplet sizes between the two techniques diminished given sufficient processing times. US emulsification consistently produced lower spans in drop size distributions and at higher viscosities could produce minimum droplet sizes at lower exposure times. However it was also found that at higher oil-phase volumes US performance was considerably compromised when emulsifying in viscous mediums.

6.1 Introduction

Mixing of two immiscible liquids is an important process operation for the production of a great variety of consumer products consisting of emulsions (Hall et al., 2011; Behrend et al., 2000). Some important industrial food applications include milk, cream, mayonnaise, butter, margarine (Behrend et al., 2000; McClements, 1998). Such systems are inherently unstable (McClements, 1998; Behrend et al., 2000; Jafari et al., 2007) which can be attenuated by addition of surface-active agents, commonly in the form of low molecular weight emulsifiers (Abismail et al., 1999). The emulsifier serves an important thermodynamic function in reducing the surface free energy by reducing the interfacial tension, γ (cohesive force maintaining drop shape) thus facilitating an increase in the interfacial area ($\Delta G = \gamma \Delta A$) (Abismail et al., 1999). It has also been well documented that emulsion droplet size (EDS) plays a fundamental role in the long-term stability of these systems (McClements, 1998; Jafari et al., 2007); lower EDS improves emulsion stability and its encapsulation capacity(Jafari et al., 2007). Many other emulsion properties, such as rheology and visual appearance also depend on EDS and size distributions (McClements, 1998). Based on EDS, emulsions can be categorised as; macro-emulsions (0.5-100 μm), and nano-emulsions (0.1-1 μm). In general for the production of emulsions energy must be supplied (McClements, 1998). A large amount of energy is required to overcome firstly the interfacial forces and secondly the viscous resistance of the droplets (McClements, 1998; Binks, 1998). A common process for providing sufficient energy is mechanical agitation. Such equipment, often in the form of high-shear rotor-stator mixers, create high-energy dissipations rates due to the kinetic energy dissipated in the small stator volume, thus creating very high shear rates causing break-up (Rueger and Calabrese, 2013).

Recent focus has been on potential of low frequency ($\leq$ 100 kHz), high power ($>$ 10 $W\ cm^{-2}$) ultrasound due to its more efficient transfer of energy for droplet disruption (Delmas and Barthe, 2015; Siddiqui, 2011).

6.1.1 Theory

6.1.1.1 Mechanical agitation

The general approach for understanding the break-up mechanism is by equating the disruptive and cohesive forces acting on a droplet. Kolmogrov's theory predicts the largest surviving stable drop size based on the kinematic viscosity and the rate of energy dissipation (Hall et al., 2011; Clift et al., 2013). The disruptive stresses acting on a droplet are related to the amount of energy per unit volume contained in an eddy, which can be related to the local energy dissipation rate ϵ and kinematic viscosity ν_ε based on the break-up regime. The break-up regime is thus based on Kolmogorov's length scale given by;

$$\eta_k = (\frac{\nu_\varepsilon^3}{\varepsilon})^{\frac{1}{4}} \tag{6.1}$$

6.1.1.2 Ultrasound

The predominant break-up mechanism in power ultrasound emulsification is through generation of cavitation bubbles resulting from pressure differentials during acoustic wave propagation (Jafari et al., 2007; Behrend et al., 2000). Generally cavitations are concentrated in the volume at the tip of the sonotrode (Martini, 2013). Acoustic cavitation at high acoustic pressure has a significant effect on the sonicated medium due to the violent collapse of micro-bubbles, giving rise to intense turbulence and shear stresses at the micro-scale in the liquid phase (Delmas and Barthe, 2015). Viscosity of the medium subjected to ultrasound treatment affects the onset of acoustic cavitation; higher viscosities imply higher attractive forces, thus the higher the threshold intensity for the onset of cavitations (Mason et al., 2015). This is because viscosity of the liquid is directly related to cohesive pressure of the liquid, which determines the resistance of the liquid to formation of cavitations due to expansion (Prebeg, 2004). Thus viscosity and the number of cavitation events, result in higher attenuation levels due to acoustic scattering (Povey and Mason, 1998; McClements, 1998).

Existing studies on the effect of continuous phase viscosity on emulsification by ultrasound where no pre-mixing stage is utilised are limited. The influence of viscosity still requires elucidation in acoustic emulsification for US to be efficiently used in mixing and emulsifying applications. This study compared the influence of continuous phase viscosity in batch acoustic and high shear emulsification.

6.2 Materials and Methods

6.2.1 Materials

The continuous phases comprised of water with varying concentrations of sucrose and non-ionic surfactant $Tween^{®}$60 (Polyoxynethylene sorbitol monoestearate, HLB = 14.9). Sucrose ($\leq$ 99.5 % (GC) and less than 0.1 % free glucose) and $Tween^{®}$60 were both purchased from Sigma Aldrich LTD (UK). The dispersed phase consisted of Rapeseed oil (sourced commercially) with a density of 920 kg/m^3 and viscosity of 63 $mPa.s$ at 20 °C.

6.2.2 Emulsification

6.2.2.1 Mechanical Agitation

High Shear Emulsification by mechanical agitation was conducted using a Silverson L4RT mixer (impeller outer diameter of 22 mm and a gap width of 1 mm) with standard emulsor screen. The emulsification time and impeller speeds were varied (4,000, 7,000 and 10,000 rpm).

6.2.2.2 Power Ultrasound

Batch Ultrasonic emulsification (Viber Cell 750, Sonics, USA) was undertaken with the ultrasonic probe centrally located with an immersion depth of ∼3 mm in the medium. The processing time (20-120 seconds) and ultrasound amplitude (20-80 %) were varied.

The results were presented in terms of the acoustic intensity (I_a) which was determined calorimetrically by measuring the heat absorbed by the liquid medium as a function of time, under adiabatic conditions. The acoustic intensity, $I_a (Wcm^{-2})$, was calculated using eqn. 6.2 as first proposed by Margulis and Margulis (2003):

$$I_a = \frac{P_a}{S_A} \tag{6.2}$$

$$P_a = mc_p(\frac{\delta T}{\delta t}) \tag{6.3}$$

where P_a is the acoustic power, S_A is the surface area of the sonotrode tip, m is the mass of the treated medium, c_p is the specific heat of the medium and $\delta T/\delta t$ is the rate of change in temperature with respect to time.

6.2.2.3 Energy Dissipation Rates

The power absorbed by the treated medium calculated from the calorimetric method was checked by measuring the input electrical power (750 W) through using a digital power meter (ABB Ltd, UK) the results from which corresponded to $\sim$ 10 % probe efficiency and were in agreement to previously reported results by Siddiqui (2011).

The available power for emulsification from rotor-stator device is a function of the rotational speed of the rotor and is calculated by eqn. 6.4. Here the rotor diameter (D) is 2.7 cm, Power Number N_P is 2.2 (Padron, 2001), N is the rotor rotational speed and m is mass of the emulsion.

$$\epsilon_{mean} = \frac{\rho N_P N^3 D^5}{m} \tag{6.4}$$

6.2.3 Emulsion Characterisation

The emulsions were characterised in terms of the average droplet size and the droplet size distributions, both obtained by means of laser diffraction (Master-sizer 2000, Malvern, UK). The average droplet sizes were expressed as the sauter mean diameter ($d_{3,2} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$), and the spread of the data values in a given droplet size distribution was characterised by 'span'. Span is the measurement of the width of the distribution and is defined by;

$$Span = \frac{d_{0.9} - d_{0.1}}{d_{0.5}} \tag{6.5}$$

where 10 % of the population lies below $d_{0.1}$, 90 % of the distribution below the $d_{0.9}$ and $d_{0.5}$ is the median of the distribution.

6.2.4 Interfacial Tension Measurements

The interfacial tension between the continuous medium and dispersed oil phase was measured using a tensiometer K100 (Krűss, Germany) equipped with a titanium Wilhelmy plate (length, width and thickness of 19.9 *mm*, 10 *mm* and 0.2 *mm*, respectively). The plate was initially immersed in a set volume of aqueous phase (20 *ml*) to a depth of 3 *mm*. Subsequently, an interface between the two phases was created by carefully pipetting 50 *ml* of the oil phase over the aqueous phase. The test was conducted over 1800 *s* at 20 °C. The interfacial tension values and the errors are reported as the mean and standard deviation, respectively of three replicate measurements.

6.2.5 Density Measurements

The density of the oil phases and the continuous phases consisting of different amounts of stabiliser were determined using a density rod principle by a tensiometer K100 (Krűss, Germany).

6.2.6 Viscosity Measurements

Viscosity measurements (Kinexus, Malvern, UK) of the continuous phases with different amounts of dissolved stabiliser was performed using a dynamic shear rheometer using a shear rate range of 0.1 to 100 s^{-1}. All measurements were performed at 20 °C using the double-gap cell geometry.

6.3 Results and Discussion

6.3.1 Influence of Continuous Phase Viscosity

The influence of continuous phase viscosity on droplet break-up by US and HS emulsifications was investigated at two seperate energy inputs. All other processing and formulation parameters were kept constant (EC = 3 %, $\varphi = 0.2$ and time = 120 s) (Fig. 6.1). It could be observed that power input was inversely proportional to $d_{3,2}$ at viscosities below 40 *mPa.s* for both emulsification techniques. However within viscosities at and above 40 *mPa.s*, the $d_{3,2}$ was independent of power input and emulsification mechanism, thus all $d_{3,2}$ were similar and sub-micron. Maximum break-up occured at highest viscosity of 160 *mPa.s* which yielded droplet sizes two and one orders of magnitude lower for HS and US respectively.

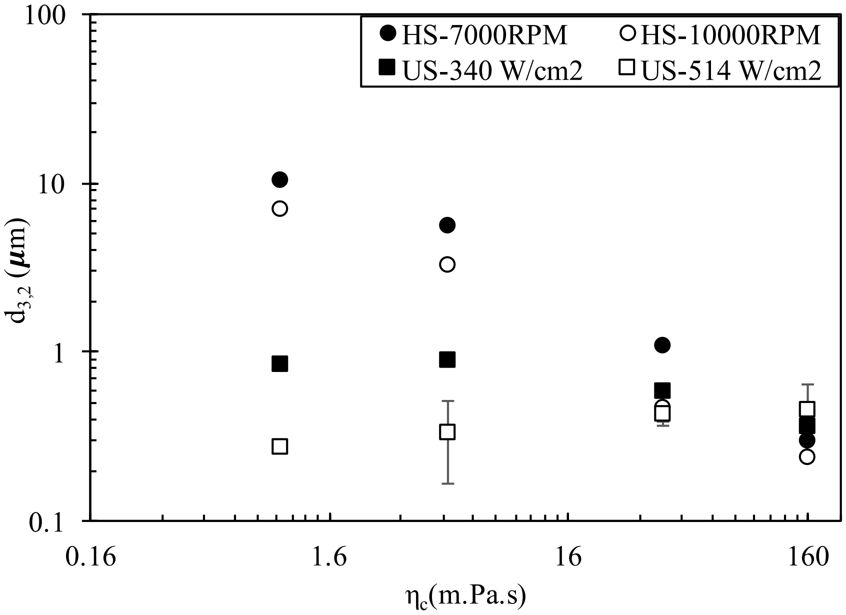


Figure 6.1: Influence of continuous phase viscosity and power input on the droplet diameters. (Systems at $\varphi = 0.2$, EC = 3 %, t = 120 s)

At viscosities below 40 *mPa.s* all EDS values for US were already within the sub-micron range and accordingly an order of magnitude lower compared to those produced by the HS emulsification. Moreover, at the highest acoustic intensity ($\sim$ 515 Wcm^{-2}) for US, droplet sizes were independent of viscosities, however there was further reduction in $d_{3,2}$ with increasing continuous phase viscosities at a lower acoustic intensity of $\sim$ 340 Wcm^{-2}. Increasing the acoustic intensity (amplitude) yielded greater ultrasonic energy transmission to the system. The acoustic power imparted to the system controls the number of bubbles; with a higher intensity (*i.e.* amplitude) generating more bubbles (Santos et al., 2009) and the unstable nature of these bubbles meant, the number of cavitation events were proportionally increased. Cavitation events result in high levels of hydrodynamic shear which act upon the dispersed phase, reducing droplet size.

Table 6.1: The effect of stabiliser addition on the physical properties of the emulsion continuous phases

Stabiliser Concentration	Physical Properties	
	Viscosity ($mPa.s$)	Density (kg/m^3)
0	1.3	998
30	5.0	1130
50	40	1235
65	160	1325

The results for the US emulsification suggested that even at highest viscosities, the conditions for the onset of cavitation were fulfilled and even smaller droplets could be produced. This is in accordance with the observations of Behrend et al. (2000), whom concluded that at high viscosities of up to 1800 $mPa.s$ cavitation was observed for continuous US emulsification. However a further trend was the reduction in $d_{3,2}$ for US emulsification at higher viscosities (reduction by 50 %). The cavitation intensity which determined the extent of break-up is influenced by the acoustic intensity (Povey and Mason, 1998). Viscosity of the treated environment has been reported to disrupt the US diffusion hence attenuating acoustic intensity (Povey and Mason, 1998). However here it was observed that higher viscosities improved emulsification by US and the extent of droplet break-up was actually increased.

All droplet sizes except for the most inviscid continuous phases (*i.e.* 1 $mPa.s$) were smaller than the Kolmogorov's length scale η_k, indicating that viscous stresses acting on the droplet were dominant. Therefore more viscous continuous phases transmitted shear stresses to the droplet interface more effectively (Hall et al., 2011). When the droplet break-up due to viscous shear forces were dominant, the viscous shear forces in the continuous phase caused a velocity gradient around the interface and deformed the fluid particle, leading to break-up (Hall et al., 2011; Padron, 2001).

6.3.1.1 Influence of Processing Time

In order to investigate the influence of viscosity on lowering cavitation intensity throughout the treated medium, the influence of exposure time on the $d_{3,2}$ was compared at the two extreme viscosities (*i.e.* 1 & 160 $mPa.s$), while maintaining constant acoustic intensity, EC and oil-phase volume. The comparison of its influence on droplet break-up ($d_{3,2}$) between US and HS emulsification is represented in fig. 6.2.

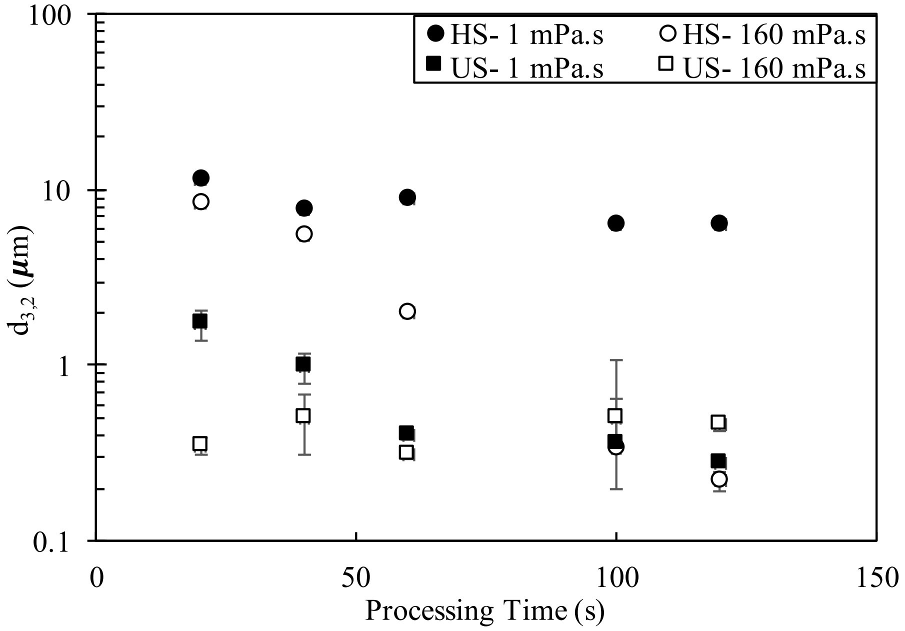
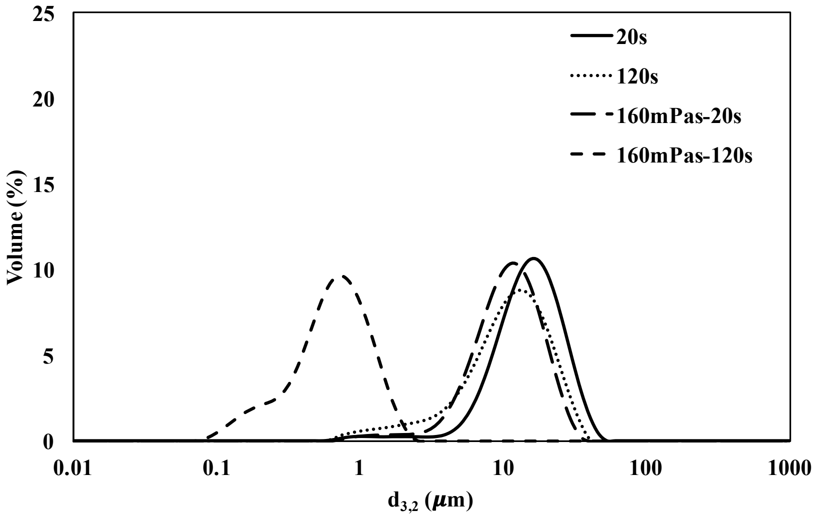
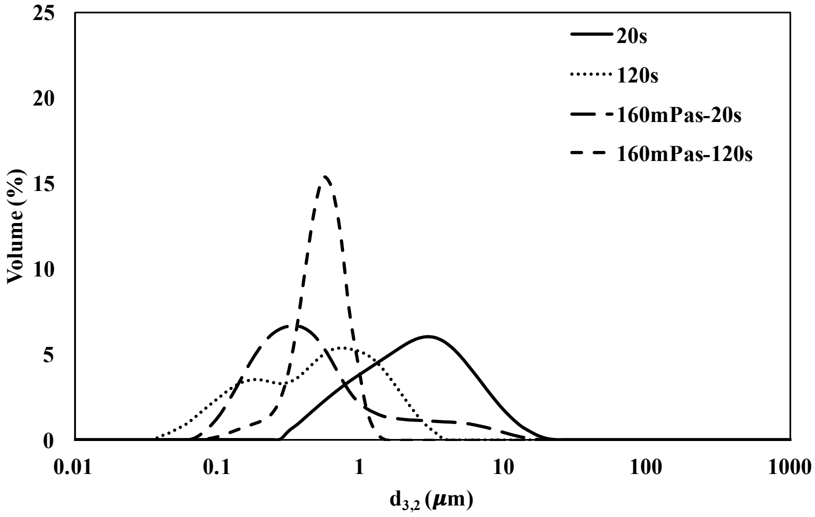


Figure 6.2: Influence of processing time on emulsification at low and high viscosities between HS and US. (Systems at $\varphi = 0.2$, EC = 3 % , acoustic intensity of 515 Wcm^{-2} for US and 10,000 RPM for HS emulsification.

The variation in droplet size for the inviscid systems (*i.e.* 1 $mPa.s$) as a function of exposure time was minimal. However increasing the continuous viscosity to 160 $mPa.s$ yielded different behaviours for both emulsifying techniques. For HS emulsification the $d_{3,2}$ were similar regardless of power input for up to 40 s, beyond which there was a sharp decrease when emulsifying at high viscosities. At high viscosities HS emulsification was capable of producing $d_{3,2}$'s comparable to US emulsions, providing the processing times were above 100 s. For US emulsified systems the difference was less significant, increasing the viscosity of the treated medium decreased the exposure time required for maximum droplet break-up to occur. A difference between the systems as a function of viscosity was only observed at exposure times lower than 40 s, beyond this period the effect of viscosity diminished and $d_{3,2}$'s became comparable. The droplet size distributions (DSD) shown in fig. 6.3 displayed wider DSD's at lower viscosities and narrower DSD (span = 0.8) at higher viscosity with 120 s of emulsifiaction. At higher viscosities the wider distributions (a span of ∼6) exhibited a shoulder of droplets at higher diameters which diminished with increased exposure times yielding lower span. However within low viscosities and low exposure times the span was lower (∼4). It can be concluded that given enough time within high viscosities, better break-up could be achieved, however at lower exposure times the acoustic intensity attenuation compromised break-up of the entire oil drop population. It must be noted that this effect was observed despite cooling of the emulsified medium. The increase in temperature and thus the decrease in viscosity of the treated medium was minimized, however temperature increases of ∼5-7 °C were unavoidable, but this did not correspond to significant decreases in viscosity of the medium.



(a) High shear emulsification



(b) Ultrasound Emulsification

Figure 6.3: The Droplet size distributions for both US and HS emulsification techniques as function of viscosity and processing time; (a) High shear emulsification and (b) Ultrasound emulsification

At highest viscosities and longer processing times, US emulsions presented much narrower distributions, however in all other cases HS emulsions exhibited lower spans. All DSD's for HS emulsification were similar with spans ~ 2-3. Viscosity of the continuous phase for both systems determined the EDS and DSD's of the final emulsions. For the high shear break-up mechanism, when the viscosity ratio of the emulsion reduced below 5 (here at 0.45), the droplet break-up mechanism shifted from turbulent inertial to turbulent viscous (Binks, 1998), which is similar to simple shear break-up in laminar flow (Windhab et al., 2005) thus maximising the break-up. However for US systems the effect of viscosity can be attributed to the findings of Behrend et al. (2000), where they concluded that if the majority of the dissipated energy is within the vicinity of the droplet surface then the disruptions are more efficient than if the dissipated energy was evenly distributed throughout the continuous phase. Thus this energy localisation although uneven throughout the process could account for why longer exposure times were required for complete break-up and more importantly why higher rates of break-up were observed in systems with higher viscosities.

6.3.2 Effect of Emulsifier Concentration (EC)

Cavitation onset as well as being dependent on liquid's viscosity also changes with interfacial tension (Povey and Mason, 1998; Prebeg, 2004). Interfacial tension also reduces the cohesive forces maintaining droplet shape, facilitating its break-up within the shear-field or the turbulent stresses of cavitation. Therefore it was deemed necessary to investigate the effect of EC on $d_{3,2}$. The influence of viscosity and EC (varied 0.1 to 3 wt.%) was investigated maintaining all other parameters constant ($\varphi = 0.2$, t = 120 s, acoustic intensity of 515 Wcm^{-2} for US and 10,000 RPM for HS emulsification).

The results shown in fig. 6.4 showed that EC within the investigated range had little effect on $d_{3,2}$ when using US emulsification and was only significant when present below concentrations of 1 wt.% for HS emulsification. The results indicated that below 1 wt.%, it was not possible to achieve sub-micron emulsions using HS rotor-stator mixers even when turbulent viscous stresses dominated. For a non-dilute system such as the ones used throughout this study (*i.e.* higher oil-phase fractions of 0.2), the droplet sizes produced were generally considered to be determined by a dynamic equilibrium between the rates of droplet breakage and coalescence (Zhang et al., 2012). The need for less surfactant for US emulsification could also be linked to the additional foam formation that occurred during HS emulsification which inevitably captured a significant portion of the emulsifier.

This result was also confirmed by Abismail et al. (1999) in their comparison of mechanical agitation to US.

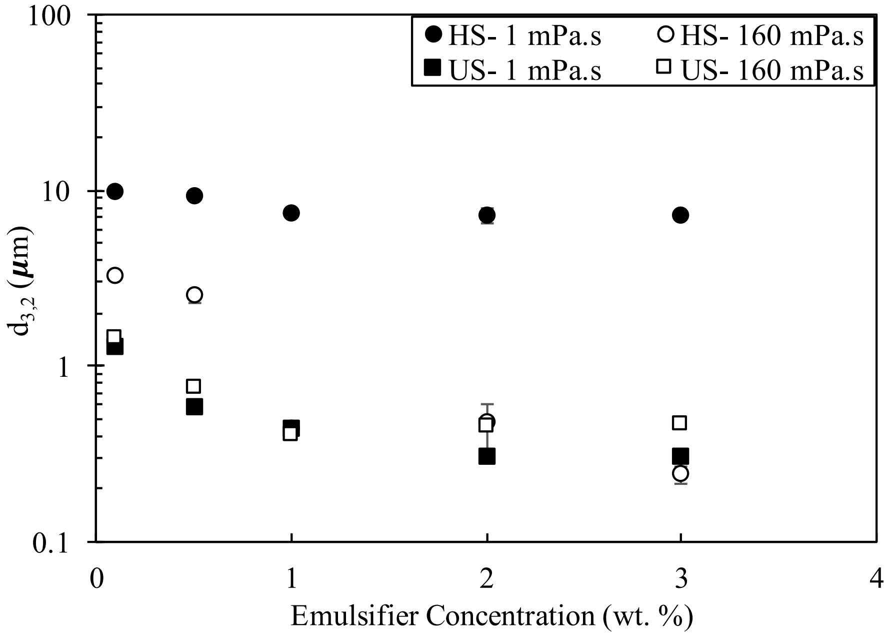
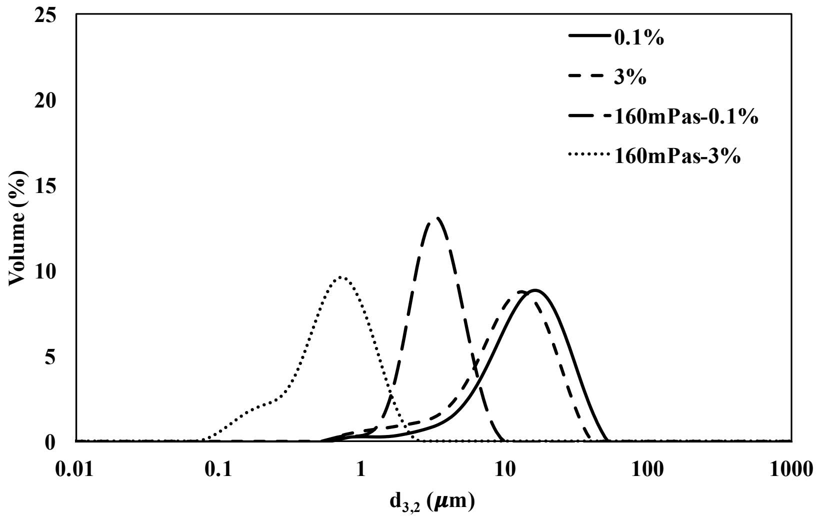
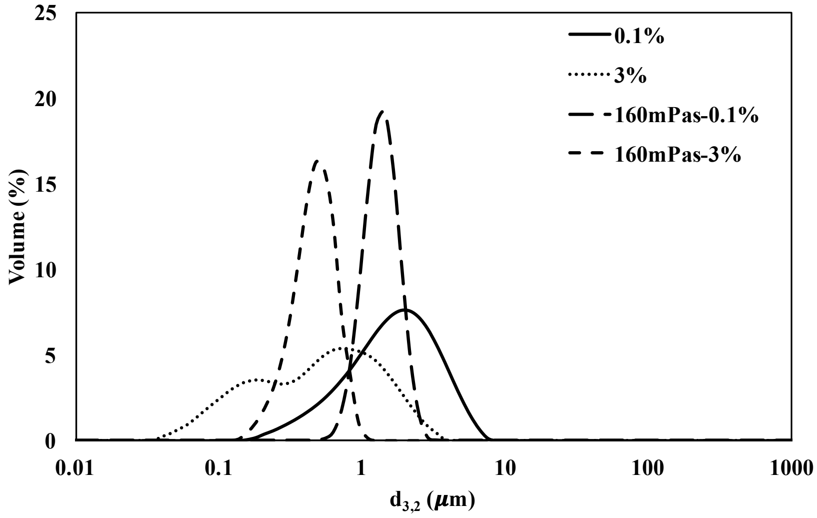


Figure 6.4: Influence of emulsifier concentration at low and high viscosities; comparison between HS and US. (Systems at $\varphi = 0.2$, t = 120 s, acoustic intensity of 515 Wcm^{-2} for US and 10,000 RPM for HS emulsification)

Moreover when inertial stresses dominate, the energy transferred within the continuous phase reduced the rate of coalescence efficiency due to the smaller time scales (Shinnar, 1960). As the viscous stresses start dominating, the smaller velocities associated lead to higher coalescence efficiency. The presence of excess surfactant increases the time needed for film thinning. Therefore when comparing the droplet diameters formed within inviscid regimes the differences between low-surfactant and surfactant laden systems was ~2 μm, compared to the viscous continuous phases where the $d_{3,2}$ could reduce by an order of magnitude.



(a) High Shear Emulsification



(b) Ultrasound Emulsification

Figure 6.5: The Droplet size distributions for both US and HS emulsification techniques as function of viscosity and surfactant concentrations. (a) high shear emulsification and (b) Ultrasound emulsification

The DSD's in fig. 6.5 showed significantly higher spans when increasing the EC in the systems for both emulsification techniques. However for US systems this was only prevalent when emulsifying in low viscosity mediums. At higher viscosities the spans were not significantly different as a function of EC and this result seemed to further stress the hypothesis about the localised energy dissipations within close proximity of the US probe due to viscosity. It has been indicated that within smaller volumes, localisation of acoustic intensity has an enhancing effect on droplet break-up (Behrend et al., 2000). Further work utilising higher emulsification volumes is required to fully investigate this effect.

The interfacial tension of the systems was investigated to further elucidate the contribution of EC on droplet break-up. Table.6.2 shows a dependency of σ on sugar and Tween 60 concentrations. At higher EC a reduction in interfacial tension was observed with additional sugar concentration.

Table 6.2: The influence of stabiliser and surfactant concentrations on the interfacial tension of the system

Stabiliser Concentration	Interfacial Tension (mN/m)	
	0.1 wt.%	3 wt. %
0	5.8 ± 0.4	4.6 ± 0.4
65	6.2 ± 0.2	2.0 ± 0.6

Based on the results in tab. 6.2, the additional break-up in US emulsification could also be as a consequence of reduced interfacial tension. Both liquid viscosity and surface tension were expected to inhibit cavitation. The higher the natural cohesive forces acting within a liquid (*e.g.* high viscosity and high surface tension) the more difficult it is to attain cavitation (Santos et al., 2009; Prebeg, 2004). Therefore lower interfacial tensions not only reduced the cohesive forces maintaining droplet shape but could also facilitate the formation of bubbles due to cavitation which in collapsing could break-up the oil drops. Here the reduction appeared to be in synergy with the concentration of Tween 60 present. The presence of sugar has also been reported to change the water structure by increasing the number of monomeric water molecules, subsequently the reduced cohesive water-water interactions lead to a change in the energy gradient at the interfaces formed. Giangiacomo (2006) reported that sugars behave as structure breakers of water clusters and the dissolving of sugar molecules leads to H-bond breakage in the cluster. This increases the distance between molecules as well as the number of free water molecules leading to the hypothesis that sugars change the structure of water by increasing the number of monomeric water molecules (*i.e.* decreasing the number of H-bonds), which subsequently leads to reduced cohesive water-water interactions and a decrease in the interfacial tension. Similar observations have been reported by Pawlik (2012), where it was shown that glucose, sucrose and fructose all reduce interfacial tension between oil and water. Other researchers have reported that sucrose slightly decreases the interfacial tension (by around 9 %) between water and olive oil (Howard and Sollman, 1924).

6.3.3 Comparison of Mean Energy Dissipation

The efficiency of the emulsification mechanisms to break-up droplets in the two flow regimes was investigated as a function of the calculated energy dissipation rates. From fig. 6.6 it could be observed that there are two distinct break-up regimes with respect to energy dissipation. At low viscosities (*i.e.* 1.3 $mPa.s$) similar rates of energy dissipation yielded droplets of two orders of magnitude lower for US emulsification compared to HS. However at 160 $mPa.s$ where droplet break-up was dominated by viscous stresses, similar rates of energy dissipations yielded $d_{3,2}$ comparable to those observed using US, thus reducing $d_{3,2}$ of the HS systems by two orders of magnitude.

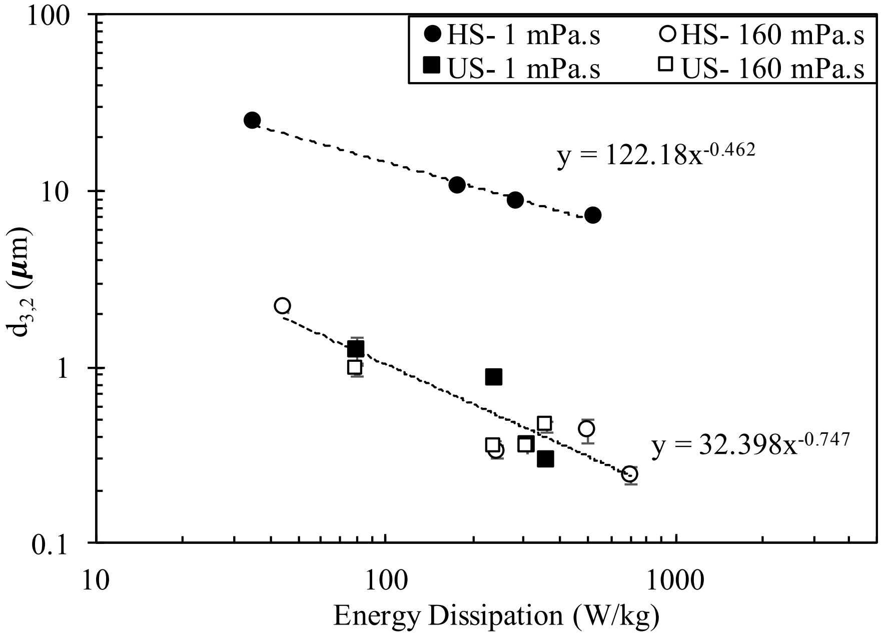


Figure 6.6: Influence of rates of energy dissipation on droplet size at low and high viscosities; comparison between HS and US. (Systems at $\varphi = 0.2$, EC = 3 % and t = 120 s)

Therefore viscosity in localising energy dissipation rates, had a much more significant effect in conventional mechanical mixing compared to US emulsification. In US emulsification, viscosity only reduced the spans of the emulsions formed (from ~2.5 to ~0.8) whilst the $d_{3,2}$ decreased by an order of magnitude for HS emulsification. The energy dissipations calculated were comparable to those reported by Siddiqui (2011) using the same equipment but only utilising low viscosity systems. The effect power P_o is inversely proportional to the Reynolds number (Re) of the systems below (Re = 10^4) (Padron, 2001). However as for more viscous systems Re was ~ 2500, higher energy dissipations were likely when using HS as the emulsification mechanism, although this only significantly affects the P_o when Re < 100 according to the standard Re / P_o correlation (Padron, 2001). The energy localisation effects were likely to describe the effects observed here, although the reduction in interfacial tensions of systems with higher sugar concentrations (shown in tab. 6.2), could also facilitate higher levels of break-up by reducing the cohesive forces resisting droplet deformation.

6.3.4 Influence of Oil-Phase Fraction, φ on $d_{3,2}$

The influence of volume fraction of oil on the droplet break-up for both US and HS emulsification techniques was compared whilst maintaining all other parameters (Fig. 6.7). It was found that viscosity in general reduced the dispersing power of in both US and HS emulsification.

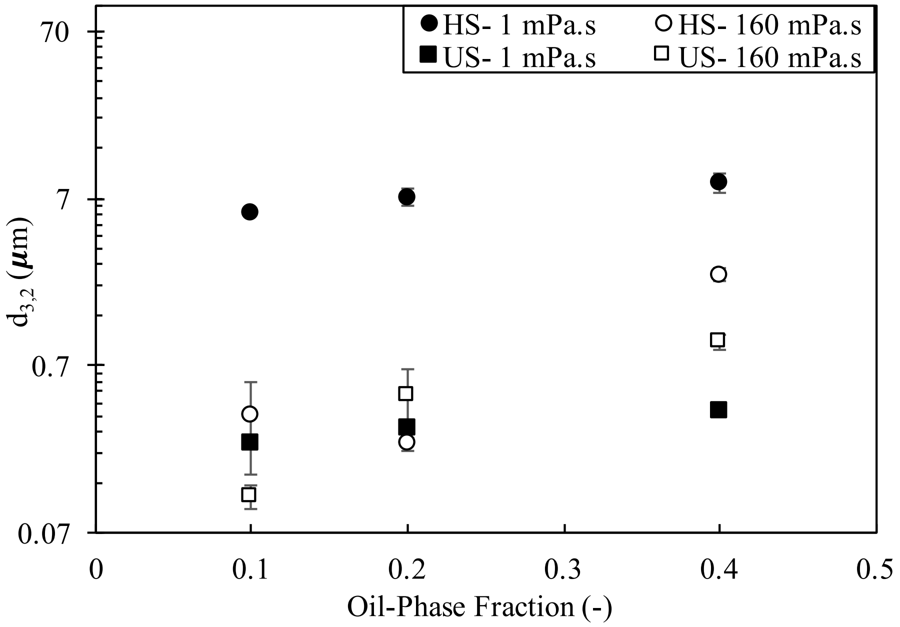
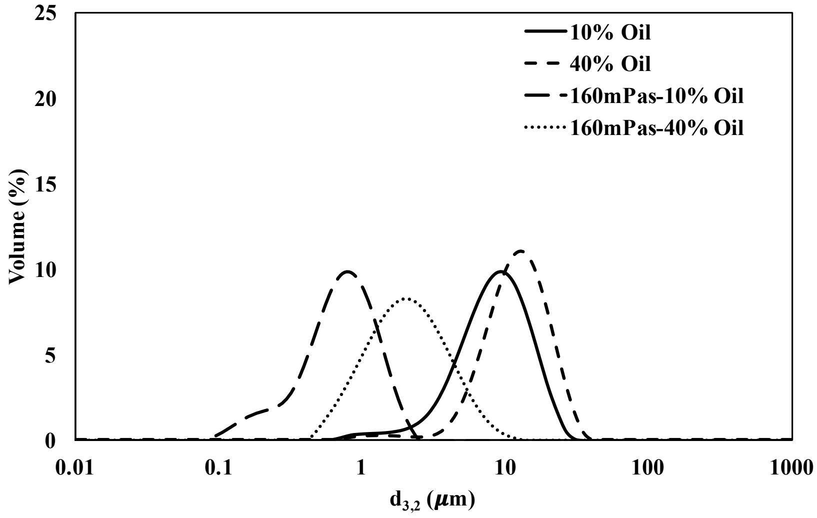
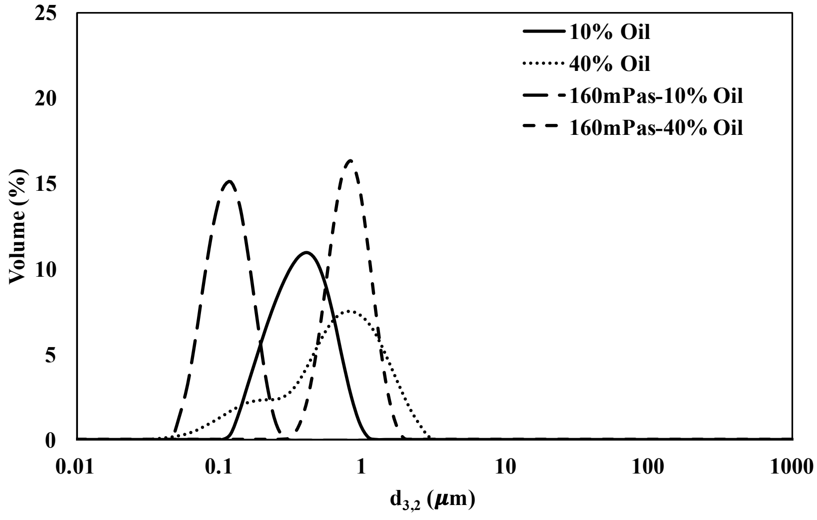


Figure 6.7: The influence of oil-phase fraction on the droplet break-up at low and high viscosities in US and HS emulsification. EC = 3 % and t = 120s, acoustic intensity of 515 Wcm^{-2} for US and 10,000 RPM for HS emulsification

Fig. 6.7 shows the $d_{3,2}$ increased by an order of magnitude for US emulsification in viscous mediums when increasing the phase fraction of oil from 0.1 to 0.4. In comparison, when the viscosity of the medium was low, then the effect of oil-phase fraction on $d_{3,2}$ was much less significant. Fig. 6.8 shows the DSD's function of the dispersed phase fraction. It was found that within HS emulsification, increasing the oil phase fraction yielded higher spans and that these spans further increased at higher viscosities (from ~1.4 to ~1.8). The opposite trend was observed when emulsification with US was utilised. At lower viscosities, the spans increased from ~1.2 to ~2.1 when increasing the oil phase fraction (from 0.1 to 0.4). However at highest viscosities, the spans were significantly lower and remained ∼0.8 regardless of the phase fraction, even when significantly higher $d_{3,2}$ was observed (in the case of 40 % oil). It could be concluded that US emulsification consistently produced narrower DSD's at higher viscosities regardless of oil-phase fraction, where as within traditional HS mixing, the spans were dependent on both viscosities and phase fractions.



(a) High Shear Emulsification



(b) Ultrasound Emulsification

Figure 6.8: The Droplet size distributions for both US and HS emulsification techniques as function of viscosity and dispersed phase volume fraction. (a) high shear emulsification and (b) Ultrasound emulsification

The transmission of energy in acoustic cavitation by the associated bubble collapse concentrates very high amounts of energy at the micron scale (Delmas and Barthe, 2015).The emulsification process by US is deemed as a successive break-up process consisting of two distinct stages; the incipient instability of the oil-water interface which produces large oil drops, and a second stage of break-up of these larger droplets by the shock-waves induced from the cavitation bubbles (Mason et al., 2015). Therefore the results could be ascribed to localisation of energy which meant attenuation of the transmission of the shock waves produced by the cavitation phenomena not allowing for the break-up of the higher fraction of oil present within the system (*i.e.* a dampening effect of the acoustic intensity not allowing break-up when there is a high fraction of oil present).

Traditional HS emulsification results from the transfer of kinetic energy from the large eddies to smaller ones down to Kolmogrov scale. However for traditional HS mixing, Rueger and Calabrese (2013) reported that at lower continuous phase viscosities where break-up is dominated by inertial stresses, the droplet diameter increases linearly with dispersed phase volume fraction. As stated earlier the final drop diameters could also be influenced by coalescence attributed to insufficient surface coverage especially given the higher phase volume fractions. As the oil phase fraction was increased to 0.4 the range of droplet diameters decreased. This indicated that at higher phase fractions of oil the collision frequency increased. If the surfactant was not transported by the turbulent eddies to the newly formed interfaces quickly enough, then the drops upon collision could attach more easily leading to coalescence. Khopkar et al. (2009) also varied phase volumes from 10% to 50% in a batch system using an oil of viscosity 60 $mPa.s$; it was found that $d_{3,2}$ was proportional to φ and the DSDs became wider with increasing φ.

6.4 Conclusion

The comparison of emulsification by conventional high shear mechanical agitation to low frequency (20 kHz) ultrasound in viscous media, showed that for limited emulsion volume; at higher viscosities, ultrasound emulsification required less processing time and less surfactant to produce sub-micron emulsions for similar rates of energy dissipation. It was also observed that at higher oil phase fractions, US was a more effective dispersing tool than conventional mechanical agitation. Moreover acoustic emulsification was more efficient at higher viscosities, requiring less time to produce maximum droplet break-up and the emulsions produced in more viscous mediums showed lower spans (*i.e.* narrower distributions) due to a more localised dissipation of energy by the ultrasound probe.

6.5 References

List of References

Abismail, B., Canselier, J., Wilhelm, A., Delmas, H., and Gourdon, C. (1999). Emulsification by ultrasound: drop size distribution and stability. *Ultrasonics Sonochemistry*, 6(1–2):75 – 83.

Behrend, O., Ax, K., and Schubert, H. (2000). Influence of continuous phase viscosity on emulsification by ultrasound. *Ultrasonics Sonochemistry*, 7(2):77 – 85.

Binks, B., editor (1998). *Modern Aspects of Emulsion Science.* Royal Society of Chemistry.

Clift, R., Grace, J., and Weber, M. (2013). *Bubbles, Drops, and Particles.* Dover Civil and Mechanical Engineering. Dover Publications.

Delmas, H. and Barthe, L. (2015). Ultrasonic mixing, homogenization, and emulsification in food processing and other applicationsmmas. In Gallego-Juárez, J. A., Graff, and F., K., editors, *Power Ultrasonics*, pages 757 – 791. Woodhead Publishing, Oxford.

Giangiacomo, R. (2006). Study of water - sugar interactions at increasing sugar concentration by nir spectroscopy. *Food Chemistry*, 96(3):371–379.

Hall, S., Cooke, M., El-Hamouz, A., and Kowalski, A. (2011). Droplet break-up by in-line silverson rotor-stator mixer. *Chemical Engineering Science*, 66(10):2068 – 2079.

Howard, R. and Sollman, T. (1924). The interfacial tension of some aqueous solutions against oils, as corrected for specific gravity. *The Journal of Physical Chemistry*, 28(12):1291–1296.

Jafari, S. M., He, Y., and Bhandari, B. (2007). Production of sub-micron emulsions by ultrasound and microfluidization techniques. *Journal of Food Engineering*, 82(4):478 – 488.

Khopkar, A. R., Fradette, L., and Tanguy, P. A. (2009). Emulsification capability of a dual shaft mixer. *Chemical Engineering Research and Design*, 87(12):1631 – 1639.

Margulis, M. A. and Margulis, I. M. (2003). Calorimetric method for measurement of acoustic power absorbed in a volume of a liquid. *Ultrasonics Sonochemistry*, 10(6):343–345.

Martini, S. (2013). *SONOCRYSTALLIZATION OF FATS.* Springer.

Mason, T., Chemat, F., and Ashokkumar, M. (2015). Power ultrasonics for food processing. In Gallego-Juárez, J. A., Graff, and F., K., editors, *Power Ultrasonics*, pages 815 – 843. Woodhead Publishing, Oxford.

McClements, D. (1998). *Food Emulsions: Principles, Practice, and Techniques.* Contemporary Food Science. Taylor & Francis.

Padron, G. A. (2001). Measurement and comparison of power draw in batch rotor-stator mixers. Master's thesis, Department of Chemical Engineering, University of Maryland.

Pawlik, A. K. (2012). Duplex emulsions for healthy foods.

Povey, M. and Mason, T. (1998). *Ultrasound in Food Processing.* Springer.

Prebeg, Z. (2004). Viscosity and cohesion pressure. *Journal of Theoretics*, 6-5.

Rueger, P. E. and Calabrese, R. V. (2013). Dispersion of water into oil in a rotor - stator mixer. part 2: Effect of phase fraction. *Chemical Engineering Research and Design*, 91(11):2134 – 2141. Mixing.

Santos, H. M., Lodeiro, C., and Capelo-Martinez, J.-L. (2009). The power of ultrasound. In *Ultrasound in Chemistry*, pages 1–16. Wiley & Co.

Shinnar, R. (1960). On the behaviour of liquid dispersions in mixing vessels. *Journal of Fluid Mechanics*, 10(2):259–275.

Siddiqui, S. W. (2011). Mixing performance of various geometries - emulsification perspective. *Procedia Food Science*, 1:131 – 137.

Windhab, E., Dressler, M., Feigl, K., Fischer, P., and Megias-Alguacil, D. (2005). Emulsion processing - from single-drop deformation to design of complex processes and products. *Chemical Engineering Science*, 60(8–9):2101 – 2113. 5th International Symposium on Mixing in Industrial Processes (ISMIP5).

Zhang, J., Xu, S., and Li, W. (2012). High shear mixers: A review of typical applications and studies on power draw, flow pattern, energy dissipation and transfer properties. *Chemical Engineering and Processing: Process Intensification*, 57-58:25 – 41.

7 | Conclusions and Recommendations for Future Work

7.1 Conclusions

In this chapter a brief summary of the main observations as a consequence of the experimental work undertaken is provided. Based on the conclusions and the shortcomings of the research, some extensions to this body of work are also proposed. Experiments that could further elucidate some of the phenomena observed through the presented effort are discussed and suggestions of further research required are put forth.

The principle objective of this thesis was the building of an understanding and clarifying the formulation interactions at a microstructural level within cake formulations with focus on derivation of novel more organic methods/processes for stabilisation of the meta-stable systems prevalent in the cake batters. This research thus provided the basis for translation of several learnings into functional, manipulate-able processes that directly correlate to either final cake or foam quality. The method of achieving these learnings was the decomposition of the microstructure, and subsequent development of an understanding of mainly starch-protein functionality as well as the mixing process' contributions to microstructural formation of cake batters. In this context, the thesis is now briefly summarised with focus on the key learnings derived.

7.1.1 Review of Thesis and General Findings

7.1.1.1 Understanding of protein-starch interactions in stabilising wet-foam systems; Influence of starch heat-treatment

- *Wet foams stabilised by a combination of hydrophobic starch and proteins produced significantly longer half-lives.*

  The physiological properties of the OSA-starch species; mainly its hydrophobicity and size were found to influence the stability of protein stabilised wet-foams. If starch species' contact angle was between 60-70° *i.e.* the optimised angle for adsorption of particulate like species at A/W interface, then their exclusion and structuring in foam films seems to be the driving force for slight stability increases due to inhibiting effects on the initial drainage. However more hydrophobic starch species ($\theta \sim$ 80-90°) were found to enhance EWP foam stability dramatically, attributed to a multitude of mechanisms, mainly increased hydrophobic interactions resulting in higher interfacial dilatation moduli and inhibition of rates of drainage.

- *Protein type was important in determining the nature of synergy with the starch species.*

  The use of unconventional and more nutraceutical protein sources such pea protein isolate as potential replacers of EWP showed drastically different foaming properties. The native protein foams were less stable with lower overruns and subsequent addition of starch species seemed to demonstrate more of an antagonistic effect rather than the synergy observed with EWP. The trial of these proteins only at pH 7.0 meant that complete dissociation of pea globulins was not possible, thus reducing their amphiphillic properties. Additionally pea protein was considerably more susceptible to presence HT starch species, lowering the stabilities of the foams comprising it or significantly reducing foaming capacity.

- *Heat-treatment of native starch enhanced its hydrophobicity in terms of contact angle* *by a hypothesised surface protein denaturation effect.*

Rice starch that was subject to heat-treatment according to Rayner et al. (2012) was found to not only demonstrate higher contact angles but when utilised in conjunction with EWP, its effect as an anti-foaming agent diminished and foam stability was enhanced. There was little evidence of any interfacial effects observed, but most likely a consequence of lamella drainage inhibition through additional hydrophobic protein-protein interactions with contribution from the exposed surface proteins of the starch granule potentially inducing a level of network formation. Additionally the surface of the HT starch suspended in water was drastically different to that of the native granule as observed by Cryo-SEM microscopy.

7.1.1.2 Influence of heat-treatment on performance of wheat flour in a model angel cake formulation

- *A combination of smaller flour particle size due to additional processing and heat-treatment of flour yielded more aerated batters and final cakes.*

The influence of heat-treatment on individual flour components could not be fully evaluated, however a combination of smaller flour particle size and heat-treatment on all flour components yielded batters with better elasticity and aeration capacity. Moreover, the HT systems observed lower decrease in both G' and δ values as a function of EWP reduction. The final cakes also showed less of an increase in the K values compared to WF flour systems which observed a marked increase in K values with EWP reduction owing to lower final cake volumes.

7.1.1.3 Evaluation of quinoa starch in angel cake formulation and effect of heat-treatment

- *Dilution of heat-treated wheat flour with starch increases the volume of angel cakes.*

Stronger EWP-starch gels were produced at higher dilution rates of the heat-treated flour, resulting in smaller more uniform final cake crumb structures with higher volumes. It was found that aeration of batter, elasticity and viscosity all increased with higher starch dilutions. Starch contributed significantly to gel strengths measured at 90 and 100 °C resulting in more porous and less hard final cake structures.

- *Use of quinoa starch in a model cake formulation showed that starch size and heat treatment influence batter aeration, visco-elasticity and viscosity.*

  Quinoa starch at high substitution levels (> 60 %) in the batter produced significantly lower SG, more extensive networks and higher viscosities. This was attributed to quinoa granule's smaller size, and the fact that heat-treatment could enhance its interactions with protein thus modifying the rheological and physical properties of the batter, which was better observable at 100 % starch substitution levels.

- *Heat-treatment had significant a effect in more aerated systems and increased gel strengths at elevated temperatures for wheat starch whilst not influencing quinoa starch's heating properties.*

  While heat-treatment of both quinoa and wheat starch granules contributed to a more extensive network formation and in the case of quinoa lower specific gravities within the batter, only wheat starch granules showed a significant difference in their gel forming capacities. The weak gels formed at 90 and 100 °C by HT wheat starch were consistently higher in strength compared to those formed by the native wheat granule. This effect was inconsistent for quinoa starch granules.

- *The weak gel model and x-ray visualisation were shown to be effective tools for predicting and assessing final cake structures respectively.*

  Rheological measurements of gel strength showed good correlation to the final cake properties. The gel strength A_F was a representative value for the strength of the formed network with z values corresponding to number of air bubbles entrapped within the batter system *i.e.* the number of interactions. X-ray CT proved a powerful tool for visual characterisation of the cake crumb structure. Good initial correlations between structural components, porosity, wall thickness and mechanical properties were found. However, with derivable parameters such as degree of anisotropy and percentage of closed and open bubbles from the scans, a more comprehensive mechanical modelling of the cake microstructure could be feasible (Gibson and Ashby, 1987).

7.1.1.4 Comparison of power ultrasound efficacy to mechanical agitation for emulsification

- *Emulsification in more viscous systems showed higher efficiency for both high shear and acoustic emulsification.*

  Smaller droplets with narrower, more uniform distributions were consistently obtained when emulsification took place at continuous phase viscosities of 160 $mPa.s$ as opposed to 1.3 $mPa.s$ This was particularly apparent at higher oil-phase fractions (0.4), where narrower distributions were obtained. This was reported as specific to emulsification in smaller volumes as theory and previous studies have demonstrated that viscosity will acts to attenuate the acoustic field as the distance from the sonotrode increases (Behrend et al., 2000). However for emulsification volumes of 100 ml viscosity localised energy dissipations and maximised break-up.

- *The emulsifier was more efficiently utilised when emulsification occurred firstly using US, secondly as viscosity increased to* $\sim$ *160* $mPas$.

  It was shown that emulsification with US requires less emulsifier for achieving submicron droplet sizes. Also less emulsification time was required for maximum droplet break-up to occur and yielded narrower drop size distributions. The increase in transmission of energy by the continuous phase through localising effects were attributed to the more efficient break-up.

7.2 Recommendations for Future Work

The following are particular areas the author feels warrant further investigation as a result of the findings presented throughout this thesis.

- *Use of quinoa starch in EWP stabilised wet-foams and acquisition of further information such as bubble size distribution and interfacial shear moduli.*

  Quinoa starch with a distinctly smaller and more mono-dispersed granule could enhance the synergy observed with the EWP. Quinoa starch was shown to enhance the aeration capacity of batters, however in a more dilute protein-starch system, the quinoa granule should prove to be a better stabilising agent compared to the larger rice starch granule. Acquisition of information on the foam bubble size distribution and interfacial shear rheology would elucidate more information about the mechanisms contributing to the increased stabilities and their interactions at the A/W interface.

- *Visualisation of heat treated quinoa starch starch within cake batter with use of confocal microscopy.*

  In a study by Dürrenberger et al. (2001) Safranin O was used as a staining probe for starch within a bread dough formulation providing the capacity to visualise its distribution using confocal laser scanning microscopy (CLSM). This principle applied to heat-treated quinoa or wheat starch could elucidate the nature of change in heat-treated starch granules functionality and thus its positioning within the batter structure. The visualisation of the starch granules will enhance the understanding of the influence of the surface effects reported here. This will provide the possibility to examine the microstructure of batters in three dimensions and also transformations during baking may be followed in situ. Simultaneous labelling of both protein and starch with probes which are specific for each component such as Acid Fuchsin (Dürrenberger et al., 2001) or alternatively, covalently bonded probes (to avoid double staining) can shed more light on the microstructure. Combining of CLSM with rheological measurements, will provide the possibility to obtain detailed microstructural information.

- *Further incremental build-up of the cake batter structure with addition of other ingredients in particular a fat/oil phase.*

  Changing the mixing process for it to be initiated by "creaming" will dramatically change the nature of the air dispersions formed. The process of creaming will require formation of largely fat stabilised air/water interfaces with large quantities of sugar providing sufficient viscosity. During baking the fat will coat the protein and the starch fractions acting as a tenderizer. Both of these function will be expected to modify the reported phenomena observed within a simpler EWP-starch foam. The potential contribution of starch as a stabilising agent as well as its water absorption capacity will be further inhibited. Meza et al. (2011) and Chesterton et al. (2015) have shown that heat treatment of flour still produces more cohesive batters with higher gel forming capacities at elevated temperatures, however the extent of the starch fraction's contribution requires validation in a purer starch based system (*i.e.* removal of other flour fractions).

- *Evaluation of ultrasound as a mixing aid and for alteration of the functional properties of proteins.*

  The setting-up and incorporation of a high power ultrasonic bath with a planetary mixer demonstrated significant effects on the rheological properties of cake batters and cakes (Tan et al., 2011). It was observed by Tan et al. (2011) that Ultrasound exposure could yield pre-bake structures with lower densities and flow behaviour indices. The higher viscosities attributed to greater aeration during mixing, were translated into final cake structures with significantly higher springiness, resilience, and lower hardness. This study demonstrated the potential of US in mixing applications and in particular for better aerating of cake batter structures. US aided mixing could be used as a route for reduction of emulsifiers by facilitating creation of more aerated structures.

Treatment of proteins with high power ultrasound (20 kHz) probe has also been shown to have a major influences on their functional properties like solubility and foam ability (Jambrak et al., 2008). Through changing the temperature and conductivity of the surrounding media of whey proteins, it was demonstrated that their foaming performance was greatly improved (Jambrak et al., 2008). Moreover the treatment of dairy proteins by high power US led to reduction in protein size and the hydrodynamic volumes taken up by them, which was attributed to the high shear forces provided by ultrasonic cavitations (O'Sullivan et al., 2014). The application of ultrasound to EWP or even alternative protein sources for use in preparation of cake batter could provide an interesting application for their enhanced functional properties.

List of References

Behrend, O., Ax, K., and Schubert, H. (2000). Influence of continuous phase viscosity on emulsification by ultrasound. *Ultrasonics Sonochemistry*, 7(2):77 – 85.

Chesterton, A., Wilson, D., Sadd, P., and Moggridge, G. (2015). A novel laboratory scale method for studying heat treatment of cake flour. *Journal of Food Engineering*, 144(0):36 – 44.

Dürrenberger, M. B., Handschin, S., Conde-Petit, B., and Escher, F. (2001). Visualization of food structure by confocal laser scanning microscopy (clsm). *LWT - Food Science and Technology*, 34(1):11 – 17.

Gibson, L. J. and Ashby, M. F. (1987). *Cellular Solids; Structure and properties.* Pergamon Press.

Jambrak, A. R., Mason, T. J., Lelas, V., Herceg, Z., and Herceg, I. L. (2008). Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. *Journal of Food Engineering*, 86(2):281 – 287.

Meza, B. E., Chesterton, A. K., Verdini, R. A., Rubiolo, A. C., Sadd, P. A., Moggridge, G. D., and Wilson, D. I. (2011). Rheological characterisation of cake batters generated by planetary mixing: Comparison between untreated and heat-treated wheat flours. *Journal of Food Engineering*, 104(4):592 – 602.

O'Sullivan, J., Arellano, M., Pichot, R., and Norton, I. (2014). The effect of ultrasound treatment on the structural, physical and emulsifying properties of dairy proteins. *Food Hydrocolloids*, 42, Part 3:386 – 396. Special Issue: Bridging the Divide between Food and Pharma.

Rayner, M., Sjöö, M., Timgren, A., and Dejmek, P. (2012). Quinoa starch granules as stabilizing particles for production of pickering emulsions. *Faraday discussions*, 158(1):139–155.

Tan, M., Chin, N., and Yusof, Y. (2011). Power ultrasound aided batter mixing for sponge cake batter. *Journal of Food Engineering*, 104(3):430 – 437.

A | Quinoa Starch Isolation

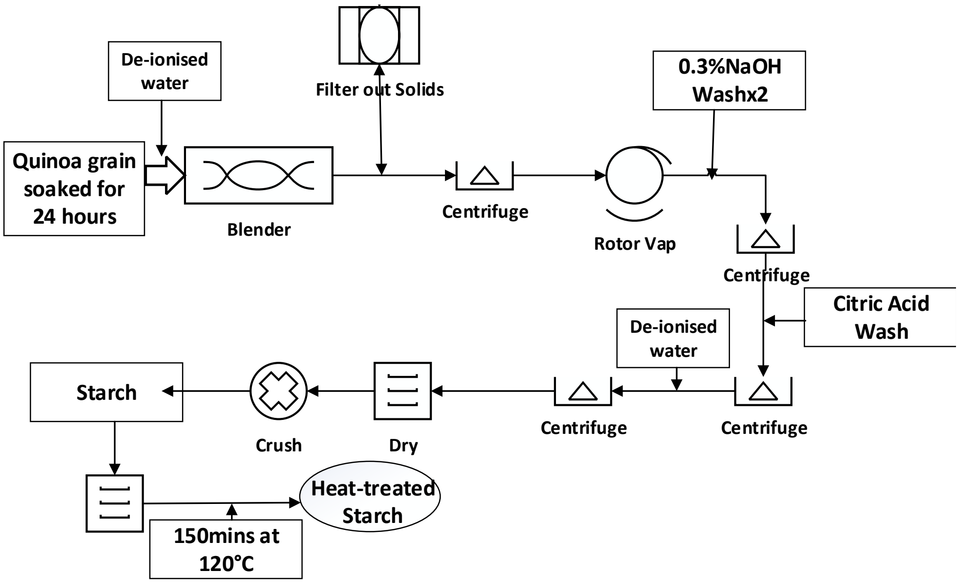


Figure A.1: Simple process flow diagram representing extraction of quinoa starch from the granule

Quinoa seeds were isolated from the grain. They were soaked in distilled water for 24 h ina fridge (4 °C) before the seeds were milled with distilled water in a blender (Philips HR7625, The Netherlands) into a smooth pulp, which was filtered through a cheese cloth and rinsed with distilled water. The starch in the permeated liquid was allowed to settle and the supernatant was removed. The settled layer was re-dispersed in distilled water and centrifuged at 3000 x g for 10 min. The water and the grey top layer, consisting of proteins and seed-coat fragments, were removed. Fresh distilled water was added to the starch, which, after settling and removal of water, was dried in a Buchi R-215 Rotary evaporator (Flawil, Switzerland). Proteins and any fibre residues in the dried starch were removed by washing the starch twice with 0.3% NaOH-solution, once with distilled water and once with citric acid (pH 4.5). Between each washing step the supernatant was removed by centrifugation at 3000 x g for 10 min. Finally, the starch was washed twice with distilled water and centrifuged. The starch was spread on stainless steel trays and dried at room temperature for at least 48 h. Before use the starch granules were disaggregated into a fine powder using (AndrewJames coffee grinder, UK).