Designing microstructures for sodium reduction

By

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Table of Contents

Acknowledgement I
Publications and Presentations II
Abstract VII
1 Introduction 1
1.1 Rationale for sodium reduction 1
1.1.1 Sodium and consumption 1
1.1.2 Role of sodium in human physiology 1
1.1.3 Consequences of overconsumption 2
1.2 Challenges and current strategies of sodium reduction 5
1.2.1 Functions of sodium in food products 5
1.2.2 Current reduction strategies 10
1.2.2.1 Reduction by stealth 11
1.2.2.2 Compensation, replacing salt and multimodal interactions 12
1.2.2.3 Product structures and salt distribution 13
1.2.2.3.1 Salt dissolution 14
1.2.2.3.2 Structural effects on perception 14
1.2.2.3.3 Inhomogeneous distribution of salt 16
1.2.2.3.4 Inert fillers 18
1.3 Experimental aim and hypotheses 20
2 Aerated foams and flavour perception 22
2.1 Introduction 22
2.2 Materials and methods 25
2.2.1 Materials 25
2.2.2 Foam preparation 25
2.2.3 Sodium concentration 26
2.2.4 Conductivity 26
2.2.5 Comparing saltiness perception of foamed samples 27
2.2.6 Measuring *in vivo* aroma release 28
2.3 Results and Discussion 30
2.3.1 Foam stability 30
2.3.2 Sodium ion release 34
2.3.3 Sensory evaluation 35
2.3.4 *In vivo* volatile release through the APCI-MS 39

2.3.4.1 Understanding release of aromas with different partition-coefficients……. 43
2.4 Conclusion 45
3 Delivering sodium using double emulsions 47
3.1 Introduction 47
3.2 Materials and Methods 53
3.2.1 Materials 53
3.2.2 Emulsification 54
3.2.2.1 Single o/w emulsions 54
3.2.2.2 Double $w_1/o/w_2$ emulsions 54
3.2.3 Microstructure imaging 55
3.2.3.1 Light Microscopy 55
3.2.3.2 SEM 55
3.2.4 Particle size 55
3.2.4.1 Emulsifiers 55
3.2.4.2 Emulsions droplets 56
3.2.5 Sodium release 56
3.2.6 Encapsulation efficiency 57
3.2.7 Total starch assay 58
3.2.8 Viscosity 59
3.2.9 Paired comparison testing of double emulsions stabilised with PPI or commercially modified OSA starch 60
3.3 Results and Discussion 61
3.3.1 Native starch 61
3.3.1.1 Native starch stabilised single emulsions 62
3.3.1.1.1 Microstructure after oral processing 64
3.3.1.1.2 Remaining starch after oral processing 66
3.3.1.2 Native starch stabilised double emulsions 67
3.3.1.2.1 Droplet size 67
3.3.1.2.2 Emulsion microstructure 68
3.3.1.2.3 Encapsulation efficiency 69
3.3.2 Commercial OSA starch 71
3.3.2.1 Commercial OSA starch stabilised single emulsions 72
3.3.2.1.1 Remaining starch after *in vitro* 74
3.3.2.2 Commercial OSA starch stabilised double emulsions 75

3.3.2.3 Comparing stability of double emulsions stabilised with commercial OSA starch or PPI 77
3.3.2.3.1 Encapsulation efficiency 79
3.3.2.3.2 Viscosity of double emulsions 80
3.3.2.3.3 Emulsion microstructure after *in vivo* and *in vitro* 81
3.3.2.3.4 Remaining starch after *in vivo* and *in vitro* 83
3.3.2.3.5 Sodium release *in vitro* 84
3.3.2.3.6 Overall saltiness perception 86
3.4 Conclusion 87
4 Optimising sodium delivery in double emulsions 89
4.1 Introduction 89
4.2 Materials and Methods 91
4.2.1 Materials 91
4.2.2 OSA starch modification 92
4.2.3 Determining degree of substitution of OSA starches 93
4.2.4 Emulsification 94
4.2.4.1 Double $w_1/o/w_2$ emulsions 94
4.2.5 Microstructure imaging 94
4.2.5.1 SEM 94
4.2.5.2 Cryo-SEM 94
4.2.6 Particle size 95
4.2.6.1 Emulsion droplets 95
4.2.7 Sodium release 95
4.2.8 Encapsulation efficiency 95
4.2.9 Total starch assay 95
4.2.10 Comparing saltiness perception of double emulsions stabilised with different degrees of modified OSA starch 95
4.2.11 Time-intensity tests 96
4.2.12 Expectorated samples 97
4.2.13 Salivary α-amylase 98
4.3 Results and Discussion 99
4.3.1 Degree of modification of waxy maize starch 99
4.3.2 Double emulsion microstructure 102
4.3.3 Remaining starch after *in vivo* and *in vitro* 107
4.3.4 Sodium release *in vitro* 108
4.3.5 Overall saltiness perception 110

4.3.5.1 Effect of the degree of OSA modified starch on overall saltiness perception....110
4.3.5.2 Comparing saltiness perception of sodium encapsulated emulsions stabilised with different levels of modified OSA starch and non-encapsulated emulsion 111
4.3.6 Temporal perception of saltiness....114
4.3.6.1 Effect of amylase on saltiness perception 116
4.4 Conclusion....118
5 Conclusions and Future Work 120
6 References 126

Abstract

The aim of this project was to develop the tools and knowledge to reduce dietary sodium by mitigating restrictions to flavour delivery and enhancing saltiness perception through sodium contrast effects in the mouth.

This is achieved by restructuring semi-solid and liquid model food systems to achieve maximum flavour delivery for enhanced perception. The project considered two model systems: stable foams and double emulsions.

Stable foams were developed to evaluate air inclusions as a potential sodium reduction strategy. Saltiness perception was enhanced as the levels of air inclusion increased and the incorporation of air also increased the delivery of a congruent mushroom aroma, ultimately this resulted in an enhanced overall flavour perception. The release of volatile aroma compounds from the aerated matrix was dependent on the hydrophobicity (Log P) of the volatile.

Double water-in-oil-in-water ($w_1/o/w_2$) emulsions were evaluated as vehicles to entrap (during storage) and then deliver sodium during oral processing, ultimately to enhance saltiness perception. The emulsions ($w_1/o/w_2$) stabilised with a commercially modified octenyl succinic anhydride (OSA) starch (NC46) were able to encapsulate sodium within the inner water phase (w_1), retaining 97 % of this sodium for up to 90 d and partially releasing the sodium during oral processing. The release mechanism was the digestion of the stabilising starch by oral α-amylase. When compared to a protein stabilised emulsion, a 23.7 % decrease in overall salt was achieved using NC46 stabilised $w_1/o/w_2$ emulsions, without compromising perceived saltiness.

To optimise the stability and delivery of sodium from the double emulsion, different levels of OSA modification were evaluated. High levels (3%) of OSA modification increased storage stability and low (0 % OSA) and intermediate levels offered enhanced saltiness. The optimised (1.5% and 2 % OSA) $w_1/o/w_2$ emulsion was stable and conferred a 15 % reduction in total sodium without compromising saltiness.

These results provide new insights into using colloidal systems to efficiently deliver sodium and aroma volatiles for perception. The sodium redistribution and contrast effects demonstrated in this work may provide new avenues to achieve sodium reduction, particularly in semi-solid and liquid systems.

1 Introduction

The present chapter is organised into three main sections. Section 1.1 reviews the rationale of sodium reduction. Section 1.2 reviews the challenges and current strategies of sodium reduction. The final part of the introduction, section 1.3, outlines and details the experimental aims and hypotheses of the research.

1.1 Rationale for sodium reduction

1.1.1 Sodium and consumption

Sodium is an essential nutrient required for maintaining normal bodily functions. It is present in many food sources and most commonly consumed as salt (sodium chloride) (Mattes and Donnelly, 1991). Salt is formed from a positively charged sodium ion and a negatively charged chloride ion (Van Der Klaauw and Smith, 1995), at a weight ratio of 40:60 (Macgregor, 1997). Both elements are essential for health and must be consumed from the diet as they are not produced within the body (Liem *et al.*, 2011, Mccarron *et al.*, 1984). However, many studies have shown that excess consumption can be harmful to human health and should be reduced (Aburto *et al.*, 2013, Brown *et al.*, 2009, Havas *et al.*, 2007, Ramsay *et al.*, 1999).

1.1.2 Role of sodium in human physiology

It is estimated that a minimum of 184 to 500 mg/d of sodium (equivalent to 0.46 to 1.25 g/d of salt) is required for normal physiological functions such as maintaining membrane potential, the concentration difference between

sodium and potassium ions across the cell membrane (Dahl, 1972, Holbrook *et al.*, 1984).

The occurrence of a sodium ion deprived state causes blood volume and pressure to decrease. This can be fatal when blood pressure falls below the normal range. An immediate and direct way to identify sodium is through taste (Mccaughey and Scott, 1998). Compounds containing sodium are often associated with saltiness and in states of rapid sodium loss, animals, including humans, will use perceived salty taste as a prompt means of recognising sodium (Delwiche, 1996).

The ability to maintain sodium levels is important and can be achieved through two processes (Geerling and Loewy, 2008). The first is reabsorption of sodium through the kidneys and other organs to conserve sodium present in the body (Morris *et al.*, 2008). The second is by sourcing sodium from the environment (food) and consuming enough to replenish the sodium that may be lost excessively through vomiting or diarrhoea (Beauchamp *et al.*, 1990). Whilst these controls are beneficial in scarce sodium environments, they are insufficient to limit sodium intake to adapt to a sodium abundant diet, which can be detrimental to human health (Meneton *et al.*, 2005). Therefore, excessive sodium consumption is suggested to be associated to a preference of taste rather than a physiological need (Dötsch *et al.*, 2009).

1.1.3 Consequences of overconsumption

The average global consumption of salt is approximately 10.21 g/d, equivalent to 4.08 g/d of sodium (Powles *et al.*, 2013). The amount of sodium consumed

far exceeds the levels required for normal physiological functions and the recommended daily intake of less than 5 g/d salt (or 2 g/d sodium) (World Health Organization, 2012).

Overconsumption of salt has been widely reported to cause adverse health (He and Macgregor, 2007). This sub-chapter will review some of the damages linked to increased salt consumption, including hypertension (high blood pressure), cardiovascular disease, kidney related diseases and stomach cancer. There is strong evidence that excess salt intake is strongly related to the development of hypertension (Dickinson *et al.*, 2007, Frisoli *et al.*, 2012, Ramsay *et al.*, 1999). Hypertension is defined as a blood pressure of ≥140 mmHg systolic and ≥90 mmHg diastolic (He and Macgregor, 2007). It was demonstrated that an increased blood pressure would increase the risk of cardiovascular mortality *(*Kannel*,* 1974*)*. Therefore, population approaches to reduce high blood pressure is believed to impact greatly on the prevalence of cardiovascular related diseases (Macmahon, 1996).

Evidence has linked high blood pressure in some individuals to impairments in the kidneys that prevent efficient excretion of sodium (Dahl *et al.*, 1974). The kidneys impaired ability to excrete sodium causes water and salt retention, which expands the extracellular fluid volume and thus stimulates several compensatory mechanisms (Guyton *et al.*, 1980). The presence of compensatory mechanisms will increase urinary sodium excretion, however these mechanisms will also cause blood pressure to rise (De Wardener *et al.*, 2004). The retention of salt and subsequent plasma volume expansion plays a fundamental role in the chronic elevation of blood pressure (Blaustein and

Hamlyn, 2010). However, the relationship between processing renal sodium and blood pressure is influenced by a combination of factors, including genetic and environmental factors, and the complete mechanism is yet to be fully elucidated (Takahashi *et al.*, 2011).

Increased salt consumption has also been associated with the development of urinary stones (Matkovic *et al.*, 1995). Urinary calcium, the main constituent of urinary stones, is increased by a high salt concentration (Massey and Whiting, 1996). Studies have shown that a reduction in salt intake can decrease calcium excretion and reduce the reoccurrence of renal stones (Cappuccio *et al.*, 2000, Lin *et al.*, 2003, Taylor *et al.*, 2009). It may be particularly beneficial for individuals with raised blood pressure to consume a reduced salt diet as it not only lowers blood pressure but can also reduce urinary calcium excretion.

A gram-negative bacterium, *Helicabacter pylori* (*H. pylori*), is present in half of the world's population and the presence of this organism in the stomach increases the risk of gastric adenocarcinoma (Wroblewski *et al.*, 2010). Various studies showed that the expression of particular strains of *H. pylori* is upregulated in high salt conditions (Beevers *et al.*, 2004, Forman *et al.*, 1991, Loh *et al.*, 2007, Takachi *et al.*, 2010). Other epidemiological studies have reported that a high salt intake increases the risk of gastric cancers (Krejs, 2010, Tsugane and Sasazuki, 2007). The lining of the stomach is irritated by high salt concentrations and this is likely to increase the risk of *H. pylori* infection, which may eventually lead to stomach cancer (Forman *et al.*, 1991). He and Macgregor (2007) suggest that a reduction in salt intake may reduce *H. pylori* infection and this may also reduce the prevalence of stomach cancers.

1.2 Challenges and current strategies of sodium reduction

Global initiatives to reduce overall sodium intake have been established as a result of the evidence of excessive sodium consumption in the diet towards increased risk of hypertension.

To date, multiple strategies have been investigated and some have been successfully applied to existing products (Angus, 2007). Despite the efforts to reduce the levels of salt consumption, targets have yet to be met and current population consumption levels remain far from ideal. This is largely due to the complications involved in reformulating food products to reduce salt.

The following subsections will explore some of the functions of salt in current foods, highlight current strategies to tackle elevated salt intake and the difficulties concerning lowering salt levels in food products.

1.2.1 Functions of sodium in food products

Limited sodium naturally occurs in foods and added salt has become the major route of sodium intake (Angus, 2007). In addition to its roles in human physiology, nutrition and health, salt has various functions in many foods and drinks. It is a multifunctional food ingredient that is widely used in the food industry and domestically. Salt is applied to food products for multiple reasons and plays an important role in providing preservation, textural and sensory properties to foods (Doyle and Glass, 2010, Durack *et al.*, 2008).

Salt acts as a preservative by reducing the water activity (a_w) (Leistner, 2000) as the growth of the pathogenic bacteria is supressed with a reduced a_w (Rodrigues *et al.*, 2003). Salt ionises in water, forming sodium and chloride ions

that interact with water molecules through ion hydration making a proportion of water unavailable for microbial growth (Davidson and Taylor, 2007). In particular, salt in combination with nitrite is essential for the preservation of cured meats as it prevents spore-forming bacteria such as *Clostridium botulinum* from growing (Gibson *et al.*, 1987). Salt concentration in cheese varies from 0.7 % to 6 % and the level of salt is chosen specifically to control microbial growth, activity of cheese starter cultures and to limit the growth of spoilage and pathogenic microorganisms (Guinee *et al.*, 2007). In addition, increasing salt content promotes solubilisation of casein, altering the rheology, texture and changes during cooking (Doyle and Glass, 2010).

Salt can affect the texture of foods and its processability by interacting with other components (Doyle and Glass, 2010). The hydration of proteins and their binding both to each other and to fats are enhanced through the addition of salt (Man, 2007). In meat products, myofibrillar proteins are solubilised and the hydration and water-binding capacity increases, which improves the texture of the products (Devlieghere *et al.*, 2009). Ruusunen and Puolanne (2005) demonstrated that the effect of salt on meat proteins is most likely caused by chloride ions being bound to the protein. This increases the negative charge of the protein causing repulsion between the myofibrillar proteins and therefore increasing the swelling and water-holding capacity (Huff-Lonergan and Lonergan, 2005).

Salt is applied to bread making as it aids flavour development and controls fermentation to produce volume and texture in breads (Belz *et al.*, 2012). Bakers have exploited the impact of salt on yeast fermentation for many years

as increasing salt levels slows down yeast fermentation, which allows the dough to increase in volume and expand uniformly (Lynch *et al.*, 2009). Salt also impacts on gluten structure which is an essential property of fermented products and creates a stable and less extensible product that is easier to handle (Man, 2007). The Chorleywood Bread Process (CBP) is a modern commercial process applied to the majority of the industrially produced breads. This process requires more yeast and salt (double the amount of traditional recipes) to develop the dough through high speed mechanical mixing that saves time and cost (Cauvain and Young, 2006). Therefore lowering salt content in bread products will not only impact on the flavour, but also affect the overall texture and volume of the product, in addition to the processability of the product.

A significant role of sodium in food is its impact on taste, saltiness is one of the five basic tastes perceived by humans and enhances liking and can reduce certain off tastes (Pangborn and Pecore, 1982). Via taste receptors in papillae on the tongue, humans are able to perceive and differentiate five basic tastes: sweet, sour, bitter, salty (Kinnamon and Cummings, 1992, Stewart *et al.*, 1997) and umami (Yamaguchi, 1991). There are three types of papillae: fungiform (found in the anterior of the tongue), foliate (located at the posterior lateral edge of the tongue) and circumvallate (at the back rear of the tongue). Depending on the type of papillae, it may contain between one and thousands of taste buds (Chandrashekar *et al.*, 2006). Each taste bud contains 50-150 taste receptor cells that can respond to all five taste sensations (Chandrashekar *et al.*, 2006, Mccaughey, 2008).

Sodium present in the saliva determines the threshold level and sodium concentrations above this level triggers a distinct set of events converting sodium into electrical signals that can be communicated through the nerves (Mccaughey *et al.*, 2007, Morino and Langford, 1978). In addition, taste intensity and quality of solutions containing sodium ions differs based on the anion present, with larger anions decreasing saltiness more than smaller ones and sodium chloride is consistently reported as one of the saltiest sodium containing compounds (Delwiche *et al.*, 1999, Schiffman *et al.*, 1980, Van Der Klaauw and Smith, 1995).

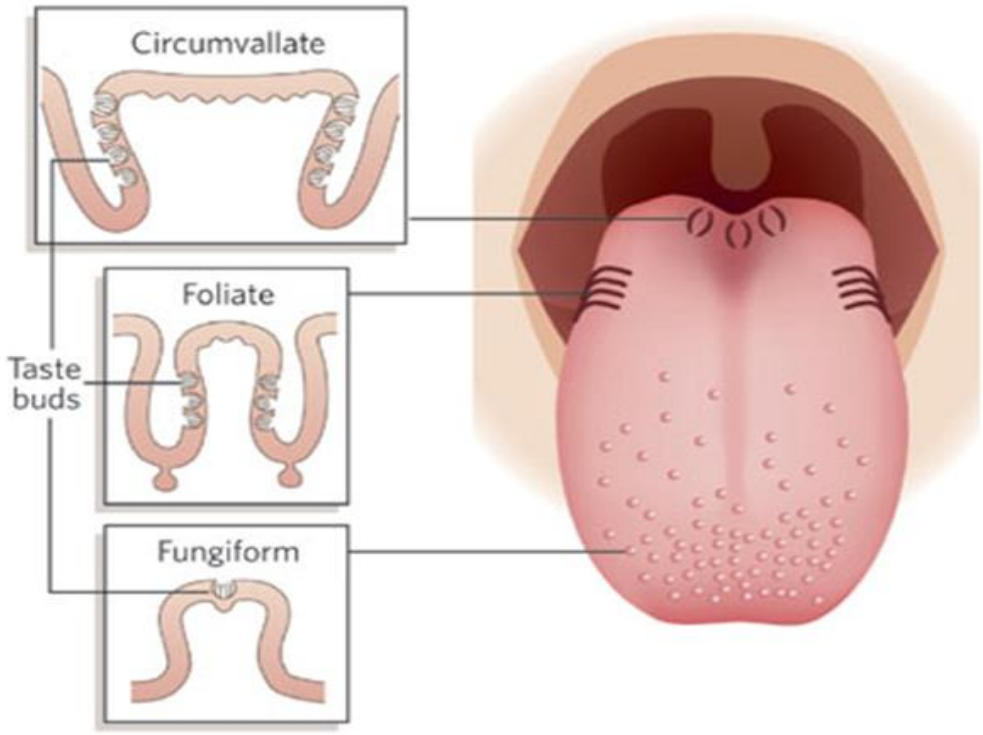

Figure 1.1 Illustration of taste bud anatomy and the distribution on the tongue (Chandrashekar *et al.*, 2006).

Specific human transduction mechanisms for salty compounds are not fully understood, however research suggests that epithelial sodium channels (ENaCs) allowing sodium ions to pass through may be responsible for saltiness

transduction (Brand *et al.*, 1985, Kinnamon, 1996, Lindemann, 1996, Mccaughey *et al.*, 2007). When salt is placed in the mouth the sodium ions must dissolve in saliva before it can be perceived as salty. As the concentration of sodium ions increase in the saliva, these ions flow passively down the concentration gradient into the receptor cells through ion channels in the apical membrane (Brand *et al.*, 1985). To detect a salty sensation, the sodium concentration must exceed that of the resting saliva, which the individual is adapted to, or residual sodium from previous stimuli (O'mahony, 1979). The depolarisation eventually leads to the release of a neurotransmitter onto a nerve that transmits a signal to the brain (Mccaughey and Scott, 1998). However, evidence also suggests that other mechanisms may be involved as sodium channels blocked by amiloride are unable to fully eliminate neural response and approximately 20 % of sodium perceptual intensity remains (Berg *et al.*, 2002, Brand *et al.*, 1985, Halpern and Darlington, 1998). This might be partially explained by sodium ions passing through tight junctions, between adjacent taste receptor cells, and then through ENaCs (Simon, 1992), whereas amiloride is unable to access tight junctions (Delwiche *et al.*, 1999). In conclusion, the mechanism of saltiness perception is not fully elucidated which increases the difficulty to produce sodium-free substitutes whilst retaining perception.

In additional to providing taste, salt also modifies flavours and has enhancing effects in foods (Man, 2007). Salt is able to enhance the flavour in savoury products (soups and bread) making them less bland and more palatable, insweet products (chocolate) to enhance sweetness (Kilcast and Den Ridder,

2007). In general, the overall flavour intensity is improved and a fuller mouth feel is achieved through the addition of salt (Hutton, 2002). One of the most noted effects of salt is its ability to suppress or mask bitter tastes, a negative attribute often undesirable in many products in food products (Kurihara *et al.*, 1994). Therefore the presence of salt is important to make food products more acceptable and palatable.

1.2.2 Current reduction strategies

Excessive dietary salt intake is a global issue and requires intervention from governments, national and international organisations and the food industry.

The Food standards Agency, Centers for Disease Control and Prevention and other government agencies around the world, have established numerous initiatives to tackle salt intake, which involve consumer education, labelling changes, intervention in public settings, food reformulation and taxation (Trieu *et al.*, 2015, Wyness *et al.*, 2012).

Although excessive salt intake is attributed to multiple sources, a large proportion of salt in the diet is reported to originate from processed foods, which contributes approximately 75–80 % of total salt intake (He and Macgregor, 2007). Therefore, the involvement of the food industry is vital and since the launch of global initiatives to reduce salt consumption, many food companies have reformulated their existing products to contain lower levels of salt per serving (Dötsch *et al.*, 2009).

Despite the success of lowering salt in a multitude of products, reduction is often limited by the complex considerations involved in reformulation, such as

costs, clean labelling policies, product-specific application approaches and the multifunctional nature of salt (Angus, 2007, Kilcast and Den Ridder, 2007).

A key concern when reformulating food products is often maintaining palatability. Sodium is an important component to maintaining food palatability where the saltiness and overall flavour is improved whilst suppressing bitterness, an undesired taste, of a food. Therefore, the salt content of a food product is a major determinant of consumer acceptance and ultimately the repurchasing and success of a product. The following subsections will highlight current sodium reduction strategies in food systems whilst retaining consumer acceptance.

1.2.2.1 Reduction by stealth

The gradual reduction of salt, often referred to as reduction by stealth, is applied in processed foods to shift consumers' sodium intake. This method relies on reducing salt in a small, stepwise process allowing consumers to adapt to the new taste before further small salt decreases are implemented (Dötsch *et al.*, 2009). Evidence demonstrates a slow and gradual reduction of salt is acceptable to consumers as the palate adjusts either a higher or lower sodium content where taste preference is adjusted as a consequence (Bertino *et al.*, 1982, Bertino *et al.*, 1986, Blais *et al.*, 1986). A product application reducing sodium content by 25 % in breads was achieved over a six week period and the change was undetected by most consumers (Girgis *et al.*, 2003). Although reduction by stealth can achieve large reductions, the process is time consuming and a level of noticeable change will inevitably be reached (Phelps

et al., 2006). Furthermore, the change will be noticed by consumers if not applied by a whole category simultaneously, which may result in significant loss of sales (Kilcast and Den Ridder, 2007). Therefore stealth reduction may be combined with other approaches to maintain acceptable perceived saltiness in foods whilst further reducing sodium content.

1.2.2.2 Compensation, replacing salt and multimodal interactions

To compensate for a loss of saltiness, addition of other flavours can be applied which relies on increasing the main components that delivers flavour in food products (Kilcast and Den Ridder, 2007). Heinz adopted this approach in many of its soups to achieve an overall reduction of salt by 24 % (Robinson *et al.*, 2007).

Another proposed reduction strategy involves the usage of salt replacers, ingredients that taste salty but do not contain sodium. The replacement of sodium cation by potassium, ammonium, calcium or lithium and by anions have been examined (Lawless *et al.*, 2003, Murphy *et al.*, 1981). Potassium chloride is the most common replacer, however acceptance is limited due to the bitter/metallic taste and aftertaste at high concentrations (Bartoshuk, 2000). However, effects have been made to eliminate the undesirable bitter and metallic tastes by introducing a mixture of salts (Van Der Klaauw and Smith, 1995). Magnesium sulphate is another potential salt replacer and provides different tastes at various concentrations, salty taste at low levels and bitter at high levels (Delwiche *et al.*, 1999). Although there are various salts available, many of these salts are associated with bitter and metallic tastes, which limits

the use of such salts as these tastes are undesired (Breslin and Beauchamp, 1995, Lawless *et al.*, 2003).

Salt enhancers are also used as a sodium reduction tool (Albarracín *et al.*, 2011). A salt enhancer is described as not significantly salty tasting but is able to enhance the perception of saltiness in salty compounds. Research related to taste-enhancing compounds is focused on materials that are often already present in food products and compounds generated from precursors during processing (Kemp and Beauchamp, 1994). Ingredients high in glutamate, ribotides and mineral salts are used as salt enhancers to compensate for sodium loss and rebalance the overall flavour profile (Kemp and Beauchamp, 1994).

Taste-aroma interaction is suggested as a viable approach to enhance saltiness (Delwiche, 2004). Odour induced saltiness enhancement (OISE) has been demonstrated to enhance model solid and aqueous foods (Djordjevic *et al.*, 2004b, Pionnier *et al.*, 2004). However, the concept of congruency is vital and only aromas associated with the tastant concerned is able to enhance the taste (Djordjevic *et al.*, 2004a).

1.2.2.3 Product structures and salt distribution

Structure alteration can be implemented from a nanoscale to macro level (Aguilera, 2006). Modifying product structure is applied as a sodium reduction tool by optimising the delivery of sodium ions to the taste buds, allowing more sodium to be perceived in the short time frame that a food is present in the mouth.

Improving saltiness delivery is an approach that is based on increasing the rate of salt delivery to receptors as it is expected that a significant proportion of sodium is not perceived before the food product is swallowed. A study conducted on salted potato crisps revealed that peak saltiness perception was reached between 20 s and 30 s, where the crisp would be consumed during normal consumption and therefore resulting in the ingestion of a large proportion of unperceived sodium (Tian and Fisk, 2012).

To develop product structures that rapidly deliver sodium, it is important to consider the interaction between the size, shape and distribution of the salt in the food and how this may influence product break down in the mouth.

1.2.2.3.1 Salt dissolution

In dry food products, salt remains as crystals and is only perceived to be salty once dissolved. A rapid dissolution rate may increase perceived saltiness and therefore reduce the levels required (Mccaughey and Scott, 1998). The crystal size and exposed surface area will influence the dissolution rate of salt in the mouth (Bravieri, 1983). Salt crystals with a smaller particle size or hollow structures, create a larger surface area, which will dissolve faster and is shown to enhance saltiness in products with a lowered salt content (Kilcast and Den Ridder, 2007, Rama *et al.*, 2013). The application of this method is limited to solid products where salt remains as a crystalline solid.

1.2.2.3.2 Structural effects on perception

The texture of a food can influence the release of flavours, which is related to the structural changes that occur when food enters the mouth.

In liquid systems a change in viscosity has been reported to alter taste perception. With increasing viscosity, taste perception generally decreased when carboxymethylcellulose (CMC) was applied to salted solutions (Moskowitz and Arabie, 1970). Although others have reported no significant effect on perception at lower concentrations, at a critical concentration, c*, taste decreased rapidly (Baines and Morris, 1987, Cook *et al.*, 2003, Yamamoto and Nakabayashi, 1999). This c* is the point where hydrocolloid chains overlap and the entangled network physically inhibits the transport of tastants to receptors (Baines and Morris, 1987).

In solid products, the effect of texture on taste perception has also been reported. Increasing the concentration of a gelling agent increased rupture stress and energy of the gel and therefore reduced the taste intensity of salted gels and a similar trend was observed with sucrose (Moritaka and Naito, 2002). Moritaka and Naito (2002) also proposed that tastant release was influenced by heat which affected melting of the gel. The perceptual difference is suggested to be related to fracture strain (Morris, 1993). Brittle gels having a lower stain at fracture, released more tastants as a result of increased surface area during fracturing when chewing (Bayarri *et al.*, 2007, Koliandris *et al.*, 2008). In addition, salt release from gels *in vitro* resulted in more salt release when gels were fragmented into smaller pieces compared to gels with larger and fewer fragments (Mills *et al.*, 2011).

The impact of structural agents on perception may also affect the physicochemical properties of the system. It is suggested that anionic hydrocolloids in salted solutions result in the decrease of saltiness perception

as the sodium ions bind to the charged biopolymers and were shown to be less mobile in ionic compared to non-ionic systems (Rosett *et al.*, 1994). An additional effect concerning the osmolarity of solutions which is influenced by the concentration of the structuring agent has been proposed (Koliandris *et al.*, 2010).

The breakdown of food products in the mouth can be optimised to maximise sodium delivery through careful selection of the structuring agent. Starch containing systems have shown partial hydrolysis via α-amylase present in saliva (Chen, 2009, Hoebler *et al.*, 2000, Janssen *et al.*, 2007) and may result in an increase in flavour and taste perception (Ferry *et al.*, 2004). Also, products that melt in the mouth may deliver tastants at a faster rate compared to those that are masticated (Frasch-Melnik *et al.*, 2010a, Malone and Appelqvist, 2003, Mills *et al.*, 2011).

1.2.2.3.3 Inhomogeneous distribution of salt

Distributing salt heterogeneously within a food matrix has been shown to alter taste perception. In solid products, this may consist of distributing salt in two different portions where one portion would contain a higher level of salt than the other (Figure 1.2). The uneven distribution of salt was shown to effectively reduce salt by 25 % in bread, without affecting consumer acceptance (Noort *et al.*, 2010). Other solid products that have a heterogeneous distribution of salt have delivered similar saltiness intensities, with less salt compared to a control (Emorine *et al.*, 2015). An additional concern when applying this method, is the migration of salt during storage of the product, which lowers the initial contrast

between the two regions in the food product. Further studies on consumer acceptance towards the contrast of such a product must be carried out to understand the viability of this application (Noort *et al.*, 2012).

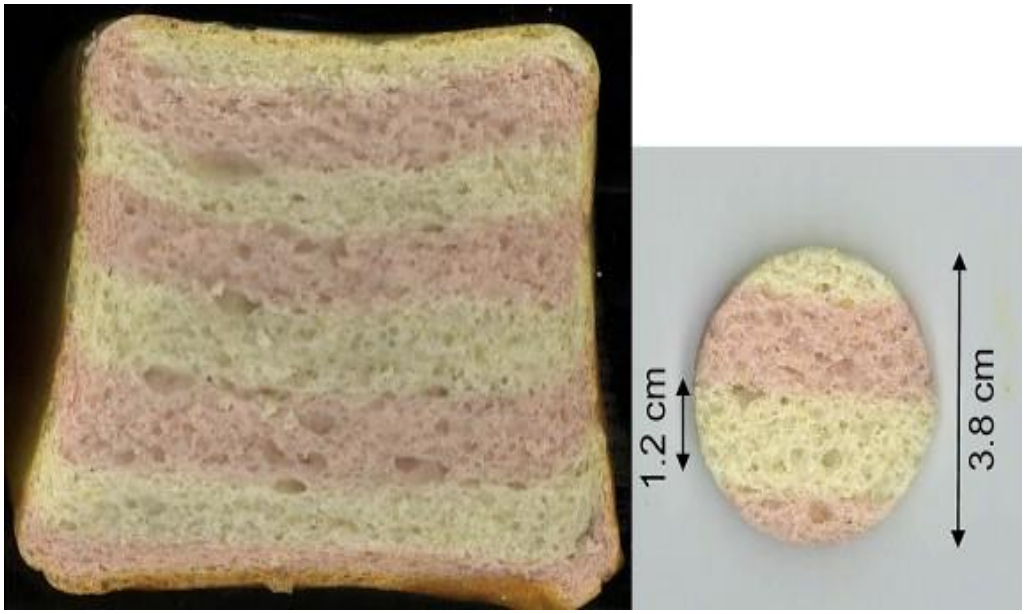

Figure 1.2 Image of bread slice and sample with heterogenous salt distribution. Red indicate regions with high salt content (Noort *et al.*, 2010).

Applying salt heterogeneously in liquid products is less directly transferred as salt is dissolved and therefore more evenly distributed (Shepherd *et al.*, 1989). In a liquid solution, a viable option to create two different salt concentrations is to encapsulate one of the liquid phases. Indeed, food grade examples using double emulsions have been shown to separate salt containing aqueous phases (Frasch-Melnik *et al.*, 2010b). However, *in vivo* and sensory response has yet to be determined.

The addition of salted particulates in a soup product has been perceived to be saltier than a soup containing the same overall sodium content with unsalted particulates (Busch *et al.*, 2010, Busch *et al.*, 2008). It is believed that the solid particulates remain in the mouth for longer than the liquid phase and the salted

particulates create a contrast in the mouth. However, this approach requires the salt to remain in the aqueous phase for a relatively short time so the salt in the particulates do not completely dissolve into the liquid phase.

Equipment, including the Gustometer and Dynataste, used to create model systems to control the delivery of different liquids to the mouth has been beneficial for studying the effects of heterogeneous salt delivery on salt perception (Busch *et al.*, 2009, Meiselman and Halpern, 1973, Morris *et al.*, 2009, Morris *et al.*, 2010). Some have reported no significant increase in perception through pulsation (Morris *et al.*, 2009, Morris *et al.*, 2010), whilst others studies have reported an increase in saltiness perception (Burseg *et al.*, 2011, Busch *et al.*, 2009, Meiselman and Halpern, 1973). It is suggested that the overall receptor response is higher through pulse delivery of the stimuli. This induces phases of high receptor response which result in an overall greater receptor response compared to a homogenously distributed system with continuous stimuli (Meiselman and Halpern, 1973, Mosca *et al.*, 2010).

Furthermore, consumer expectation may influence perception as consumers expect a product with evenly distributed ingredients within a phase and the anticipation of a product to be consistent in taste may allow producers to manufacture products to contain less overall salt (Woods *et al.*, 2010).

1.2.2.3.4 Inert fillers

Fillers are applied in aqueous solutions to replace part of the aqueous phase volume and therefore less salt is required to achieve the same salt concentration.

Multiple studies have used oil as a filler, forming oil-in-water (o/w) emulsions, replacing a proportion of the aqueous phase to increase saltiness perception as a result of increasing the salt in the aqueous phase (Metcalf and Vickers, 2002, Yamamoto and Nakabayashi, 1999). The application of oil as a filler is limited as it gives a mouth coating effect. Equal salt concentrations were reported to be less salty in the presence of oil, and the extent of saltiness reduction is dependent on the level of oil used, with more oil reducing perception further (Malone *et al.*, 2003, Metcalf and Vickers, 2002). However, depending on the type of emulsifier used to stabilise the emulsion, the mouth coating effect of oil can be controlled (Dresselhuis *et al.*, 2008).

Other fillers, including solids and air, have been applied to aqueous systems. The application of tomato solids was shown to reduce overall taste perception and was thought to be due to a reduced rate of tastants reaching the receptors (Kokini *et al.*, 1982). Air is another filler that has been investigated and a study found that the overall taste was dependent on the salt concentration in the aqueous phase rather than the volume of air (Goh *et al.*, 2010).

Although some fillers are able to reduce sodium content, careful consideration is needed as sensory properties may be altered including the appearance and texture, limiting the effect of this approach (Goh *et al.*, 2010, Minor *et al.*, 2009).

Despite the efforts to tackle sodium intake, average consumption remains above recommended levels (World Health Organization, 2012). It is evident that further understanding is required to develop new approaches, including

adapting existing product and category specific approaches, for the continual reduction of sodium in the diet.

1.3 Experimental aim and hypotheses

The main aim of this project was to develop the tools and knowledge to reduce dietary sodium by mitigating restrictions to flavour delivery and enhancing saltiness perception through sodium contrast effects in the mouth. This is achieved by restructuring semi-solid and liquid model food systems to achieve maximum flavour delivery for enhanced perception. This thesis examines two different microstructures, foams and double emulsions, and the mechanisms of sodium delivery for perception using instrumental and sensory analysis. The specific hypotheses to be tested in this research project were:

Utilising inert fillers to displace sodium (Chapter 2)

1. Saltiness perception is enhanced when displacing water with air inclusions due to an increase in concentration of sodium chloride in the aqueous phase of a fixed volume of sample.
2. Overall perception is increased in aerated hydrogels where aroma compounds are rapidly delivered to receptors and aroma compounds with lower Log P values are released more rapidly.

Developing an emulsion system to target sodium delivery (Chapter 3)

3. Saltiness perception is enhanced when encapsulated salt within starch stabilised emulsions is rapidly released in the presence of α-amylase, hence more sodium is available in the continuous phase for perception.

Optimising sodium delivery in double emulsions (Chapter 4)

4. Increasing the degree of OSA modification will produce more stable emulsions and less encapsulated sodium is released when a more OSA modified starch is utilised to stabilise the water-in-oil-in-water ($w_1/o/w_2$) emulsion.

This research examines a semi-solid (foam) and liquid (double emulsion) food system to improve saltiness perception by redistributing the sodium in the matrix. In the following chapter of the thesis, Chapter 2, examines the effect of air inclusions towards taste and aroma perception. Chapter 3 investigates the effect of contrasting sodium concentrations in a double emulsion on saltiness perception. The effect of modifying the external emulsifier towards the release of sodium for perception is examined in Chapter 4.

2 Aerated foams and flavour perception

2.1 Introduction

This chapter focussed on how air can be used as an inert filler to enhance both taste (specifically saltiness) and aroma perception (octanol used as a model savoury aroma). Here, the methods, results and discussion regarding the development of the model foam system and *in vivo* aroma release from the foams are presented, in combination with sensory characterisation of both saltiness and overall flavour.

The incorporation of inert fillers which structurally alters food products has shown promise in optimising the delivery of tastants and aromas for perception. Studies have explored oil-in-water (o/w) emulsions, using oil droplets as fillers replacing the water phase within the system (Bayarri *et al.*, 2007, Drewnowski and Schwartz, 1990, Yamamoto and Nakabayashi, 1999). The addition of oil replaces water which causes an increase in taste perception as the concentration of tastant increases in the aqueous phase. However, an increase in oil can causes a mouth coating effect (Yamamoto & Nakabayashi, 1999) and additional oil in products is undesirable for health, therefore limiting its application**.**

Control over the temporal delivery of tastants from the bolus to saliva has been shown previously to control taste perception (Rama *et al.*, 2013, Tian and Fisk, 2012) and could be used as a method for enhancing the perception of sodium in food products. It has also been shown that heterogeneously increasing the concentration of a tastant within a specific phase of a food can increase

perceived taste intensity. Therefore, techniques where tastant concentrations are increased through substitution of the water phase could be applied as an appealing sodium reduction method. A previous study, using a foamed system, reported that overall taste perception was dependent on the concentrations of tastant in the aqueous phase and had no correlation with the volume fraction of the air inclusions (Goh *et al.*, 2010).

Foams are a type of colloidal system formed through aeration, a process by which gas (often atmospheric air) is introduced throughout an aqueous continuous phase. Depending on the volume fraction of gas bubbles, the bubbles in a foam system may exist in spherical or polyhedral shapes and the inclusion of air in foods provides unique structures and textures (Murray and Ettelaie, 2004). Foamed food products include: whipped cream, breads and soufflés (Campbell *et al.*, 1999). Furthermore, implementation can reduce product cost (Campbell and Mougeot, 1999).

Foams are considered lyophobic colloidal dispersions, where the dispersed phase and continuous phase are thermodynamically more favourable as two completely separate phases (Walstra, 1996). Foam dispersions do not form spontaneously and require mechanical energy to increase the interfacial area (Aksenenko *et al.*, 2006). The thermodynamically unstable nature of foams is associated with the gas-liquid interface and the two phases rapidly separate to minimise the interfacial contact area through liquid drainage and disproportionation (Gonzenbach *et al.*, 2006, Kinsella, 1981, Monsalve and Schechter, 1984).

In foams, coalescence of bubbles occur when liquid draining causes the thinning of the lamella film, this is the liquid film of the continuous phase that separates the air bubbles, and rupture of the film. The rate of film drainage is shown in Equation 1.

$$\boldsymbol{V = 2h^3(\Delta P)/3\mu R^2} \qquad \textbf{Equation 1}$$

where h is lamella film thickness; μ is dynamic viscosity; R is the radius of the bubble; ΔP is the difference between the capillary hydrostatic pressure and the disjoining pressure between the interfaces of the lamella.

It is possible that the rate of liquid drainage can be decreased by increasing the viscosity and disjoining pressure.

Disproportionation, also known as Ostwald ripening, in foams is the process which refers to the diffusion of gas between bubbles that have different internal pressures (Murray and Ettelaie, 2004). The bubbles is foams are polydisperse and the net diffusion is often from small to large bubbles, due to the greater Laplace pressure of the smaller bubbles. The movement of gas between bubbles leads to coarsening of the foam. In addition, the pressure within the bubbles may depend on the interfacial elasticity of the adsorbed film around the bubbles and the extent of their shrinkage (Damodaran, 2005). Acceleration of liquid drainage and coalescence of the foam can occur as a result of foam coarsening (Murray and Ettelaie, 2004).

These processes may be partially delayed by careful selection of surface active agents such as surfactants, proteins and particles.

Despite the prevalence of foams in food products, extensive literature remains focused on the stability and structure of foams while the role of air inclusions in terms of perception has yet to be fully understood.

A main objective was to better understand the impact of using air as filler particles and correlate the impact of sodium displacement on the temporal delivery of salt and congruent aroma on overall perception. In addition, the *in vivo* delivery of different aroma volatiles from aerated foams was investigated to obtain a better understanding of the impact of aroma hydrophobicity.

2.2 Materials and methods

2.2.1 Materials

All materials used in the foams were food grade. Whey protein was donated by Davisco (Minnesota, USA). Type B bovine skin gelatine, sodium chloride, 1-octan-3-ol, 3-Heptanone, dichloromethane (99.8 %,), 2,5-Dimethylpyrazine, Ethyl butyrate, Ethyl hexanoate, Limonene were purchased from Sigma-Aldrich Ltd. (Dorset, UK). Deionised water, with a resistivity of 15MΩ/cm was used for the preparation of all solutions. Plain crackers (99 % Fat Free, Rakusen's, UK) and mineral water (Evian, France) were purchased from a local supermarket.

2.2.2 Foam preparation

An aqueous solution containing 2 g/100 mL type B bovine gelatine, 5 g/100 mL whey protein and between 0.6 g/100mL and 3.3 g/100mL sodium chloride was stirred and treated to reach a specific temperature (25 °C, 40 °C, 60 °C, 80 °C

and 100 °C). Aroma volatiles were added to produce a concentration of 8 µL/L. The solution was immediately placed into an ice bath and sheared at 3000 rpm using a high shear overhead mixer (L5M, emulsor screen, Silverson, Chesham, UK). Different durations were used to achieve the required volume. Air inclusion fraction was calculated by subtracting the original volume of the solution before shearing from the volume of the solution after shearing. The foams were stored at 4 °C, all samples were prepared and analysed within 24 h.

2.2.3 Sodium concentration

Flame photometry (Sherwood Scientific Ltd., Model 410, Cambridge, UK) was used to evaluate sodium concentration, sodium standards (0, 2, 4, 6, 8 and 10 mg/L) were prepared for calibration. The calibration curve demonstrated repeatability ($R^2>0.99$), and linearity up to 1 mg/L, wavelength 589 nm. The sodium data was collated in triplicate and converted to sodium chloride concentration by multiplying the detected sodium content by 2.5.

2.2.4 Conductivity

The rate of dissolution of sodium from a 6 mL sample to a beaker of deionised water (200 mL) was evaluated every 10 s over 150 s and a conductivity meter (Hanna Instruments, Michigan, USA) was used to determine the concentration of ions released over time in triplicate. The temperature was maintained at 37 °C using a water bath and the solution was stirred at 500 rpm using a magnetic stirrer.

2.2.5 Comparing saltiness perception of foamed samples

96 untrained panellists (aged 19-61; 55 female and 41 male) were recruited to conduct a series of paired comparison (PC) tests after signing consent forms which complied with local ethical procedures (BS EN ISO 5495:2007). The same volume of sample (6 mL) was presented individually on plastic spoons and left to equilibrate at room temperature (25 ˚C). For each sample, panellists were required to place the sample in their mouth for 10 s, the tongue was moved up and down three times before swallowing. The samples were presented in pairs on plastic spoons each labelled with a random three-digit code and panellists were asked to select the sample they perceived as saltiest overall, each sample was evaluated against every other sample in a balanced design resulting in three PC tests. The sensory samples are shown in Table 2.1. The first set of samples, S1, S2 and S3, contained equal concentrations (6.6 g salt/L) of salt in the aqueous phase and 0 %, 40 % and 80 % air inclusions respectively, (S1, S2 and S3 contained total sodium contents of 198 mg, 118.8 mg and 39.6 mg respectively). The following series of samples, S1, S4 and S5, contained increasing air inclusions from 0 % to 40 % and 80 % respectively whilst overall weight amount of salt was kept constant (198 mg). Again each sample was evaluated against each other resulting in a further 3 PCs. Finally, panellists were asked to perform PCs selecting the sample that was perceived to be most intense in overall flavour, where a mushroom aroma volatile was applied. Samples S6, S7 and S8 were used in these tests and contained the same concentration of 1-octen-3-ol aroma volatile (8 μL/L) and the same overall salt

concentration (6.6 g/L) in all samples (samples varied in air inclusion, 0 %, 40 % and 80 % respectively).

The test was used in forced-choice mode, so panellists were required to give an answer even if the perceived difference was negligible. Plain crackers (99 % Fat Free, Rakusen's, Leeds, UK) and mineral water (Evian, France) were supplied for panellists to palate cleanse between samples, rest breaks were given between every three pairs. All tests were carried out within individual sensory booths under northern hemisphere lighting and controlled temperature and humidity. Consensual answers were compared to data tables to determine significance (BS EN ISO 5495:2007), α=0.05 for difference testing, α=0.2, β=0.05 and p_D=30 % for similarity testing.

Table 2.1 Formulation of foam samples.

Sample code	Air inclusion (% v/v)	Solution (mL)	Salt (mg)	Air inclusion (mL)	Total volume (mL)	Salt concentration in aqueous phase (g/L)	1-octan-3-ol (ppm)
S1	0	30	198	0	30	6.6	0
S2	40	18	118.8	12	30	6.6	0
S3	80	6	39.6	24	30	6.6	0
S4	40	18	198	12	30	11	0
S5	80	6	198	24	30	33	0
S6	0	30	198	0	30	6.6	8
S7	40	18	118.8	12	30	6.6	8
S8	60	6	39.6	24	30	6.6	8

2.2.6 Measuring *in vivo* aroma release

Gas chromatography – mass spectrometry (GC-MS) analysis was used to quantify 1-octen-3-ol (Log P=2.73) (Cook *et al.,* 2003). The concentration of 1-octen-3-ol in all samples was compared to ensure volatile concentration was comparable after preparation, thereby removing concentration differences of aroma volatiles as a variable of perceivable difference. An internal standard, 3-

Heptanone, was used and 15 mL of dichloromethane was added to the spiked sample, and left on a roller mixer to equilibrate for 3 h. Once the phases were separated,

2 mL of the solvent was pipetted into 2 mL GC vials (SLS Ltd, Nottingham, UK) and 1 µL of the sample was injected into the injector port of a Trace 2000 Series GC (Thermo Scientific, Massachusetts, USA) using an AS 3000 autosampler (Thermo Scientific, Massachusetts, USA). The column used was ZB WAX, 30 m x 0.25 mm i.d. x 1 µm film thickness (Phenomenex, Macclesfield, UK). The temperature programme for the oven was maintained at 50 °C for 1 min after injection and then ramped at 10 °C/min to 250 °C over 3 min. Analytes were detected in triplicate using a DSQ II mass spectrometer (Thermo Scientific, Massachusetts, USA) operating in full scan mode from 50 to 250 *m*/*z* at 1.6 scans/s. 1-octen-3-ol peak area was compared to that of the internal standard, 2-methyl-3-heptanone, to calculate the concentration of 1-octen-3-ol, and a two-tailed t-test showed no significant differences between the samples.

Real-time gas phase release of 1-octen-3-ol was measured using the atmospheric pressure chemical ionisation mass spectrometry (APCI-MS-NOSE) (Fernández-Vázquez *et al.*, 2013). Seven panellists were recruited from the University of Nottingham (aged 19-30; four female and three male) and asked to follow a fixed predefined oral processing protocol; panellists consumed two different samples (S6, and S8 in Table 2.1) containing equal concentration of 1-octen-3-ol. Panellists were asked to place their nose on a sampling tube, breath in, consume the whole sample, close their mouths, breath out and in at specific

times, swallowing the sample when instructed (10 s) and were asked to continue breathing out and in as indicated. Exhaled aroma was monitored at m/z+1, 129. Area under the curve was used to compare release rates and the relative abundances of 1-octen-3-ol within the gas phase were calibrated against known standards prior to and at the end of each analysis run. Data was collected in triplicate.

The aroma release of four additional aroma volatiles with different partition coefficients (Log P), which determines the ratio of concentrations of a compound in a mixture of two immiscible or slightly miscible phases at equilibrium, was measured (Table 2.2). The samples were analysed using the same methods as 1-octen-3-ol. To determine if significant difference of aroma release was present between the two aeration levels a two tailed T-test was performed (SPSS, IBM, Version 22, USA). Analysis was based on 95 % confidence limit.

Table 2.2 The partitioning coefficient and molecular weight of each aroma compounds.

Compound	Partition coefficient (Log P)	Molecular mass (m/z)
2,5-Dimethylpyrazine	0.63	108
Ethyl butyrate	1.85	116
Ethyl hexanoate	2.83	144
Limonene	4.83	136

2.3 Results and Discussion

2.3.1 Foam stability

To achieve the aims of this study, the development of a stable foam system was crucial for the scope of this work. Here the results of a model foam system formed using whey protein are presented.

While extensive research has been conducted to create extremely stable foams, food-grade materials remain limited (Dickinson, 2010, Du *et al.*, 2003, Subramaniam *et al.*, 2006). Hence, proteins are still commonly used to stabilise foams for food applications. In this study, whey protein isolate (WPI) and gelatine, were selected for their foaming capacity and stability (Davis and Foegeding, 2004, Kuropatwa *et al.*, 2009)

As previously mentioned, a main cause of foam destabilisation is drainage. Drainage is the thinning of the liquid film which results in liquid separating from the foam (Saint-Jalmes, 2006). Therefore, the rate of liquid drainage from the foams was used as an indication of foam stability.

Heat treatment was applied prior to foaming to understand the effects of heat on foam stability. The amount of liquid drainage from the foams heated to different temperatures (25 ˚C, 40 ˚C, 60 ˚C, 80 ˚C, 100 ˚C) prior to foaming was measured for 10 h (Figure 2.1A). Thermal treatment affected the level of drainage and the greatest loss of liquid from the foams was observed when heat treatment of 100 ˚C was applied and the final drainage at 10 h was significantly greater than the other four treatments (p<0.05), followed by treatment at 80 ˚C. However, heat treatment at 40 and 60 ˚C improved the stability of the foams and treatment at 60 ˚C was the most stable foam system with minimal drainage after 10 h (Figure 2.1B).

Previous studies have demonstrated that presence of protein aggregates can improve a protein's foaming properties (Davis and Foegeding, 2004, Unterhaslberger *et al.*, 2007). The formation of protein aggregates via heat denaturation of WPI has been reported to occur between 60 ˚C and 90 ˚C (Bals

and Kulozik, 2003). However, protein aggregates alone are unable to improve foaming capabilities but rather complement non-aggregated proteins to improve foam stability (Nicorescu *et al.*, 2009, Nicorescu *et al.*, 2010, Zhu and Damodaran, 1994). The decrease in stability which was observed at higher heat treatments (80 ˚C and 100 ˚C) could be attributed to the formation of excessive proportions of protein aggregates. Protein aggregation created high molecular weight aggregates with decreased adsorption rates preventing them from adsorbing at the relevant time scales causing the foam to collapse (Damodaran, 2005, Davis and Foegeding, 2004, Nicorescu *et al.*, 2009).

Although thermal treatment at 40 ˚C is below the suggested denaturation of WPI, thermal treatment at this temperature improved stability when it was compared to no heat treatment, suggesting that some protein aggregates may be present. The formation of such aggregates could be aided by the presence of salt in the mixture which increases the formation of aggregates during thermal treatment (Schmitt *et al.*, 2007). Treatment at 60 ˚C was deemed optimal and was selected for subsequent experiments.

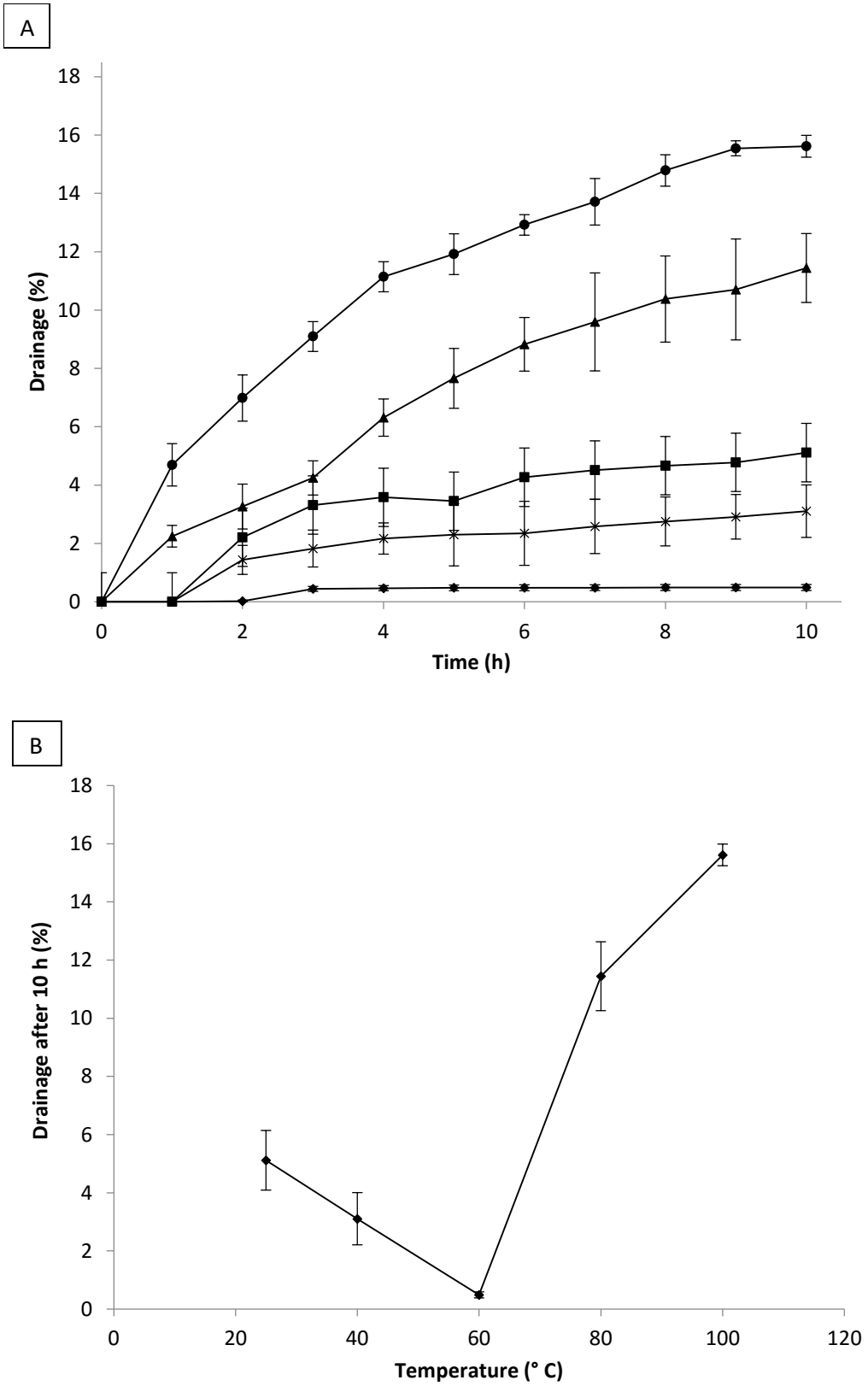

Figure 2.1A) Drainage over time when samples were not heated (25 °C) (■), heated to 40 °C (✳), heated to 60 °C (◆), heated to 80 °C (▲) and heated to 100 °C (●) prior to aeration and equal weights of salt. B) Drainage after 10 h of different temperature treatments. Data are means of three replicates ± standard deviation.

2.3.2 Sodium ion release

Conductivity measures the concentration of dissolved sodium which has been ionised in a solution (Ge *et al.*, 2007). Conductivity readings of samples were measured at 37 °C (Figure 2.2). The conductivity of the samples increased, indicating that the sodium and chloride ions are moving from the sample into the continuous phase. As air inclusion increases, the rate of dissolution increased and samples containing air regardless of volume inclusion, reached peak conductivity faster than the sample with no air included. As air inclusion increased, the surface area within each sample increased proportionately. Assuming that the samples performed in a similar manner in the mouth, in the short time frame of oral consumption this would increase the amount of sample that is in contact with the saliva. This potentially would allow sodium ions to reach the taste receptor cells at a faster rate and subsequently increase perception at a faster rate. It is also important to consider differences in the mouth, such as the surface roughness of the tongue and palate and level of saliva which can contribute to the extent of structure disruption and perception.

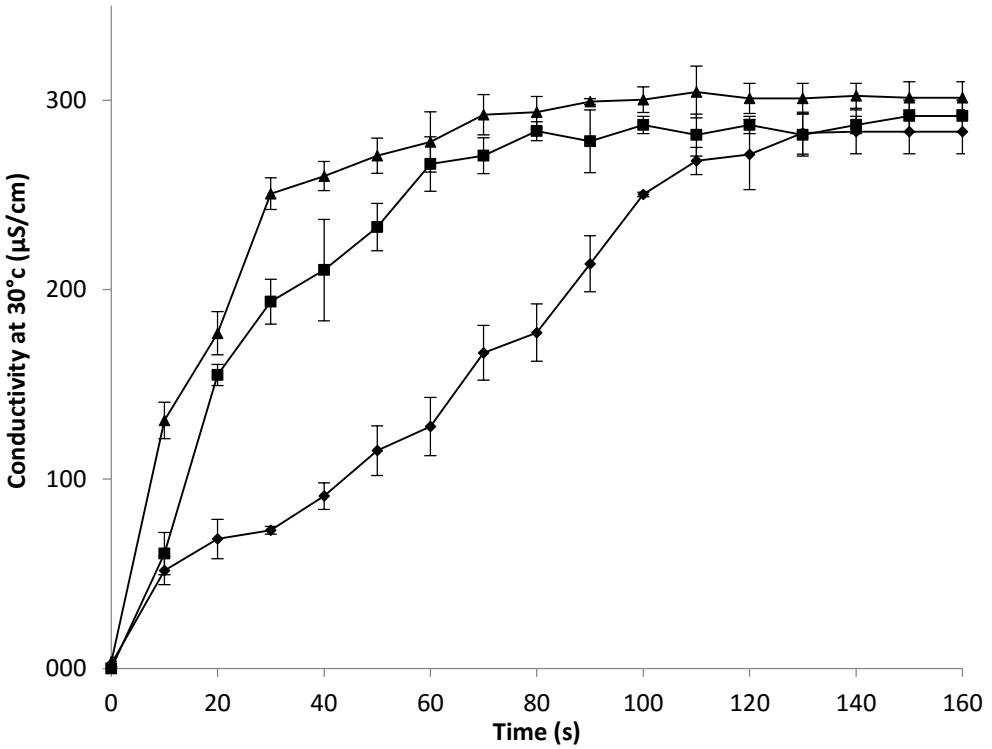

Figure 2.2 Conductivity over time of samples with 0 % (◆), 40 %(■) and 80 %(▲) air inclusion and equal weights of salt. Data are means of three replicates ± standard deviation.

2.3.3 Sensory evaluation

The results of the paired comparison tests for overall saltiness with equal concentrations of salt in the aqueous phase (Figure 2.3) indicated no significant difference in salt perception (*p>0.05*). Indeed, evaluation of results provided sufficient evidence to conclude that there was a similarity between the samples despite the highly significant reduced concentration of total sodium (198 mg reduced to 39.6 mg). It was therefore shown that less salt per product volume was required to maintain overall perception of saltiness after the inclusion of air as a filler particle. This suggests that salt perception is not driven by the amount of salt in the total volume but rather the concentration of salt in the continuous phase and that inclusion of air did not obstruct taste perception of the samples. The results contradicted the findings from previous studies where

oil and solid fillers were applied which resulted in oil coating the mouth and the solid filler obstructeing the diffusion of tastants in terms of perception (Kokini *et al.*, 1982, Yamamoto and Nakabayashi, 1999). It is suggested that the air bubbles rupture during consumption, removing any physical barrier, and thus allowing tastants to successfully reach the taste buds and be perceived (Goh *et al.*, 2010). Panellists were asked to follow a specific protocol, including specific tongue movement, however if actions including free movement and chewing was permitted, the disruption of the structure and consequently perception is presumed to increase further.

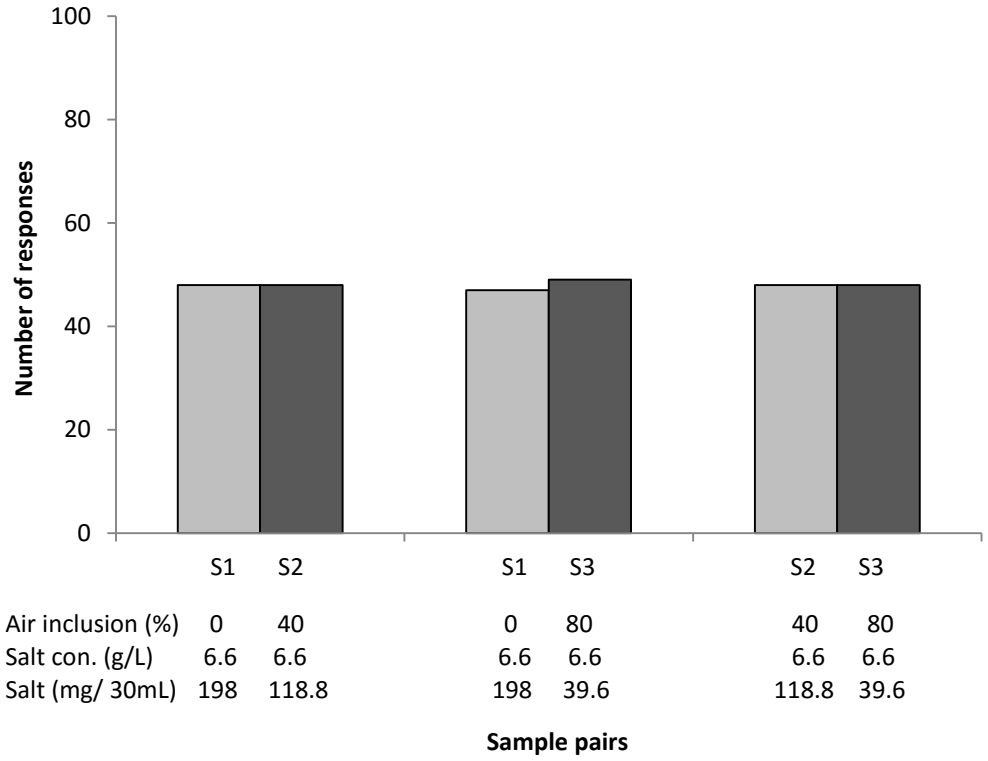

Figure 2.3 Results of the two-sided paired comparison tests for salt taste where samples contained equal salt concentrations in the aqueous phase.

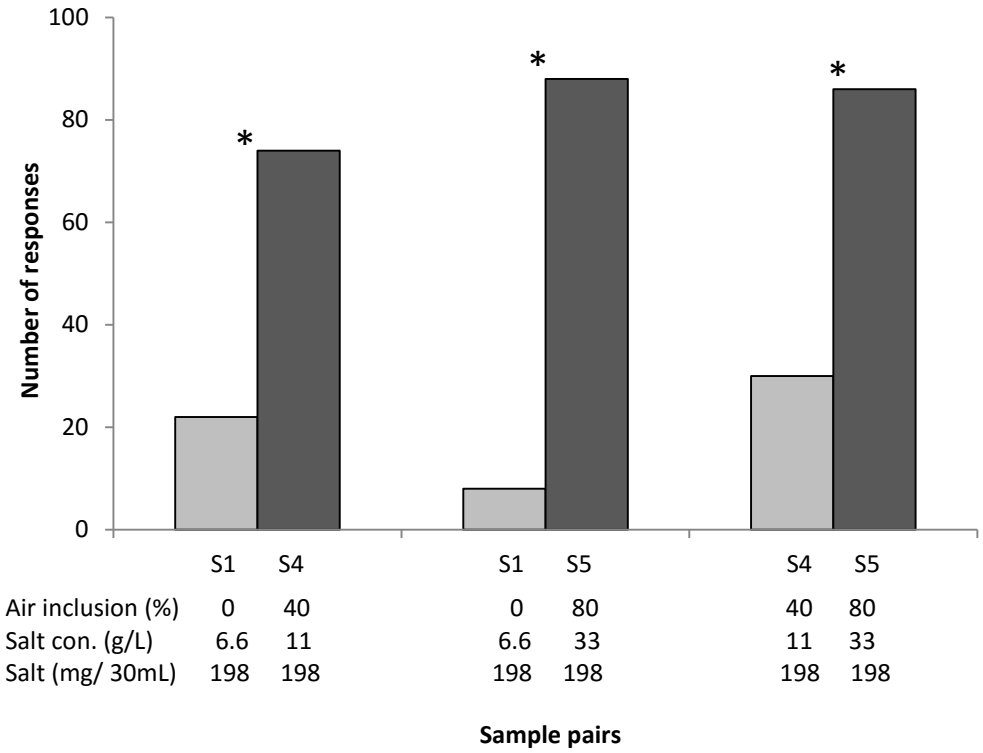

Figure 2.4 Results of the two-sided paired comparison tests for salt taste where samples contained equal weight amounts of salt, * p<0.05.

The overall saltiness perception of samples with equal weights of salt and different air inclusions was determined (Figure 2.4). The results indicated that samples containing air inclusions were perceived to be significantly saltier when compared to the samples containing no air (p<0.05). Despite having the same overall weight of salt, the perception of saltiness increased significantly with increasing air inclusions.

Air replaced the aqueous phase, making the salt in the aqueous phase more concentrated. Thus samples with more air inclusions were perceived as overall more salty which supports similar results published previously (Goh *et al.*, 2010).

Taste-aroma interactions have been reported between some aromas and tastes and these odour-induced taste enhancements are dependent on the specific aroma (Djordjevic *et al.*, 2004b, Frank and Byram, 1988, Hort and

Hollowood, 2004). In addition, aroma perception is also influenced by texture and an increase in hardness reduces both taste and aroma intensities (Baines and Morris, 1987, Kälviäinen *et al.*, 2000). Therefore, to examine the impact of aeration on overall perception, a congruent aroma was applied to the foam systems.

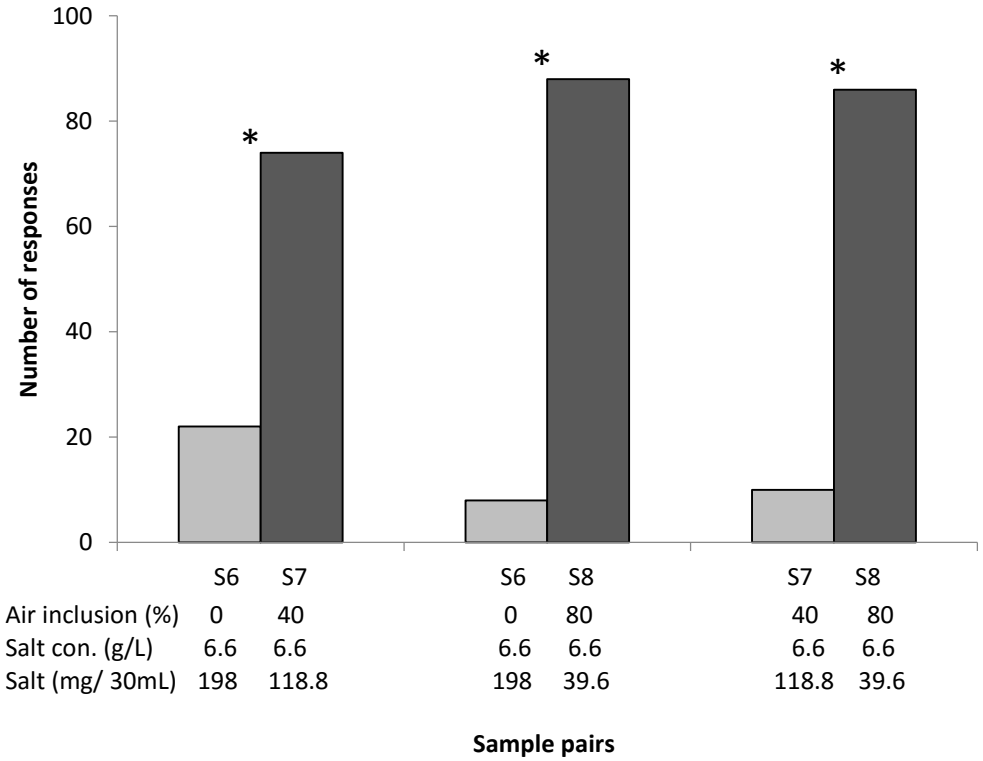

Figure 2.5 Results of the two-sided paired comparison tests for overall flavour perception for samples containing equal concentrations of salt and 1-octan-3-ol, * p<0.05.

Naïve panellists were asked to rate the overall flavour intensity of the samples containing 1-octen-3-ol. 1-octen-3-ol was selected as it is commonly encountered in many salty food products and can be considered congruent with saltiness (Cook *et al.*, 2003). Samples were comparable to those presented in Figure 2.3 but contained 1-octen-3-ol at 8 µL/L. Whilst it was previously shown that there was no difference in saltiness across the three samples, the aeration (40 % and 80 %) of samples containing 1-octen-3-ol resulted in a

significant (p<0.05) enhancement in flavour intensity (Figure 2.5). The concentration of salt in the continuous phase was constant across all samples presented in Figure 2.5, therefore the difference in flavour intensity is proposed to be due to the enhanced delivery of 1-octen-3-ol.

The addition of air altered the appearance and texture of the samples. Visual differences were observed between the samples, as samples with 0 % air inclusion were noted to be clear compared to samples with air inclusions which were opaque foams. Texture differences were also reported in a previous study, although minimal influence on taste perception was reported (Goh *et al.*, 2010). In this study, panellists were instructed to focus only on either overall saltiness or total flavour intensity, although multimodal interactions involving appearance and textural variation could not be ruled out. It is important to consider the type of product this method of salt reduction could be utilised for, such that appearance and texture differences due to air inclusions do not impact on the consumer acceptance of the product whilst allowing the enhanced mixing offered by the bubble collapse to maximise sodium perception.

2.3.4 *In vivo* volatile release through the APCI-MS

Prior to measuring *in vivo* release of the aroma compounds, the concentration of aroma volatile in samples consisting of 0 % and 80 % air inclusion must have a similar aroma concentration for comparison. This was checked before and after preparation and sheering of the solution to ensure incorporation as air had no significant effect on the relative abundance of 1-octen-3-ol ($p>0.05$).

The aroma concentration, prior to consumption, within the two systems was comparable.

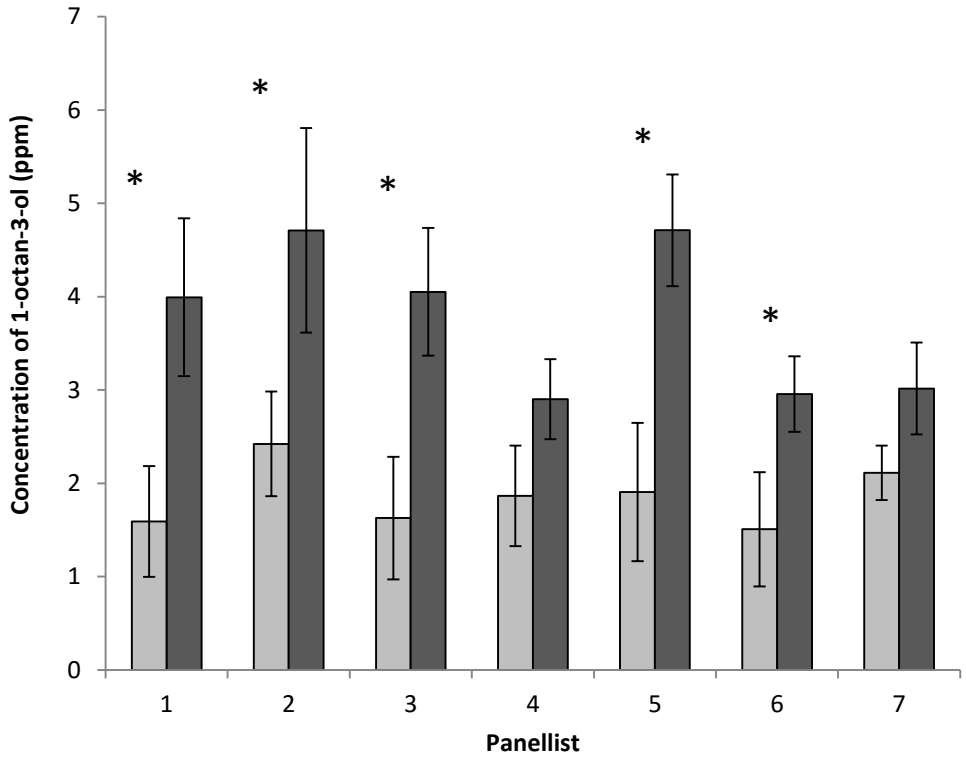

Figure 2.6 Maximum 1-octen-3-ol aroma release from the nose during consumption of samples consisting 0 % (□) and 80 % (■) air inclusion. Data is expressed as means of three replicates ± standard deviation. * p<0.05.

MS-Nose was used to measure the concentration of 1-octen-3-ol in exhaled breath during consumption. The maximum in nose concentration occurred after swallowing samples containing 0 % and 80 % air inclusion (Figure 2.6). Although individual variations were observed, similar trends were observed for all panellists and the maximum in nose concentration was greatest in the samples containing 80 % air inclusion. The maximum in nose concentration was significantly higher in samples with 80 % air inclusion when compared to 0 % air inclusion (p<0.05). A possible explanation for the maximum in nose concentration being greater in the aerated sample could be due a faster liberation of aroma volatiles as the sample is less dense resulting in a quicker

breakdown of the matrix (Mestres *et al.*, 2005, Wilson and Brown, 1997). This subsequently leads to a greater flux of aroma compounds being delivered retronasally to the oronasal cavity (Buettner *et al.*, 2001).

The total release (total area under the curve) of 1-octen-3-ol was recorded (Figure 2.7) and the overall release of 1-octen-3-ol was significantly higher for the sample containing 80 % air inclusion (p<0.05). The increase in aroma release could be due to an increased surface area between the sample and oral cavity where air is introduced to the sample creating air pockets, which increased volatile release (Yu *et al.*, 2012). The action of movement of the tongue followed in the protocol further increased the exposed surface area enhancing aroma volatile release. The microstructure of the foam may allow aroma volatiles to equilibrate within the air pockets and the volatile therefore is rapidly released during mastication (Zúñiga and Aguilera, 2008), thus accelerating aroma release from the matrix prior to swallowing. Diffusion rates in air are much faster than those shown in solid/ semi-solid gels, this could additionally contribute to an increased rate of release of 1-octen-3-ol from the aerated samples.

The work presented demonstrates enhancement of both salt and flavour perception solely by increasing the proportion of air inclusions, this is consistent with previous reports by Goh *et al.* (2010). Similar results have also revealed that by increasing the oil phase volume in oil-in-water emulsions (Bayarri *et al.*, 2007, Drewnowski and Schwartz, 1990, Malone *et al.*, 2003, Yamamoto and Nakabayashi, 1999); the limitation of the mouth coating effects shown previously to suppress saltiness are not observed using air inclusions.

This study demonstrates the rate of diffusion of tastant increased with increasing air inclusion, contributing to higher concentrations of salt being made available at the receptor and therefore an increased perception of saltiness. The presentation of a complimentary aroma was able to enhance total flavour perception in samples with increasing air inclusions. The rupture of the air bubbles during oral processing is believed to increase delivery of aroma and tastants and consequentially saltiness and flavour perception (Minor *et al.*, 2009, Weel *et al.*, 2002). Aerated food gels have been shown within this work to have the potential to control tastant and aroma delivery and are directly applicable to food applications (Zúñiga and Aguilera, 2008).

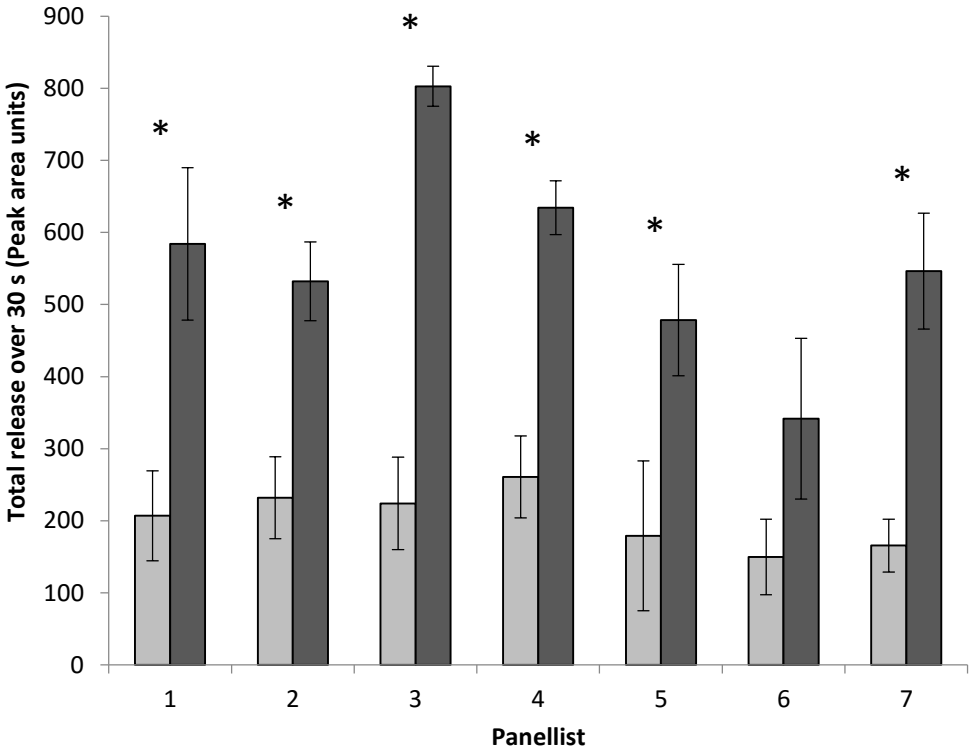

Figure 2.7 Total release of 1-octen-3-ol aroma release from the nose during consumption of samples consisting 0 % (□) and 80 % (■) air inclusion. Data is expressed as means of three replicates ± standard deviation. * p<0.05.

This would be especially applicable in multiphase foods containing hydrogels however careful consideration of the nature of the volatile, tastant and matrix is important for future research or industrialisation of the results.

2.3.4.1 Understanding release of aromas with different partition-coefficients

Aromas with different physical properties were selected to understand the release of aroma volatiles from foams with different levels of aeration. The interaction between an aroma, specifically a congruent aroma, and taste can affect the overall perception, therefore the partitioning of the aroma volatile from the matrix and availability of the aroma during consumption was studied. The effect of total *in vivo* aroma release of five aromas with different partition-coefficients (Log P) from different air inclusion levels (0 %, 40 % and 80 %) was measured (Figure 2.8).

The total release of aroma decreased with increasing Log P value, regardless of air inclusion. When the sample is placed in the mouth, volatiles may remain in the sample or partition into the gas phase above it. During exhalation, the gas phase is expelled and the volatile concentration is measured. Once the foamed or non-foamed sample is swallowed, the cavity is coated with the volatile containing sample and the aroma may also be present in the gas phase.

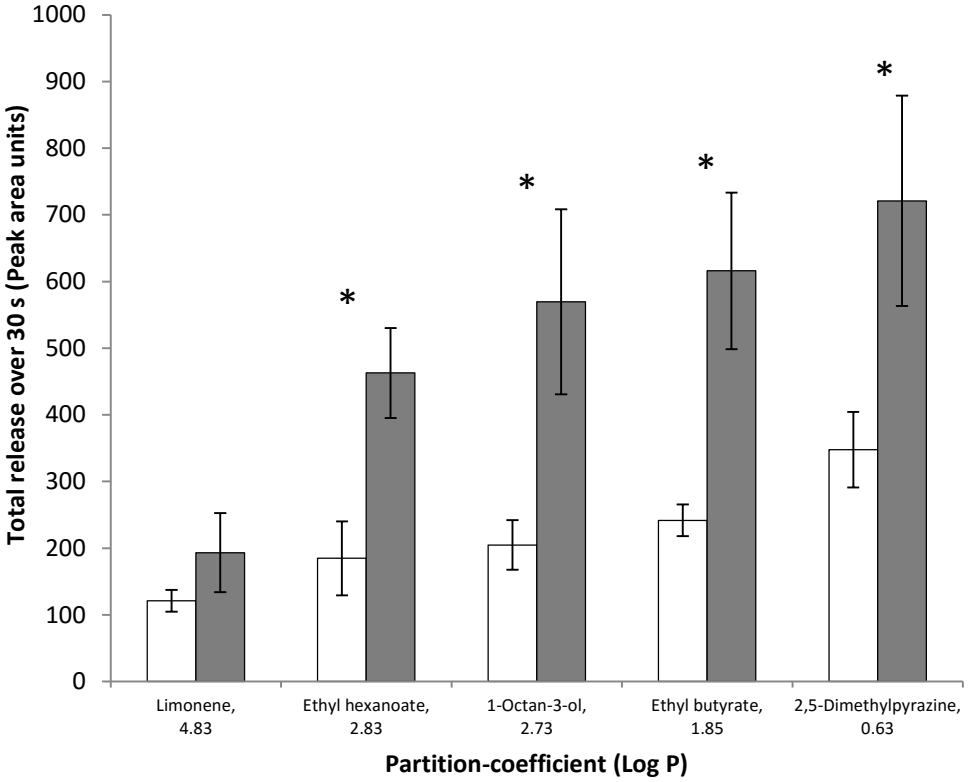

Figure 2.8 Total release in 30 s of aroma volatiles from the nose during consumption of samples consisting 0 % (□) and 80 % (■) air inclusion. Data is expressed as means of three replicates per panellist ± standard deviation. * =significant difference between aeration levels (p<0.05).

The overall detection of aromas with higher Log P values were less as they are less persistent compared to low Log P aromas (Linforth and Taylor, 2000). This trend is observed as aroma molecules with a lower Log P are more hydrophilic and the aromas present in the residual sample after swallowing gradually repartition into the gas phase for detection. In contrast, compounds with a higher Log P values are less likely to be localised within the aqueous phase and less aroma is likely to be present in the residual sample. Aromas with a lower Log *P* may interact with the saliva in the oral cavity which may also increase persistence.

In addition, total aroma release increased significantly with more air inclusions, with the exception of limonene (Log P=4.83) and no significant differences were present between the two different air inclusions ($p>0.05$).

An increase in release of aroma compounds from more aerated systems can be explained by the difference in structure. Samples containing no air inclusion display an increased density of the gel network and more aroma molecules are likely to be entrapped within the network (Hansson *et al.*, 2003).

Furthermore, increasing the level of air within the foam creates a less rigid structure and the samples are likely to rupture during oral processing, refreshing the surface area and aiding the release of all aroma compounds, compared to that of a gel, with no air inclusion.

2.4 Conclusion

In this chapter, the development of a stable foam system using WPI was achieved when heated to 60 ˚C and was used as the model foam system for all subsequent experiments. The impact of sodium reduction on taste has been mitigated through the use of air inclusions. Air inclusions were also shown to enhance sodium perception for samples containing equal amounts of salt, as salt concentration in the aqueous phase increases. This was proposed to be due to an increase in sodium concentration in the aqueous phase as air was used to displace the aqueous phase.

However, it should be highlighted that the displacement of the aqueous phase with air would affect the energy density within a specific volume. Therefore, careful consideration is required when applying to products, particularly staple food products, as calorie intake may be affected.

The understanding of tastant and aroma delivery through air inclusions may be adapted into existing foods (including mousse, whipped cream and during

restaurant dining experiences) or incorporated into foods that may currently not have air to create a new category of food products where the enhance perception of flavour can be exploited through the inclusion of air.

Air inclusions were able to further enhance delivery and the perception of a congruent aroma. In conclusion, the use of air inclusions has been shown to increase both the delivery and perception of salt and aroma, in addition to increasing overall flavour perception.

When investigating other aroma compounds, the inclusion of air increased the total level of aroma released. However, the relative effectiveness was dependent on the aroma compounds physical properties (Log P). Therefore, it is important to consider such aroma properties when applying these findings to real systems.

In addition, knowledge gained from this study may potentionally be adapted towards other tastes, such as sweetness. Finally, this study has demonstrated the use of air fillers as a potential sodium reduction strategy.

3 Delivering Sodium using double emulsions

3.1 Introduction

This chapter explores the use of double water-in-oil-in-water ($w_1/o/w_2$) emulsions as vehicles to deliver enhanced perception of saltiness. The results specifically detail the development of the model $w_1/o/w_2$ emulsion, evaluation of emulsion stability, sodium release *in vitro*, *in vivo* and saltiness perception.

The use of contrasting levels of stimulus delivered across an eating event is a promising route to enable the reduction of the total concentration of a stimulus whilst maintaining perception without compromising acceptability. One example successfully applied as a sodium reduction strategy (Noort *et al.*, 2010), was achieved by structuring sodium in regions of a dry bread product offering contrasts of sodium during eating. The tastant is delivered to the receptor in varying concentrations thereby increasing taste perception (Meiselman and Halpern, 1973). Currently this strategy only applies to products where the sodium remains localised and is not able to diffuse across. However, through careful structural design, this strategy could be applied to liquid systems.

Other research groups have shown the theoretical potential of this approach in liquid systems through pulsed delivery (Busch *et al.*, 2009, Morris *et al.*, 2010). The success of this approach appears to very much depend on the timing of short and intense stimulus delivery and the overall length of the experimental protocol. One group of researchers using 15 s as a delivery profile

for salty water concluded that saltiness perception was proportional to the overall amount of salt delivered within that period of time (Morris *et al.*, 2010). Another study, delivering 5 s and 2 s pulses in sequential 10 s intervals with the same overall salt concentration, found an increased perception of saltiness when delivered in 2 s pulses compared to a constant delivery (Busch *et al.*, 2009). However, the mentioned studies delivered the tastant through a pump which does not reflect how food products are consumed therefore this approach needs to be validated in real food products.

To deliver regions of concentrated sodium in a liquid food system, the tastant requires some form of entrapment and controlled delivery. Emulsions are colloidal systems whereby both the dispersed phase and continuous phase are liquids and may be considered as a viable option in separating different dispersed sodium in a liquid system.

Emulsions are dispersions which consist of two immiscible liquids such as oil and water. Emulsions are often described as single or double/complex/multiple emulsion. In single emulsions, the oil can be dispersed in an aqueous phase forming oil-in-water (o/w) or water droplets dispersed in oil to produce water-in-oil (w/o) systems (Mcclements, 2004). Two main types of double emulsion can be noted: water-in-oil-in-water ($w_1/o/w_2$) emulsions, which consists of a w/o emulsion dispersed in an aqueous phase, and oil-in-water-in-oil ($o_1/w/o_2$) emulsions, consisting of an o/w emulsion dispersed in an oil phase (Garti, 1997).

The formation of an emulsion is not spontaneous as the contact between the continuous and dispersed phase are thermodynamically unfavourable and can be characterised by the interfacial tension between the two liquids (Israelachvili, 1992). When an emulsion is homogenised, the interfacial area between the continuous and dispersed phase increases, this results in an increase in free energy. In all systems the lowest free energy state is favourable and therefore emulsions are thermodynamically unstable systems. However, a free energy barrier must be acquired before moving from a high energy state to a lower energy state. When the free energy barrier is high, the system may remain in a thermodynamically unstable state for a length of time and the emulsion system is said to be kinetically stable (Mcclements, 2004). To create an emulsion that is kinetically stable for a considerable length of time, an emulsifier such as surfactants, proteins or particles may be applied to improve emulsion stability (Damodaran, 2005).

Stability of an emulsion refers to the ability of an emulsion to resist change over time. A number of physiochemical changes may render an emulsion instable. It is important to understand which of the mechanisms are important in the system under consideration prior to implementing strategies to improve emulsion stability. Physical changes responsible for emulsion instability include creaming, sedimentation, flocculation, coalescence and phase inversion (Mcclements, 2004). Creaming and sedimentations are forms of gravitational separation (Dickinson, 2009, Walstra, 1996). Creaming describes the upward movement of droplets as they have a lower density than the surrounding liquid,

whilst sedimentation describes the movement of droplets downwards as they have a higher density compared to the surrounding liquid. Flocculation and coalescence are two types of droplet aggregation that occur in food systems (Dickinson and Stainsby, 1982). Flocculation occurs when two or more droplets come together to form an aggregate and the droplets retain their integrity. Coalescence refers to merging of two or more oil droplets to form a single large droplet and is an irreversible process. Extensive coalescence can eventually lead to phase separation. Phase inversion is when a system changes from an o/w emulsion to a w/o emulsion or vice versa (Mcclements, 2004). The implementation of an appropriate emulsifier may significantly slow down the process of such changes and should be considered when producing emulsions.

As sodium is dispersed in the aqueous phase, a double $w_1/o/w_2$ emulsion containing two aqueous phases may be ideal in separating two different sodium concentrations, where the sodium content is higher in the internal water phase of an emulsion. Whilst $w_1/o/w_2$ emulsions have been evaluated for their potential as a control mechanism for targeted release of water-soluble or oil-soluble actives during digestion (Jiménez-Colmenero, 2013, Lakkis, 2008), only a few studies have looked at $w_1/o/w_2$ emulsions for their ability to control tastant release (Frasch-Melnik *et al.*, 2010b, Spyropoulos *et al.*, 2011). To date, the potential of encapsulating tastants using $w_1/o/w_2$ emulsions is not fully understood.

There are two prerequisites of a double $w_1/o/w_2$ emulsion system as a method of controlled delivery 1) its stability during storage, and ability to retain sodium

within its internal phases prior to consumption and 2) its ability to release sodium during consumption. Particle stabilisation could be one approach to enabling effective stability during storage.

Particle stabilised emulsions or Pickering emulsions have received substantial interest and have been reported to form highly stable emulsions (Aveyard *et al.*, 2003). Furthermore, Pickering emulsions are often regarded to be more stable than surfactant stabilised emulsions as once adsorbed the particle is considered irreversibly adsorbed and desorption energies are high (Binks and Lumsdon, 2000).

Both native and modified starches can be used as particle stabilisers to stabilise oil-in-water (o/w) emulsions (Haaj *et al.*, 2014, Li *et al.*, 2013, Matos *et al.*, 2013, Nilsson and Bergenståhl, 2006, Rayner *et al.*, 2012, Tan *et al.*, 2014, Timgren *et al.*, 2011). In addition, α-amylase, present in the mouth, has the potential to hydrolyse starch, and altering the emulsion structure (Chen, 2015, Janssen *et al.*, 2007). Starch with varying modified surface chemistry is therefore proposed as a hydrophilic emulsifier that may selectively destabilise during oral processing and release internalised sodium. In addition, these emulsions may destabilise in the mouth to deliver encapsulated sodium for perception within timescales relevant to normal food consumption.

The anticipated pathway of oral destabilisation of a starch stabilised $w_1/o/w_2$ emulsion is shown in Figure 3.1. The interfacially adsorbed starch is hypothesised to be weakened through the action of salivary amylase and two scenarios of emulsion breakdown are proposed. The interface will destabilise

and droplets coalesce (Figure 3.1A) releasing the high sodium entrapped water phase into the oral cavity. In this process, surface active salivary proteins may adsorb at the droplet interface. Furthermore, manipulation between tongue and palate in combination with the emulsifying action of salivary proteins may lead to new interfaces being formed and phase inversion (Figure 3.1B). This hypothesis is based on the knowledge that fat continuous spreads and chocolate "phase invert" during oral processing into an o/w emulsion, the microstructure of which directly impacts mouthfeel and flavour release (Carvalho-Da-Silva *et al.*, 2013, Norton *et al.*, 2009).

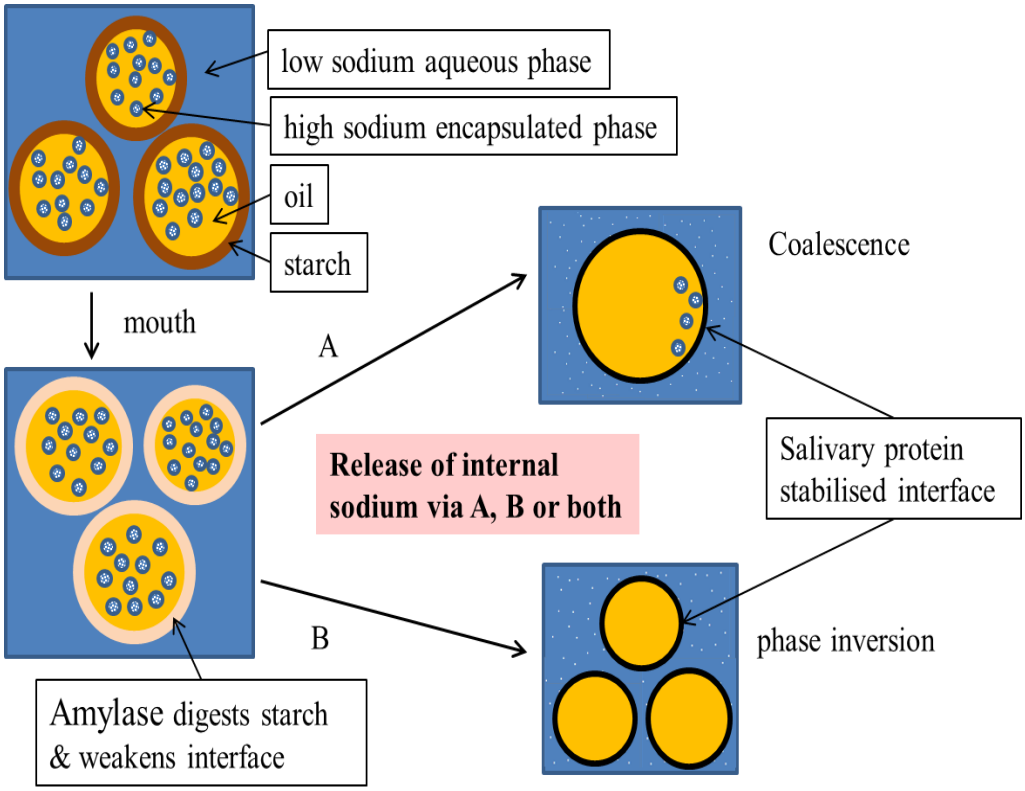

Figure 3.1 Schematic of the anticipated pathway of oral destabilisation of a starch stabilised $w_1/o/w_2$ emulsion.

The main objectives of the study were to develop a double $w_1/o/w_2$ emulsion system that is stable during storage but destabilises during oral processing, using starch particles as the external emulsifier and to understand the effects

of saltiness perception through the release of encapsulated sodium in the mouth.

3.2 Materials and Methods

3.2.1 Materials

All materials used to prepare the emulsions were food grade. Sunflower oil (KTC, Wednesbury, UK), table salt (Saxa, London, UK), plain crackers (99 % Fat Free, Rakusen's, Leads, UK), mineral water (Evian, France) and Green apple (Granny Smith variety) were purchased from a local supermarket. Polyglycerol polyricinoleate (PGPR) was donated by Danisco (Dorset, UK) and the octenyl succinic anhydride (OSA) waxy maize starch, N-creamer 46 (NC46) was provided by Univar (Widnes, UK). Pea protein isolate (PPI) was obtained from Myprotein (Manchester, UK) and was selected as an emulsifier that is not susceptible to α-amylase hydrolysis. Sodium hydroxide (NaOH) was obtained from VWR International Ltd. (Lutterworth, UK). Rice starch, potato starch, calcium chloride ($CaCl_2$), ethanol, hydrochloric acid (HCl), 4-morpholinepropanesulfonic acid sodium salt (MOPS sodium salt), phenolphthalein, porcine α-amylase and sodium azide were obtained from Sigma-Aldrich (Gillingham, UK). Sodium azide was used as an antimicrobial agent and was only added to samples that were not intended for sensory analysis. Amyloglucosidase, D-glucose, standardised regular maize starch and thermostable α-amylase were provided as part of the Megazyme total starch assay kit purchased from Megazyme, Co. (Wicklow, Ireland). Deionised water, with a resistivity of 15MΩ/cm was used for the preparation of all solutions.

3.2.2 Emulsification

3.2.2.1 Single o/w emulsions

Single oil-in-water (o/w) emulsions of 40 % sunflower oil were prepared in a high shear mixer (Silverson L5M, Chesham, UK). The aqueous phase contained NaCl ranging between 0 and 0.171 mol/L. Emulsifiers, native starch, modified starch or pea protein, were added to the aqueous phase to achieve concentrations between 1 and 6 % w/w and were mixed for 1 min at 4000 rpm, sunflower oil was added to the aqueous phase and mixed for a further 2 min at 4000 rpm.

3.2.2.2 Double $w_1/o/w_2$ emulsions

Water-in-oil (w_1/o) emulsions consisting 30 % water were prepared using the high shear mixer (Silverson). An aqueous phase consisting of NaCl, with concentrations between 0 and 0.171 mol/L was prepared. Initially, sunflower oil was mixed with 2.8 % w/w PGPR for 1 min at 4000 rpm with the aqueous phase added to the oil phase and mixed for 2 min at 4000 rpm. Water-in-oil-in-water ($w_1/o/w_2$) emulsions contained 50 wt% w_1/o emulsions. The external aqueous phase was mixed for 1 min at 4000 rpm, containing between 0 and 0.171 mol/L NaCl and an emulsifier (native starch, modified starch or pea protein) was added to obtain concentrations between 1 % and 6 % w/w. Once the emulsifier was incorporated, the w_1/o emulsion was added and was mixed for a further 2 min at 4000 rpm.

3.2.3 Microstructure imaging

3.2.3.1 Light Microscopy

A drop of each sample was placed onto a glass slide, covered with a cover slip and then imaged using a digital inverted transmission light microscope (EVOS®FL, Life Technologies Ltd., Paisley, UK) fitted with a 20 x bright field, long working distance objective (AMEP4634, Life Technologies Ltd., Paisley, UK).

3.2.3.2 SEM

To obtain scanning electron microscopy (SEM) micrographs, a small amount of sample was distributed onto carbon tabs (Agar Scientific, Stansted, UK), coated with carbon (Agar turbo carbon coater) and placed on the stage of a FEI Quanta 3D 200 dual beam Focused Ion Beam Scanning Electron Microscope (FIB-SEM; FEI, Hillsboro, USA). Micrographs were acquired using secondary electron imaging at an accelerating voltage of 5–15 kV.

3.2.4 Particle size

3.2.4.1 Emulsifiers

Size distributions of the unmodified starches (rice, potato and waxy maize starch), modified starch and PPI suspensions were measured with a laser diffraction particle size analyser (LS 13 320, Beckman Coulter, High Wycombe, UK) fixed with an aqueous dispersion cell (Universal liquid module, LS13 320, Beckman Coulter , Wycombe, UK). Each sample was measured in triplicate. The Fraunhofer diffraction model located on the particle size analyser software was used to analyse the data.

3.2.4.2 Emulsions droplets

Droplet size distributions of emulsion droplets were acquired by processing images captured after emulsion processing. This method was modified from a published protocol (Frasch-Melnik *et al.*, 2010b) where the light microscope micrographs were processed with public domain image analysis software (ImageJ, NIH, Bethesda, USA) and 600 droplets were measured from each sample. The surface area mean also known as Sauter mean diameter ($d_{3,2}$) was calculated using Microsoft Excel. The mean and standard deviation for each formulation was reported based on three independent samples and was an indication of emulsion droplet size.

To determine if significant differences in emulsion droplet diameter occurred between the emulsifiers after emulsification a one-way analysis of variance (ANOVA) was performed and where appropriate, Tukey's HSD multiple comparison tests was performed (SPSS, IBM, Version 22, USA) (p=0.05).

To determine if significant difference in droplet size was present between the first day of emulsification and the last day of storage of each sample a two tailed T-test was performed (SPSS, IBM, Version 22, USA). Analysis was based on 95 % confidence limit.

3.2.5 Sodium release

Levels of sodium in the continuous phase of the emulsions were measured using a sodium ion specific electrode with a measurement range of -1999.9 to +1999.9 mV (Jenway, Stone, UK) and measurements were recorded every 1 s. Sodium chloride solutions between 0 and 1.5 mol/L were used to create a

standard curve based on which the conductivity data of the emulsions was converted to amount of salt in the continuous phase.

Sodium chloride release from the double $w_1/o/w_2$ emulsions was quantified *in vitro* using the methodology as previously adapted form literature (Al-Rabadi *et al.*, 2009). The emulsion (10 mL) was mixed on a magnetic stirrer at 37 °C with 10 mL of 1 M aqueous solution containing carbonate buffer at pH 7. Porcine salivary α-amylase was added under continuous stirring. The final solution had an enzyme level of 50 U/mL. Measurements were recorded for 20 s to monitor the release of sodium from w_1 to w_2. After 20 s, 1 mL of 2 M HCl was added to the sample to inactivate the enzyme and 0.02 % sodium azide was mixed into the sample to prevent microbial spoilage. The amount of released salt was expressed as the percentage of salt in the continuous phase using Equation 2.

$$Salt\ released\ (\%) = \frac{m_{external}}{m_{total}} x100 \qquad \textbf{Equation 2}$$

where m_{total} is total mass of salt that was originally present in the internal phase; $m_{external}$ is mass of salt that has moved to the external water phase.

3.2.6 Encapsulation efficiency

Sodium release from the entrapped aqueous phase to the continuous phase was measured over time using the same sodium ion specific electrode. The probe was placed in the $w_1/o/w_2$ emulsion and a final measurement after 20 s was recorded and converted into NaCl. Encapsulation efficiency (*EE*) was defined as the percentage of salt still entrapped within the inner aqueous phase (w_1) using Equation 3.

$$EE\ (\%) = 100 - salt\ released\ (\%)$$ **Equation 3**

3.2.7 Total starch assay

Following sodium release measurement, the emulsions were further analysed for total starch to ascertain the degree of starch digestion by difference to added starch in the formulation. A standard published protocol (AOAC Method 996.11, Megazyme International Ireland Ltd.) was followed which required the initial preparation of morpholinepropanesulfonic acid (MOPS) sodium salt and sodium acetate buffers. MOPS sodium salt buffer was prepared by dissolving 11.55 g of MOPS sodium salt in 900 mL of water, then adjusting to pH 7.0 by the addition of 1 M HCl dropwise. Calcium chloride (0.74 g) and 0.2 g of sodium azide was dissolved in the solution and the total volume adjusted to 1 L. The sodium acetate buffer was prepared with 11.6 mL of glacial acetic acid to 900 mL water adjusted to pH 4.5 by 1 M sodium hydroxide solution, 0.2 g of sodium azide was dissolved and the volume was adjusted to 1 L.

The digested and undigested emulsion samples (100 mg) were mixed with 5 mL of aqueous ethanol (80 % v/v), and incubated at 80 °C for 5 min. An additional 5 mL of 80 % v/v aqueous ethanol was added and the sample was then centrifuged for 10 min at 1,800 x g and the supernatant was discarded. The pellet was re-suspended in 10 mL of 80 % v/v aqueous ethanol, stirred on a vortex mixer, and centrifuged as previously described. The supernatant was poured off and immediately 2 mL of DMSO was added to the pellet and stirred on vortex mixer. The content was placed in a boiling water bath for 5 min. Thermostable α-amylase (3 mL) and 50 mM MOPS buffer (90 mL) was previously mixed and added to the heated content. The mixture was heated in

boiling water for an additional 6 min. Sodium acetate buffer (4 mL) and 0.1 mL amyloglucosidase (20 U) were added to the samples followed by mixing and incubation at 50 °C for 30 min. The entire content was transferred to a 100 mL volumetric flask and the volume was adjusted to 100 mL using distilled water. An aliquot of the solution was centrifuged at 1,800 x g for 10 min. The concentration of glucose in the clear filtrate was then measured using a glucose analyser (Analox GM9 Analyser, London, UK).

To determine if significant difference in total starch was present before and after digestion a two tailed T-test was performed (SPSS, IBM, Version 22, USA). Analysis was based on 95 % confidence limit.

To determine if significant differences in remaining starch was present between the different emulsifier concentration a one-way analysis of variance (ANOVA) was performed followed by Tukey's HSD multiple comparison tests (SPSS, IBM, Version 22, USA) (p=0.05).

3.2.8 Viscosity

A MCR 301 rheometer (Anton Paar, Messtechnik, Stuttgart, Germany), with a double gap geometry (DG26.7) was used for performing rheological measurements. The $w_1/o/w_2$ emulsion samples (50 mL) were placed in the measuring system and left for 1 h prior to measuring. All the measurments were performed at 25 °C. Shear rates from 0.1 to 500 s^{-1} were applied and apparent viscosity (Pa·s) was recorded as a function of shear rate

3.2.9 Paired comparison testing of double emulsions stabilised with PPI or commercially modified OSA starch

Saltiness perception was evaluated using the method of paired comparison (PC) tests (BS ISO 5495:2007). Assessors (aged 19-57; 78 female, 42 male) were recruited from students and staff of the University of Nottingham and signed informed consent was obtained from each panellist before the study commenced. The description of the sample sets included in the paired comparison tests to determine overall perceived saltiness between $w_1/o/w_2$ emulsions is included in Table 3.4. The external emulsifier used to stabilise the emulsions was pea protein isolate (PPI) or N-creamer 46 (NC46) and the emulsions contained varying levels of salt. The sample (10 mL) was presented to the panellists in randomised, balanced order across the panel in containers labelled with a random three-digit code. Sensory evaluation was conducted 1 d after sample preparation. For each PC, assessors were required to test the samples in the order presented and following a set test protocol; place the whole sample in the mouth, press the tongue against the palate three times, hold the sample for 10 s prior to swallowing, and then indicate which of the two samples they perceived to be saltier. Assessors were instructed to taste the samples in the order presented and identify the sample they perceived to be saltier. Panellists were also instructed to cleanse their palate before and between samples with green apples, unsalted crackers and mineral water. The test was used in forced-choice mode, so panellists were required to give an answer even if the perceived difference was negligible and panellists were given the opportunity to comment on the samples. Results were compared to

tables A.2 and A.3 in BS EN ISO 5495:2007 to determine difference and similarity respectively (British Standards Institution, 2007).

3.3 Results and Discussion

3.3.1 Native starch

Emulsion stability was crucial to retain the separation of phases hence oil-in-water (o/w) emulsions were formulated using three different starches (rice starch, potato starch and waxy maize starch) to observe droplet stability. Particle size measurements were performed to determine the particle size of the three starches and the distribution are presented in Figure 3.2. All three starches were different in particle size distributions, with rice starch having the smallest mean particle diameter (6.76 μm), followed by waxy maize starch (18.97 μm) and potato starch with the largest mean particle diameter (39.12 μm).

In particle stabilised emulsions, particle size has been shown to affect the stability and droplet size of particle stabilised emulsions with stability being linear to particle size (Hunter *et al.*, 2008), hence the differences between the starch samples may influence droplet size and emulsion stability.

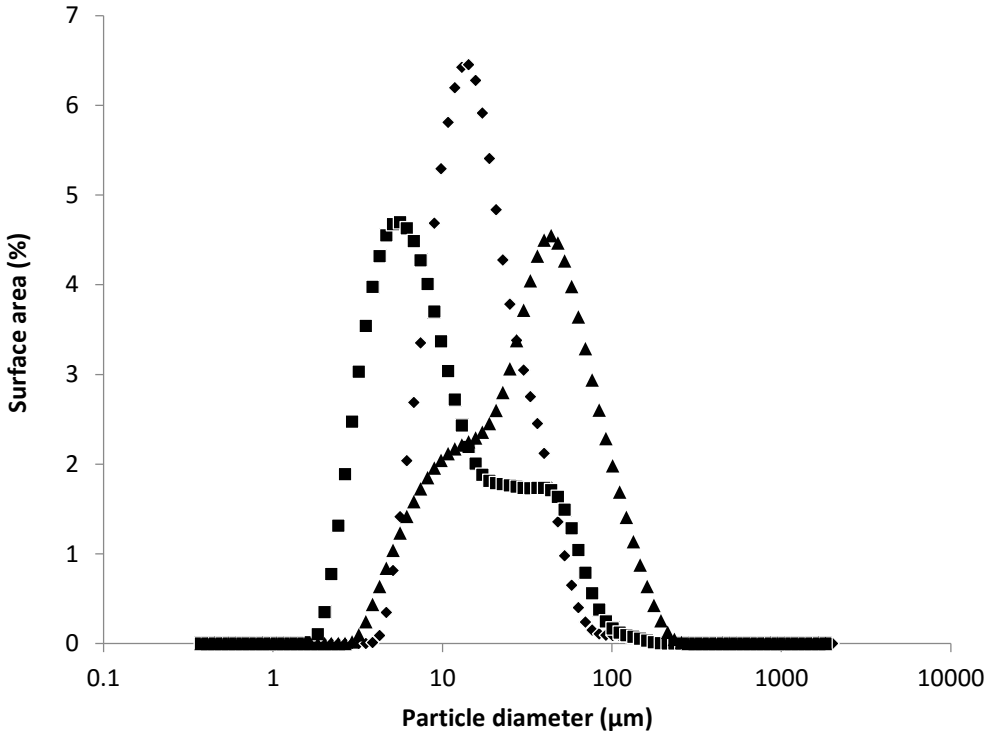

Figure 3.2 Particle size distributions of rice starch (■), waxy maize starch (◆) and potato starch (▲). Data points are the average of three independent replicates.

3.3.1.1 Native starch stabilised single emulsions

Droplet size of the single o/w emulsions was measured daily and the mean droplet surface area was calculated which reflected the size of the starch particles. The smallest surface area mean was observed when rice starch was used as the stabiliser and potato starch stabilised emulsions with the largest mean emulsion droplets (Table 3.1). After emulsification, the mean droplet surface area of potato starch stabilised emulsion was significantly larger (p<0.05) than rice and waxy maize stabilised emulsion droplets. Rice starch particles were shown to be smaller than waxy maize starch although no significant difference (p>0.05) was found between the mean droplet size of the two emulsion samples.

To attain effective Pickering stabilisation, the average size of the particles should be at least an order of magnitude smaller than the emulsion droplet size

(Dickinson, 2012). However, comparing the mean diameter of the starch particles and the emulsion droplets formed with the relevant starch particles, the average size of the emulsion droplets were indeed similar to the starch particles used to form the emulsions. Gould *et al.* (2013)also observed a size distribution overlap between cocoa particles and its stabilised emulsions. It was postulated that the starch particles present at the interface are a small sized fraction that was not detected by the particle sizing equipment.

Table 3.1 The effect of storage time on the mean surface area droplet size of the starch stabilised o/w emulsions. The mean is the average of three independent replicates. ± represent standard deviation. ND=not determined as coalescence was observed.

Type of starch used to stabilise emulsion	Droplet surface area mean (μm) at day			
	0	1	2	3
Rice	16.4 ± 0.7	17.1 ± 0.4	33.1 ± 0.6	ND
Waxy maize	17.0 ± 0.5	18.6 ± 0.3	34.2 ± 0.8	ND
Potato	25.9 ± 0.3	ND	ND	ND

Daily images of the emulsions were captured (Figure 3.3) and once a visual oil layer was observed, the droplet measurements of surface area ceased as this phase separation was indicative of emulsion instability. The emulsions formed with potato starch, the largest mean particle size in the sample set, destabilised shortly after emulsification and coalescence was observed in Figure 3.3B. The results presented differ from the observations presented by Li *et al.* (2013) where some native starches, including rice and waxy maize, were able to form stable o/w emulsions for weeks. However, the starch concentration presented here was lower in comparison. The starch particles selected as emulsifiers were unable to stabilise o/w emulsions over 2 d and phase separation was found to be present in the examined emulsions, see Figure 3.3D. This is hypothesised to be attributed to the hydrophilic nature of native starches

which would reduce the stability of the particle at the interface (Aveyard *et al.*, 2003). Furthermore, the small proportion of the small sized fraction of starch particles present at the interface may have resulted in a low surface coverage. This may have contributed to emulsion instability, as increasing starch concentration demonstrated improved stability. Pickering stabilisation requires particles to accumulate at the interface to form a steric barrier and the low surface coverage and hydrophilic properties of the starch may have rendered the steric barrier less effective in preventing the emulsion droplets against coalescence (Dickinson, 2012).

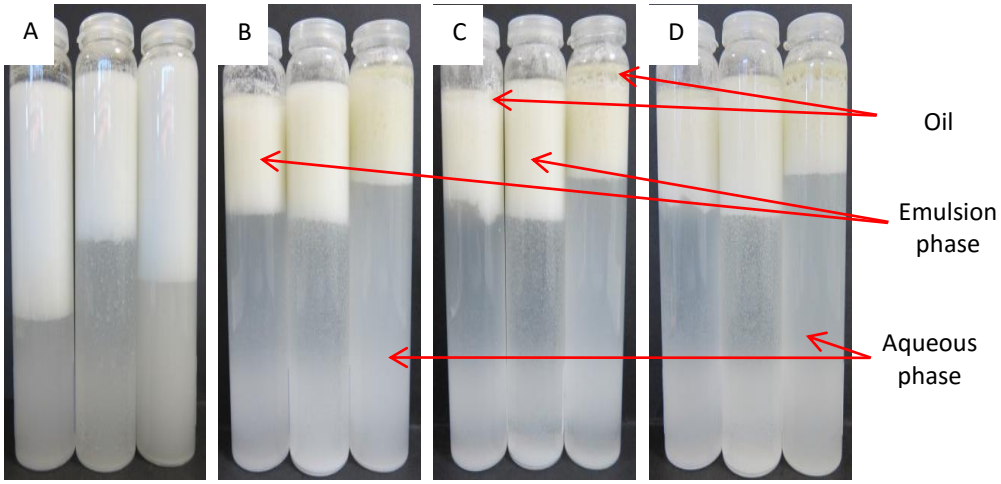

Figure 3.3 Images of single o/w emulsion stabilised by waxy maize (left), rice (centre) and potato starch (right), captured after emulsification (A), 1 d (B), 2 d (C) and 3 d (D).

3.3.1.1.1 Microstructure after oral processing

The starch stabilised emulsions were subjected to porcine α-amylase at a concentration present in human saliva for 20 s. Micrographs captured before and after porcine α-amylase are shown in Figure 3.4. It was evident from the micrographs that a change occurred when the starch was exposed to the

enzyme. The droplet size of all three starch stabilised emulsions increased post porcine α-amylase exposure and the emulsions were unstable to coalescence. Porcine α-amylase appears to have a more prominent effect on emulsions stabilised with larger starch particles. However, this may be related to the inherent instability of the emulsions when stabilised with larger starch particles, as shown previously.

In vitro observations of the emulsions may potentially reflect *in vivo* events as porcine and human α-amylase have been found to be structurally similar (Butterworth *et al.*, 2011, Gilles *et al.*, 1996, Ramasubbu *et al.*, 1996). The α-amylase is able to hydrolyse α-1,4-glycosidic bonds within amylose and amylopectin and studies have shown enzyme-induced changes to the food, such as reducing starch viscosity, were within times relevant to an eating event (Ferry *et al.*, 2004, Patel *et al.*, 2014).

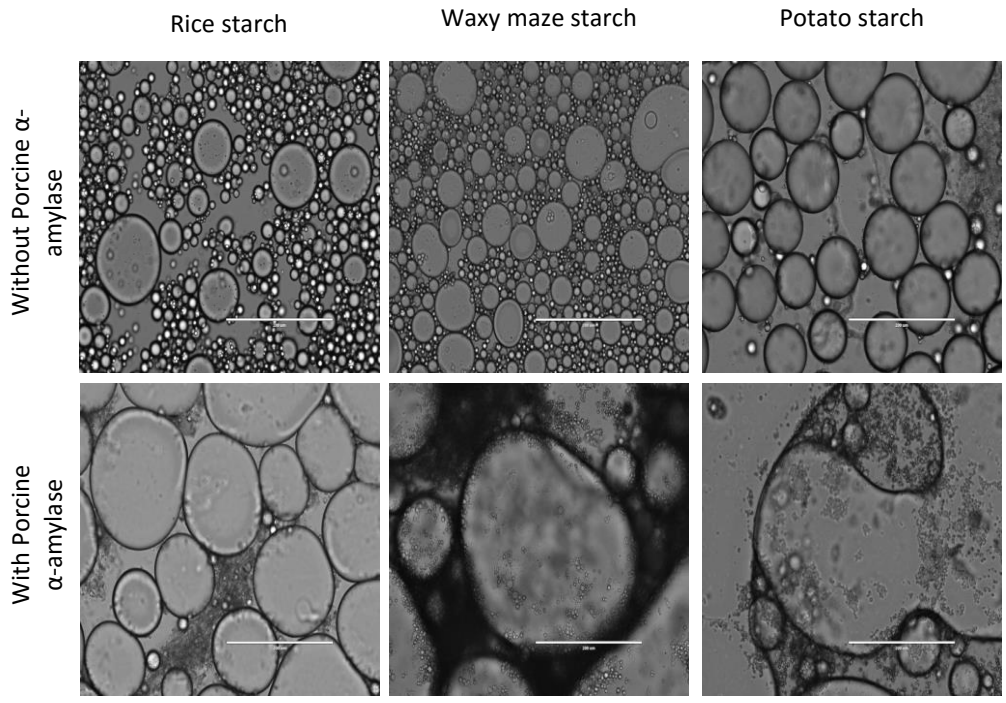

Figure 3.4 Micrographs of o/w emulsions stabilised with rice, waxy maize and potato starch before (1) and after subjected to porcine α-amylase (2). Scale bar = 200 μm.

3.3.1.1.2 Remaining starch after oral processing

To confirm the effect of both porcine and salivary α-amylase and the extent of hydrolysis that occurred during the exposure time, total starch was analysed before and after hydrolysis (Figure 3.5). There was a decrease in total remaining starch after porcine and salivary α-amylase exposure. However, salivary α-amylase was more efficient at hydrolysing starch and the loss was greatest in the waxy maize starch sample, with a reduction of 22.9 % compared to decreases of 13.7 % and 11.26 % for the emulsions stabilised by rice and potato starch, respectively. It is assumed that the hydrolysis of the starch reduces its emulsification properties and results in emulsion coalescence.

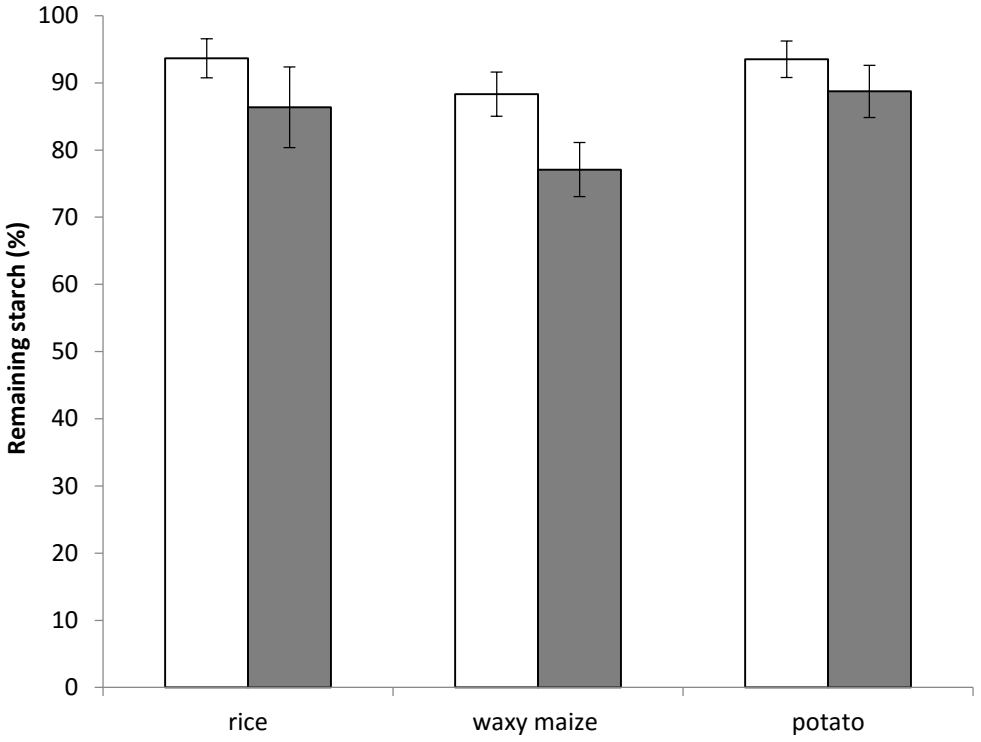

Figure 3.5 Remaining starch in o/w emulsion stabilised with rice, waxy maize and potato starch after *in vitro* (□) and *in vivo* (■) digestion.

3.3.1.2 Native starch stabilised double emulsions

3.3.1.2.1 Droplet size

The three starches previously used as an emulsifier to produce single o/w emulsions were used to form water-in-oil-in-water ($w_1/o/w_2$) emulsions. The external emulsifier, starch, stabilised the o/w phase with polyglycerol polyricinoleate (PGPR) as the internal w/o emulsifier. PGPR was selected for its effective water-in-oil (w/o) emulsifying properties that are well characterised and is frequently used by the food industry (Wilson *et al.*, 1998).

The internal (w_1) and external (w_1/o) droplets of the $w_1/o/w_2$ emulsion were measured and surface area mean was calculated daily until coalescence was observed in the emulsion samples (Table 3.2).

The initial surface area mean of the internal and external droplets of the emulsion was smallest when rice starch was used as the external emulsifier, followed by waxy maize and potato starch. The external droplet mean was significantly larger in the potato starch sample (p<0.05). Similar to the single o/w emulsions, the droplet surface area mean reflect starch granules size.

Table 3.2 The effect of storage time on the mean surface area droplet size of the starch stabilised $w_1/o/w_2$ emulsions. The mean is the average of three independent replicates. ± represent standard deviation. ND=not determined as coalescence was observed.

Type of starch used to as external emulsifier	Measured droplets	Droplet surface area mean (μm) at day		
		0	1	2
Rice	**External (w_1/o)**	27.2 ± 3.1	34.4 ± 3.1	ND
	Internal (w_1)	5.8 ± 0.7	6.6 ± 1.0	ND
Waxy maize	**External (w_1/o)**	30.2 ± 3.3	31.5 ± 2.9	ND
	Internal (w_1)	6.1 ± 0.5	7.4 ± 0.7	ND
Potato	**External (w_1/o)**	94.2 ± 5.5	ND	ND
	Internal (w_1)	10.8 ± 0.9	ND	ND

The surface area mean of the external droplets was larger compared to single o/w emulsions with an additional phase encapsulated within the oil phase. Overtime, the mean surface area of both internal and external droplets increased in all samples and was unstable after 3 d. In addition to the previously mentioned rationale for emulsion instability in Section 3.3.1.1, the additional phase in a $w_1/o/w_2$ emulsion adds to the complexity as they are thermodynamically unstable systems (Garti, 1997).

3.3.1.2.2 Emulsion microstructure

Micrographs of the emulsions were captured and all samples displayed $w_1/o/w_2$ emulsion structures after the emulsion was initially formed (Figure 3.6). After 1 d, the potato starch sample displayed mostly single o/w emulsion and oil separation was present in the emulsion. At 2 d, single o/w droplets were

also evident in rice and waxy maize samples. Micrographs of all emulsions display emptying of the entrapped aqueous phase overtime and an oil layer was observed in all emulsions within 2 d. The $w_1/o/w_2$ emulsions with rice, waxy maize and potato starch as the external emulsifier were deemed unsuitable for storage.

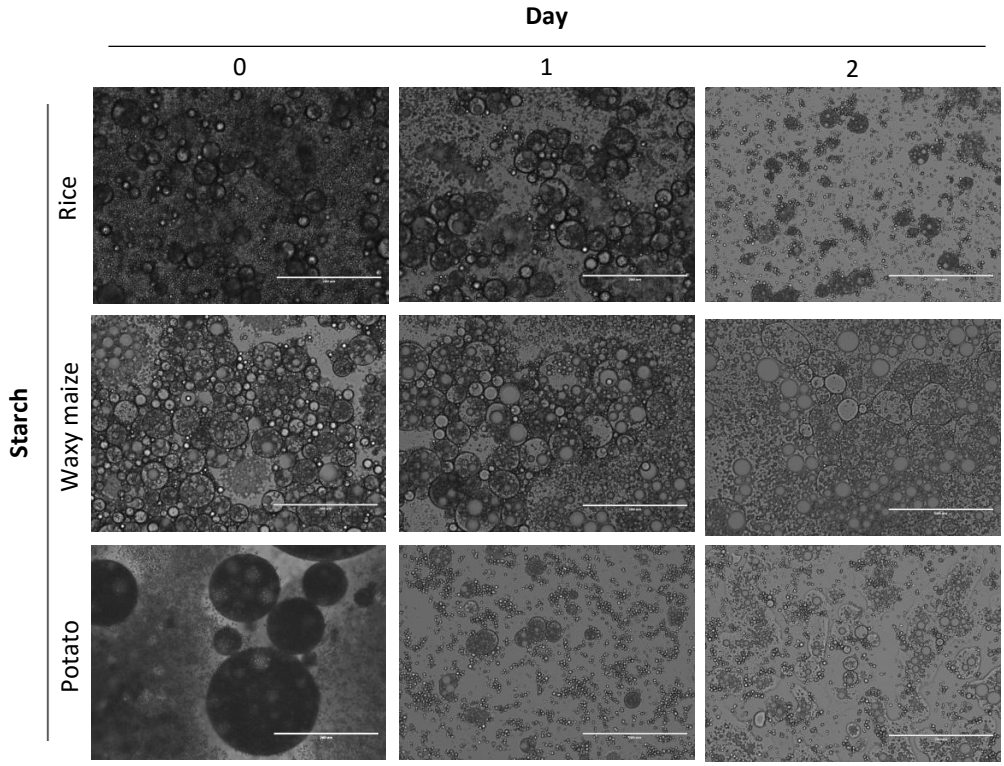

Figure 3.6 Micrographs of $w_1/o/w_2$ emulsions captured over time, when PGPR was used as the internal w/o emulsifier and rice, waxy maize and potato starch was used as the o/w emulsifier. Scale bar = 200 µm.

3.3.1.2.3 Encapsulation efficiency

The sodium content of the continuous phase was measured to assess the sodium encapsulation efficiency (EE) of the emulsions. Sodium was only applied to therefore sodium detected in the external continuous phase would indicate internal water moving towards the external phase.

Initial EE was highest using rice starch as the external emulsifier (89.7 %), followed by waxy maize (87.3 %) and lastly with 35.7 % encapsulation using

potato starch (Figure 3.7). The level of encapsulant decreased similarly for both rice and waxy maize starch, where encapsulant was not detected after 4 d. However, no detectable encapsulant was found after 3 d in the potato starch stabilised emulsion. This is likely to be attributed to multiple factors including low initial encapsulation efficiency and large droplet formation after emulsification as observed in the micrographs (Figure 3.6).

The EE of the emulsions complemented emulsion micrographs shown previously, as the level of encapsulation decreased the $w_1/o/w_2$ emulsion structures observed also decreased during storage.

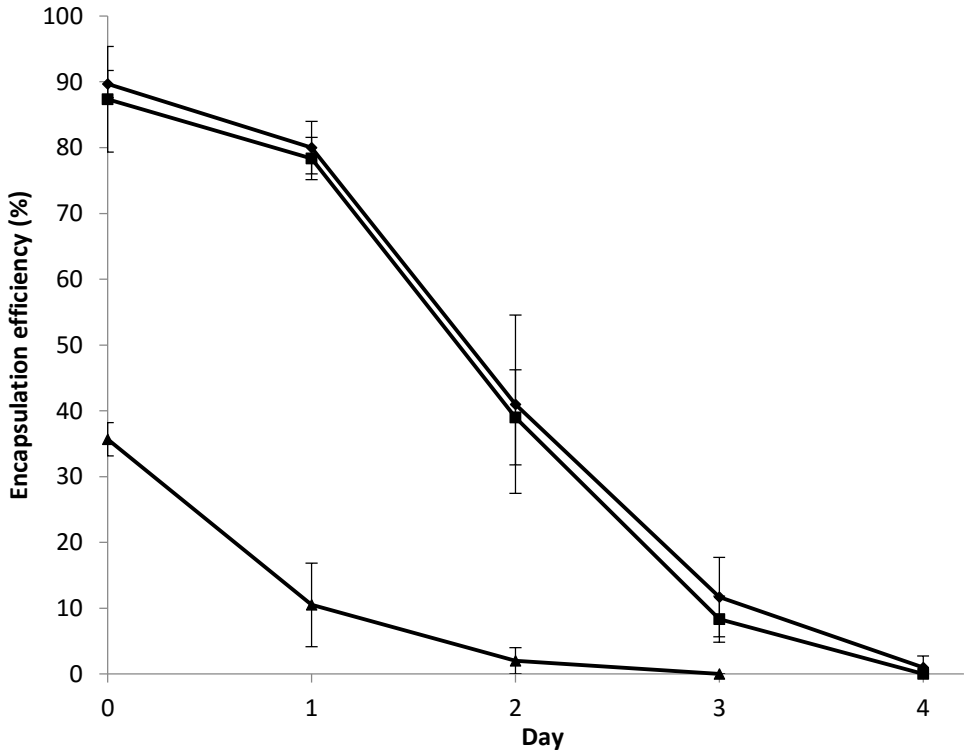

Figure 3.7 Encapsulation efficiency of $w_1/o/w_2$ emulsions stabilised with rice (◆), waxy maize (■) and potato (▲) starch over time. Data points are the average of three independent replicates. Measurements were conducted daily until encapsulation was no longer detected within the emulsion.

It was demonstrated that starch stabilised emulsions were able to destabilise when exposed to α-amylase. This enables the release of encapsulated sodium to the continuous phase, where the sodium becomes available for perception.

However, using native starches to stabilise $w_1/o/w_2$ emulsions proved to be unstable even over a short period of time. A main factor for the ineffectiveness of native starch to stabilise emulsions is its inherently hydrophilic nature and without further modification the starch is unlikely to adsorb to the interface of oil and water to stabilise the emulsion (Rayner *et al.*, 2012, Tesch *et al.*, 2002).

3.3.2 Commercial OSA starch

It was important to obtain an alternative emulsifier that both stabilised $w_1/o/w_2$ emulsions during storage and then destabilised when subjected to α-amylase. The hydrophilic nature of native starches resulted in unstable single (o/w) and double ($w_1/o/w_2$) emulsions over a long period of time. Therefore, modified starches with modified surface chemistry containing hydrophobic regions were tested and octenyl succinic anhydride (OSA) treated starch was chosen as it is food grade.

A commercially modified OSA waxy maize starch, N-creamer 46 (NC46), was selected and the particle size distribution was measured (Figure 3.8). Particle size distribution was analysed to observe the range of emulsion droplets that would be formed during emulsification and the starch granules ranged from 3.2 to 309 µm. Observations of SEM images of NC46 (Figure 3.9) reflect granules shown in Figure 3.8. It is proposed that the large measurements of the particles correlate to aggregation of granules, highlighted in Figure 3.9, rather than individual granules.

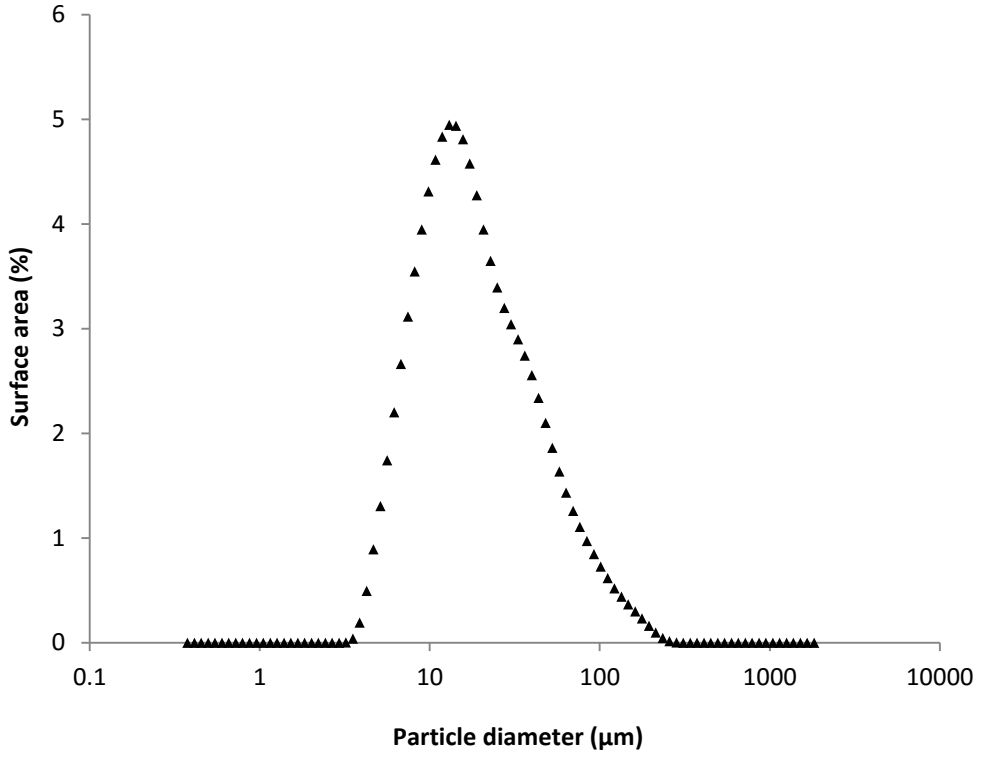

Figure 3.8 Particle size distributions of commercial OSA starch.

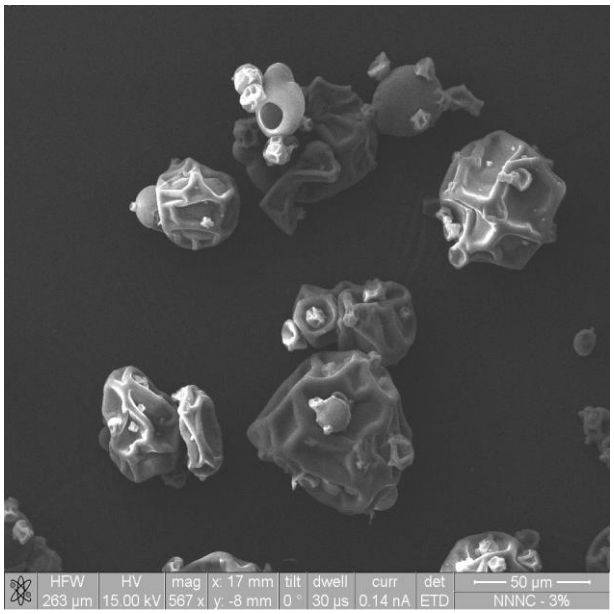

Figure 3.9 SEM micrograph of NC46. Scale bar = 50 µm.

3.3.2.1 Commercial OSA starch stabilised single emulsions

Emulsion samples were stabilised with four different concentrations of NC46. Increasing the concentration of NC46 improved the overall stability of the

emulsion with no significant changes between 0 d and 60 d when using 4 and 6 % w/w NC46 to stabilise the emulsion (*p>0.05*) (Figure 3.10). In addition, increasing NC46 concentration produced smaller droplets. However, emulsions containing 1 % and 3 % w/w NC46 were not stable over 60 d with droplet size increasing significantly during storage (*p<0.05*). The reduced stability at lower NC46 concentrations would suggest insufficient starch was available, to achieve the minimum surface coverage required at the interface to fully stabilise the emulsion droplet. The results presented here were in agreement with previous studies where droplet size was dependent on starch concentration with minor changes at higher concentrations (Matos *et al.*, 2013, Nilsson and Bergenståhl, 2006, Rayner *et al.*, 2012, Tesch *et al.*, 2002, Timgren *et al.*, 2013, Viswanathan, 1999, Wang *et al.*, 2010, Yusoff and Murray, 2011).

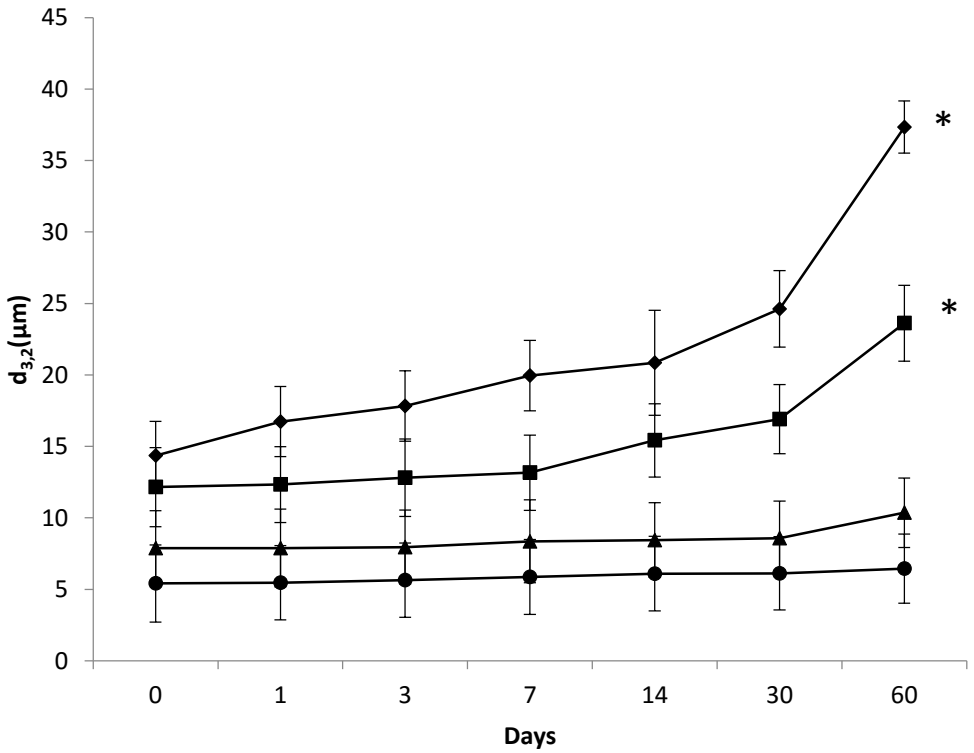

Figure 3.10 Effect of storage time on surface area mean ($d_{3,2}$) of single o/w emulsion droplets prepared with 1 %w/w (◆), 3 %w/w (■), 4 %w/w (▲) and 6 %w/w (●) NC46. Error bars represent standard deviation (n=3). * =significant difference from 0 d (*p*<0.05).

3.3.2.1.1 Remaining starch after *in vitro*

All samples were subjected to porcine α-amylase of 50 units/mL, which is an activity level present in humans (Kivelä *et al.*, 1997, Mandel *et al.*, 2010) and the remaining NC46 at each concentration is shown in Figure 3.11. The results suggest that the modified starch is indeed being broken down by α-amylase within 20 s with a minimum average decrease of 15.6 % OSA starch. The amount of OSA starch remaining after porcine α-amylase digestion was between 80.6 % and 84.4 % and no significant difference was present between the different OSA starch concentrations ($p>0.05$).

Increasing the level of NC46 did not affect the amount of starch being digested. However, the level of NC46 did change the droplet size of the emulsion. From these results, the concentration of 4 % starch was ideal as it remained stable over a period of two months with no significant difference in droplet size compared to a higher concentration of 6 % and had a similar rate of digestibility, resulting in emulsion destabilisation, as those of lower concentrations.

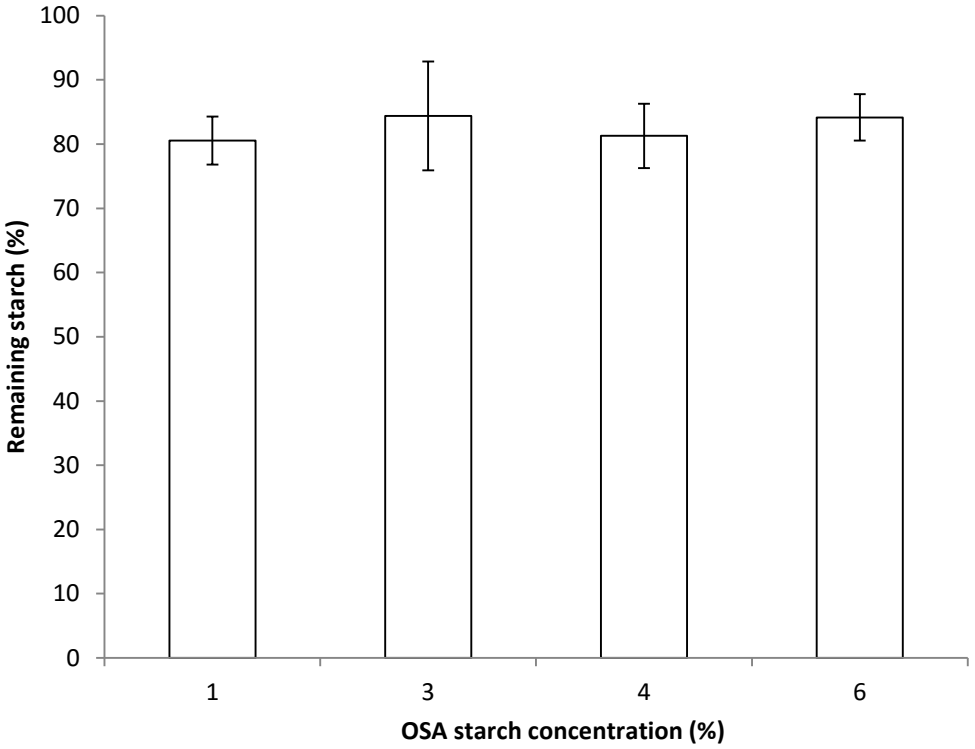

Figure 3.11 Remaining OSA starch after mixing with porcine α-amylase. Error bars represent standard deviation (n=3).

3.3.2.2 Commercial OSA starch stabilised double emulsions

Double emulsions were successfully formulated using 1 %, 3 %, 4 % and 6 % w/w NC46 as the external emulsifier. Micrographs of the emulsions captured after emulsion formation show larger droplets forming at lower NC46 starch concentrations (Figure 3.12).

The external droplets of the $w_1/o/w_2$ emulsions were measured and the surface area mean of the external droplets was calculated (Figure 3.13). The surface area mean increased over time for all concentrations and the surface area mean after 60 d of storage was significantly greater than after processing at 0 d for samples containing 1 % and 3% w/w NC46 starch (p<0.05).

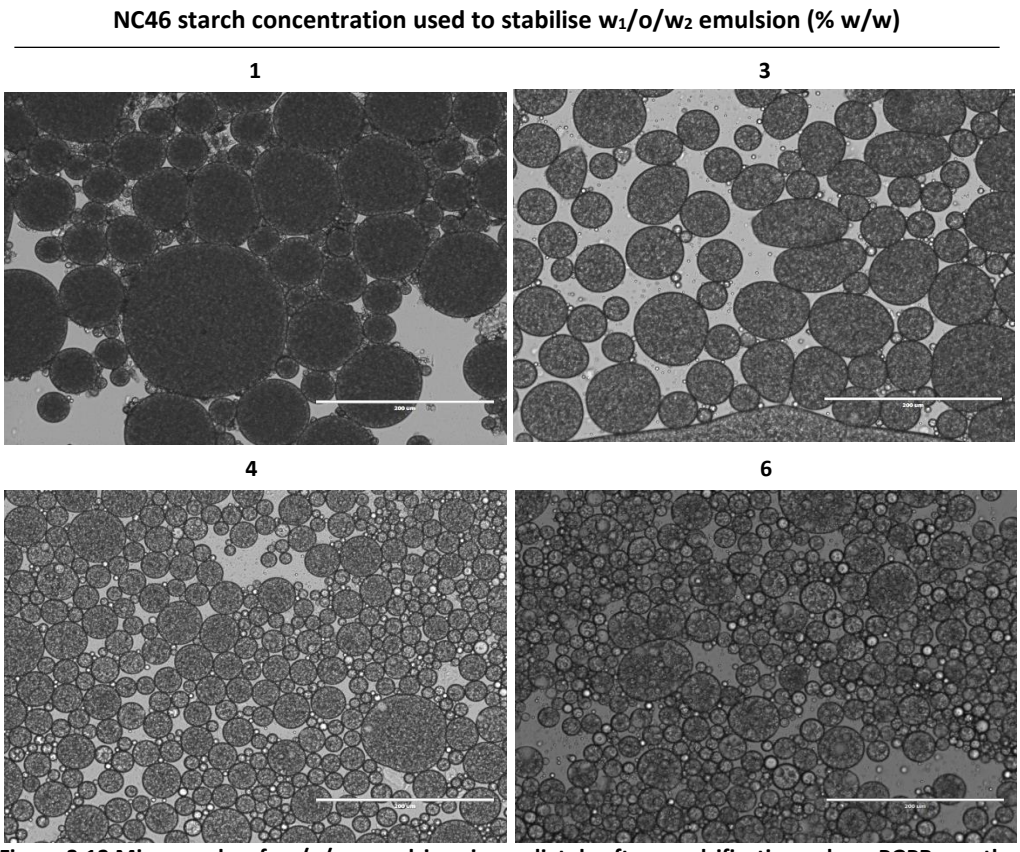

Figure 3.12 Micrographs of $w_1/o/w_2$ emulsions immediately after emulsification, where PGPR was the internal w/o emulsifier and difference concentrations of NC46 starch was added as the external o/w emulsifier. Scale bar = 200 µm.

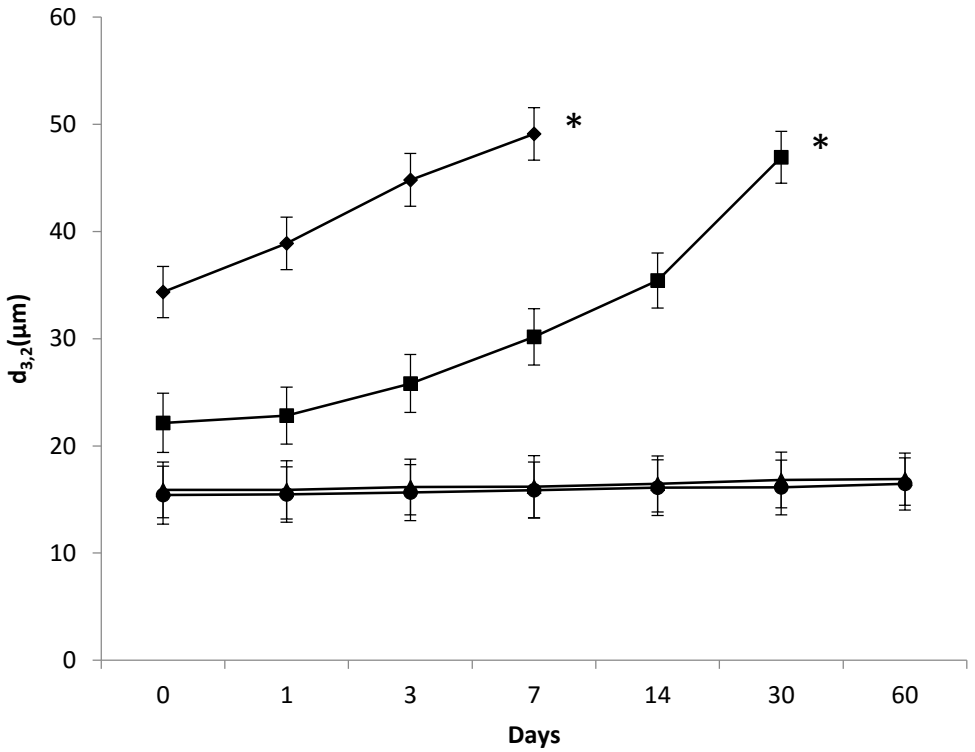

Figure 3.13 Effect of storage time on surface area mean ($d_{3,2}$) of $w_1/o/w_2$ emulsion droplets prepared with 1 %w/w (◆), 3 %w/w (■), 4 %w/w (▲) and 6 %w/w (●) NC46. Error bars represent standard deviation (n=3). * =significant difference from 0 d (p<0.05).

It was therefore concluded that a concentration of 4 % w/w starch was applied to subsequent emulsion formulation, as no difference in surface area mean was identified at higher starch concentrations (p>0.05).

3.3.2.3 Comparing stability of double emulsions stabilised with commercial OSA starch or PPI

NC46 was used to stabilise $w_1/o/w_2$ emulsions at a concentration of 4 % w/w and compared to Pea protein isolate (PPI), as a negative control to α-amylase. Distribution of the sodium chloride and choice of stabiliser had no impact on the surface area mean of the included w_1 phase droplets or the w_1/o droplets, as shown in Figure 3.14. The mean diameter ($d_{3,2}$) of the w_1/o droplets in all of the six $w_1/o/w_2$ emulsions ranged between 14.7 and 16.5 µm and there were no statistically significant differences (*p>0.05*). The Sauter mean diameter of the internalised water droplets was between 3.2 and 4.7 µm and again, across the sample set there was no statistically significant differences (*p>0.05*). Hence, it is valid to assume that droplet size does not represent a factor in these $w_1/o/w_2$ emulsions that would impact on sodium release and saltiness perception after 1 d of storage.

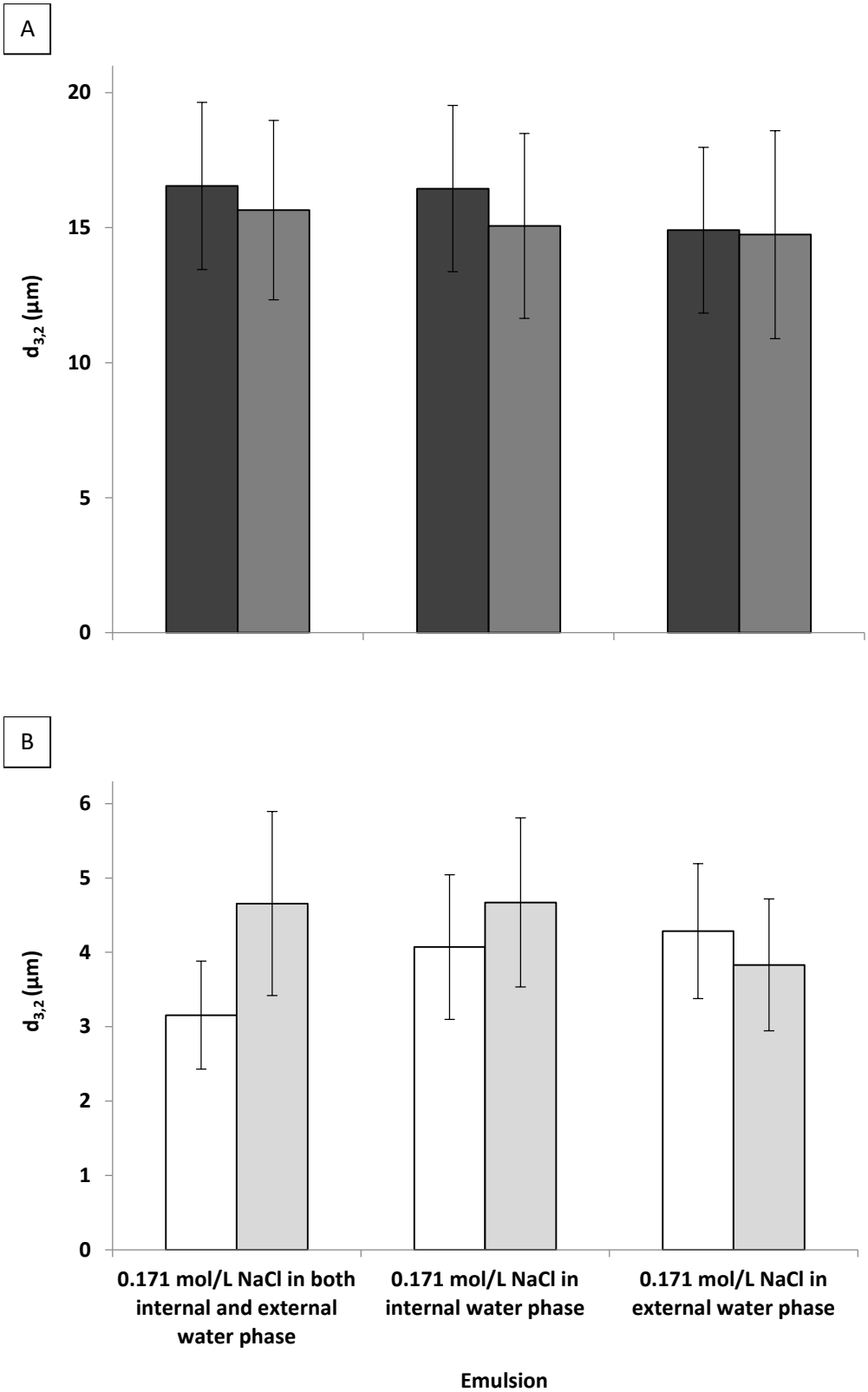

Figure 3.14 Sauter mean diameters ($d_{3,2}$) acquired by image analysis after 1 d at 20°C. A) External (w_1/o) droplets stabilised with NC46(■) and PPI (■). B) Internal (w_1) droplets in NC46 stabilised emulsion (□) and PPI stabilised emulsion (□).

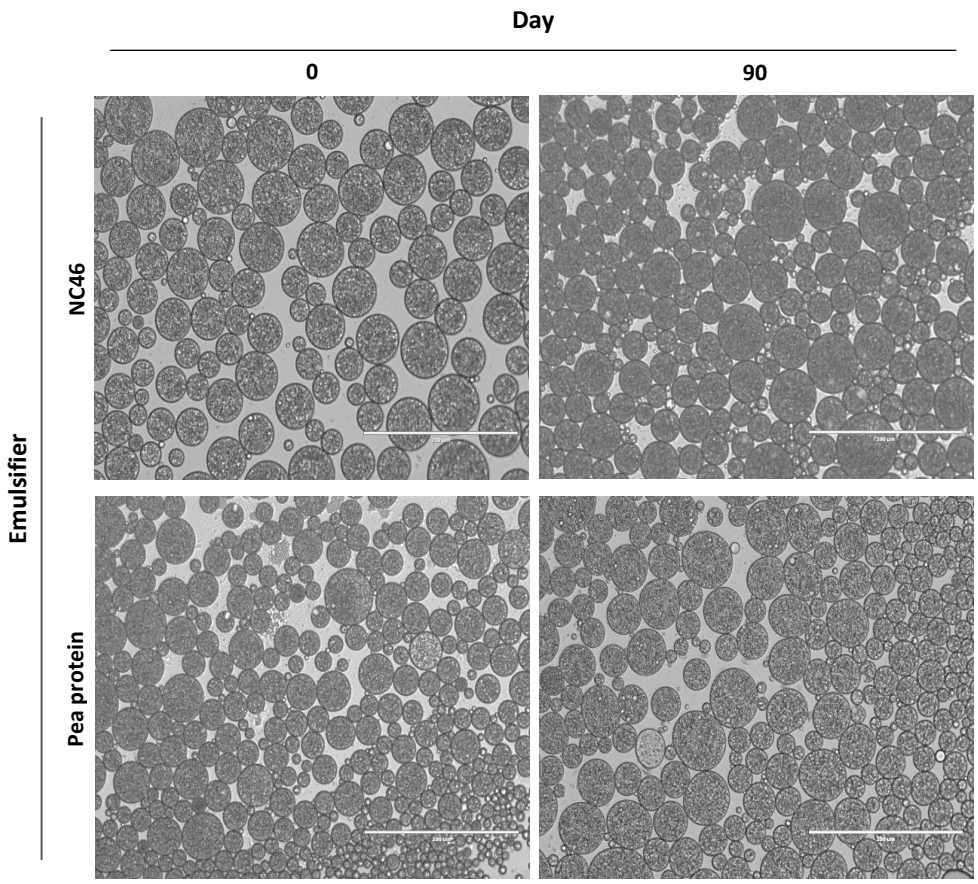

Figure 3.15 Micrographs of $w_1/o/w_2$ emulsions stabilised with NC46 and PPI captured after emulsification and at 90 d. Scale bar = 200 µm.

Microscopic evidence capturing $w_1/o/w_2$ emulsions after emulsification and three months after emulsification with NC46 and PPI as external emulsifiers were comparable and therefore it was concluded to be stable over storage (Figure 3.15). The droplet-in-droplet microstructure and dark appearance of the oil droplets typically observed for this microstructure were clearly recognisable (Garti *et al.*, 1994, Lad *et al.*, 2012, Pawlik *et al.*, 2010, Rojas *et al.*, 2008).

3.3.2.3.1 Encapsulation efficiency

Encapsulation efficiency (EE) was acquired for each emulsion over time by measuring sodium present at the continuous phase (Table 3.3). The initial EE of both emulsion samples was over 97 % and another study using modified OSA

quinoa starch also reported high EE above 98.5 % immediately after emulsification (Matos *et al.*, 2013). The formulation to attain $w_1/o/w_2$ emulsions in this two-step emulsification process exploits mixing resulting in the rupture of some droplets, resulting in the sodium in w_1 to move to the continuous phase (Kumar *et al.*, 2012). The EE values for both emulsion systems remained above 60 % throughout the observation of 90 d. No significant difference of EE during storage and between the two systems was found (*p>0.05*) and the two systems were suggested to be comparable up to 7 d.

Table 3.3 Encapsulation efficiency of $w_1/o/w_2$ emulsions stabilised with commercial OSA starch (NC46) and PPI over time.

	Encapsulation efficiency (%) after day							
Emulsifier	0	1	2	3	7	14	30	90
NC46	97.7 ± 0.43	97.7 ± 0.42	97.7 ± 0.42	97.7 ± 0.43	97.6 ± 0.46	97.5 ± 0.48	97.5 ± 0.44	96.5 ± 0.40
Pea protein	97.7 ± 0.36	97.1 ± 0.31	96.7 ± 0.31	95.6 ± 0.32	94.1 ± 0.46	89. 1 ± 0.53	78.9 ± 0.51	60.5 ± 0.57

3.3.2.3.2 Viscosity of double emulsions

Rheological measurements were performed on the NC46 and PPI stabilised $w_1/o/w_2$ emulsions to validate if the viscosity of these samples were comparable prior to sensory analysis. The viscosity curves of both $w_1/o/w_2$ emulsion samples were similar over the range of shear rates (Figure 3.16) and no statistical differences were found between the two emulsion systems (p>0.05).

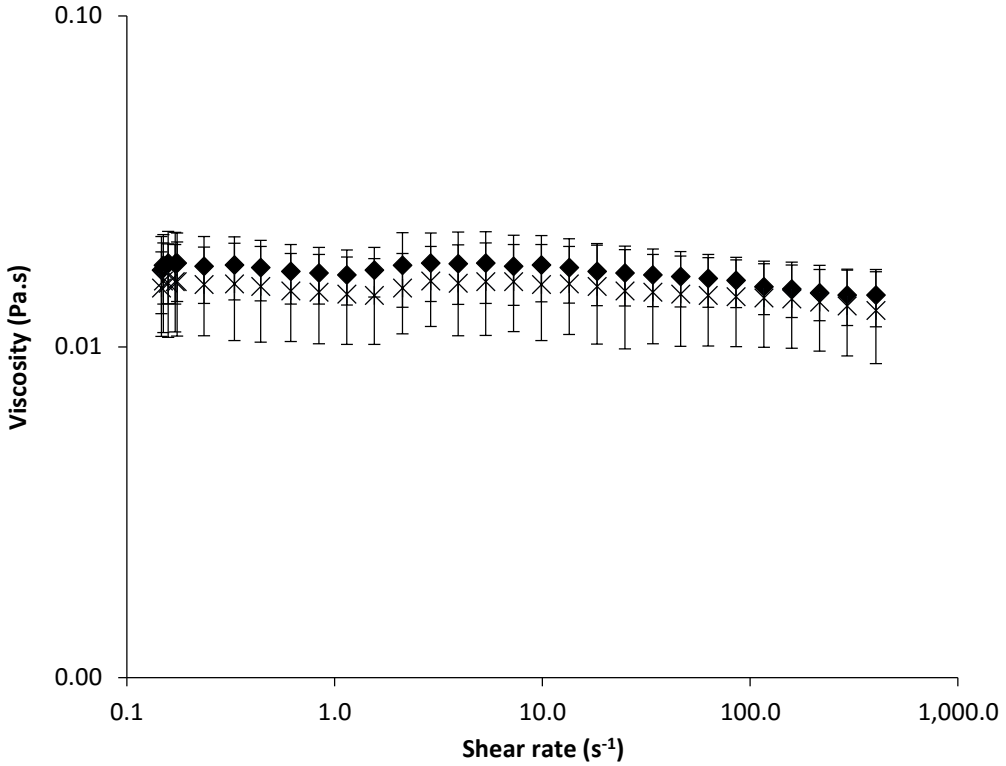

Figure 3.16 Viscosity (Pa.s) versus shear rate (s^{-1}) of $w_1/o/w_2$ emulsions with 0.171 mol/L in w_1 stabilised with NC46 (×) or PPI (◆).Error bars represent standard deviation (n=3).

3.3.2.3.3 Emulsion microstructure after *in vivo* and *in vitro*

Both PPI and NC46 stabilised $w_1/o/w_2$ emulsions were tested for amylase mediated destabilisation using *in vitro* and *in vivo* digestion over 20 s. The changes in microstructure are shown in Figure 3.17.

For the NC46 stabilised emulsion there are substantial microstructure changes after *in vitro* and *in vivo* digestion whereas there were no changes to gross microstructure in the PPI stabilised emulsion. In the case of the NC46 stabilised emulsion, digestion has led to destabilisation of the oil droplet interface causing the oil droplets to coalesce, this is evident by the presence of larger droplets found in the digested samples compared to before digestion. The larger internalised droplets recognisable in the *in vitro* digested sample suggest partial coalescence of the w_1 droplets. The coalescence processes have led to

the release of the internalised aqueous phase as indicated by the presence of void oil droplets seen in the digested samples.

This implies that oral shear combined with salivary digestive enzymes and emulsifiers are effective at imparting partial release of the internal water phase of the NC46 starch stabilised double $w_1/o/w_2$ emulsions. Other proteins in saliva include immunoglobulins, proline-rich proteins and mucins may also alter emulsion stability. However, the PPI stabilised emulsion showed no clear evidence of this type of instability process occurring during *in vitro* and *in vivo* digestion as the original emulsion microstructure is largely retained.

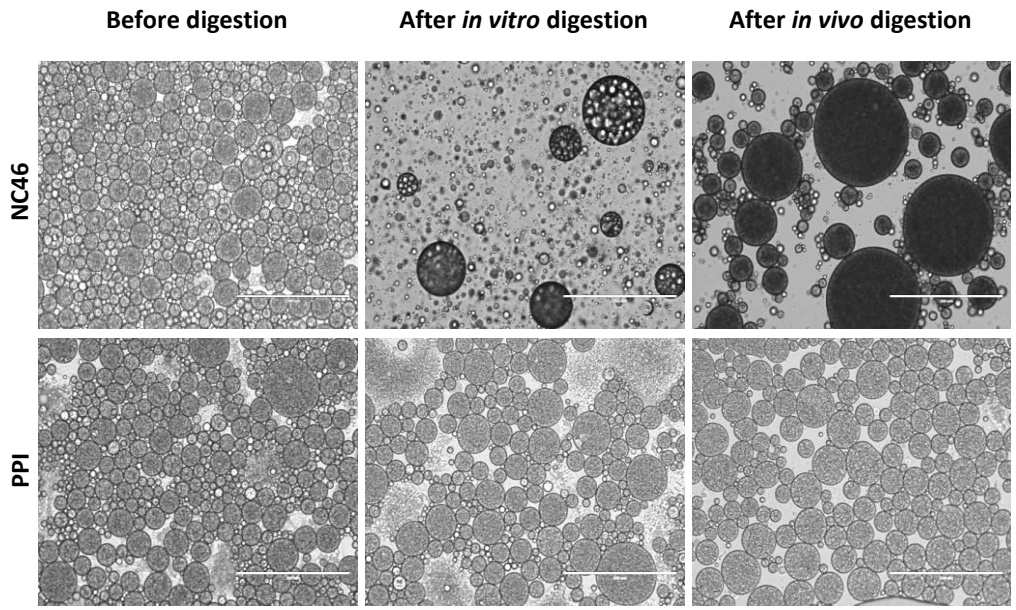

Figure 3.17 Micrographs before and after *in vitro* and *in vivo* digestion of $w_1/o/w_2$ emulsions stabilised with 2 % NC46 and PPI. The internal and external aqueous phase of both types of emulsion contains salt at 0.171 mol/L. Scale bar = 200 µm.

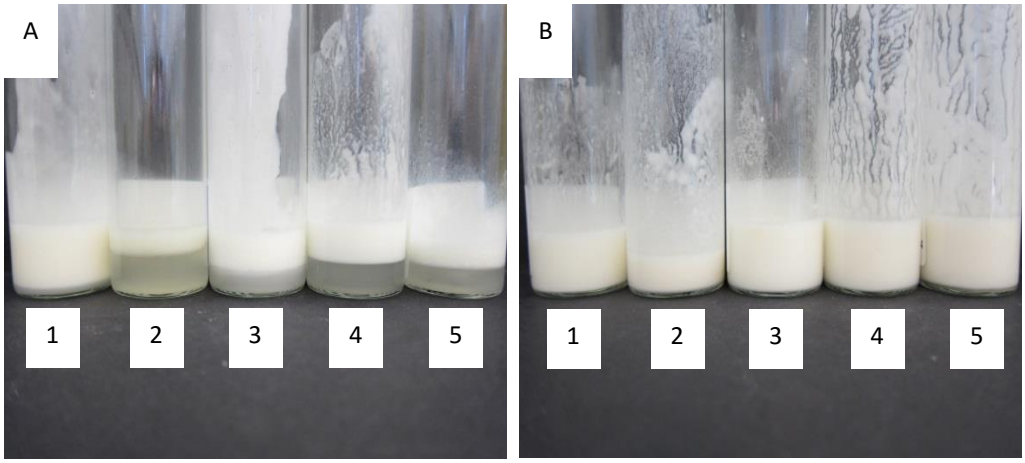

Figure 3.18 Images of $w_1/o/w_2$ emulsions stabilised with commercial NC46 (A) or PPI (B) before (1) and after *in vivo* (2-5 demonstrate expectorate from different volunteers).

Images of the expectorated samples from four panellists are presented in Figure 3.18. Sample A1 and B1 display samples prior to consumption and these were stabilised with NC46 and PP, respectively. From the samples it was evident that the expectorated NC46 stabilised emulsion was visually different after consumption, with creaming and some oil separation present in the expectorated samples. However, no such observation was found when emulsion samples were stabilised with PPI. The visual change in the NC46 stabilised emulsion may reflect the micrographs presented in Figure 3.17. From the results, both emulsions were stable during storage over a three month period. However, the stability of the emulsion when placed in the mouth was largely determined by the external emulsifier present.

3.3.2.3.4 Remaining starch after *in vivo* and *in vitro*

Starch digestion through the action of the porcine amylase or salivary amylase was analysed using a total starch assay. *In vivo* digestion resulted in significant (p<0.05) reduction of total starch (2.14 g total starch/100 g was reduced to 1.69 g total starch/100 g) whereas a smaller but still significant (p<0.05) reduction

was found after *in vitro* digestion (to 1.9 g total starch/100 g). It should be noted that the reduction was lower during *in vitro* digestion indicating that enzymes present orally may be more effective at digesting the OSA starch (Makinen, 1989), and the more intense mechanical action during oral processing compared to the *in vitro* protocol may have additionally contributed to the enhanced digestion of the starch. Another factor influencing starch hydrolysis could be attributed to higher enzyme activity present in some volunteers.

The OSA treatment involves esterification of OSA at select free hydroxyl groups mainly at the surface of the starch granules. The esterification process has been previously shown to be spatially heterogeneous on the surface and throughout the granule (Zhang *et al.*, 2011). Modified starches may contain granules with more octenyl succinic groups whilst some may have less or no modification (Bai *et al.*, 2009, Shogren *et al.*, 2000, Wetzel *et al.*, 2010). OSA starch treatment is limited to 3 % OSA modification of starch for food use and OSA loading has been shown to be proportional to resistance to digestion in a suspended (non-emulsified) state (Han and Bemiller, 2007, Viswanathan, 1999). The presented results confirm the digestibility of interfacially adsorbed commercially relevant OSA starch, NC46, on a timescale appropriate to the consumption of emulsion based foods which impacts stability and causes phase separation.

3.3.2.3.5 Sodium release *in vitro*

The amount of sodium released to the continuous phase was measured *in vitro* for emulsions prepared with no sodium in the continuous phase (Figure 3.19).

Initial detection of sodium indicates that during emulsion preparation some of the internal sodium containing water phase was released into the external water phase.

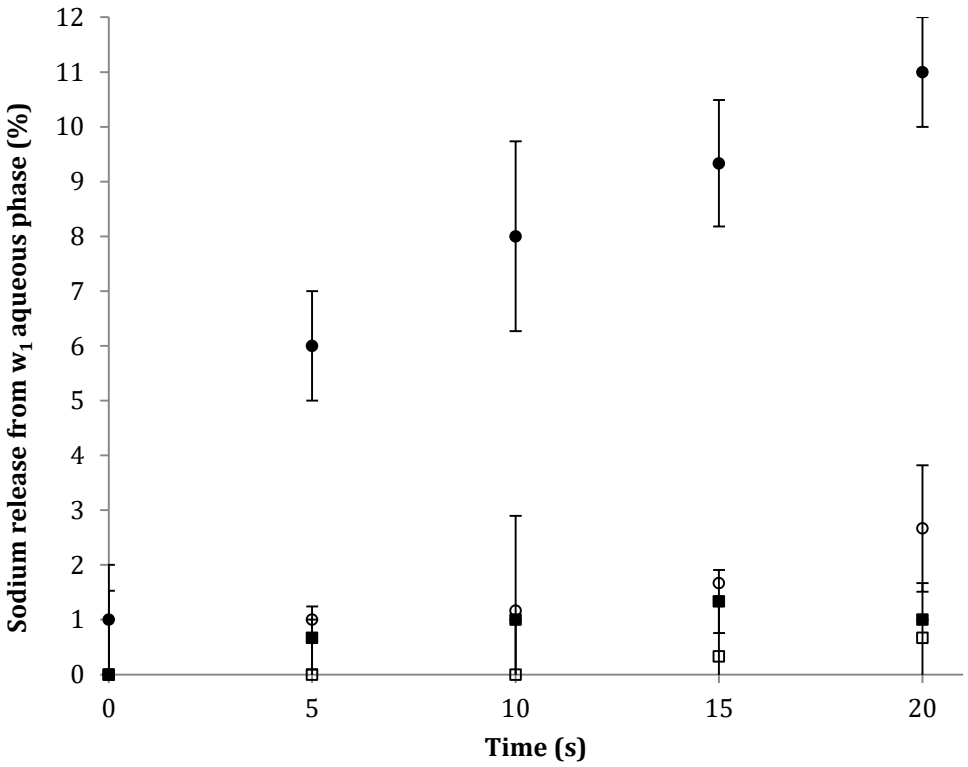

Figure 3.19 Sodium release from w_1 phase, initially containing 0.171 mol/L salt and w_2 not containing any salt, following the addition of α-amylase to the emulsion stabilised with NC46 or PPI and holding for 20 s at 37 °C. NC46 stabilised emulsion with (●) and without (○) α-amylase enzyme, PPI stabilised emulsion with (■) and without (□) α-amylase enzyme.

Sodium was rapidly released from the NC46 stabilised emulsion when in the presence of amylase, the NC46 emulsion was stable without the enzyme and the PPI stabilised emulsion was stable both with and without the enzyme. This suggests that partial release of sodium can be achieved through enzymatic digestion. It is expected that the *in vivo* release would be greater although this cannot be verified within the current experimental design.

3.3.2.3.6 Overall saltiness perception

Paired comparison tests were conducted to validate release of encapsulated sodium from $w_1/o/w_2$ emulsions to enhance saltiness perception (Table 3.4). Complete removal of the internal (w_1) sodium from the stable PPI emulsions had no impact on saltiness perception (Test 1). NC46 stabilised emulsions were perceived as saltier when compared directly to PPI stabilised emulsions containing equivalent external and internal salt concentrations (Test 2).

The higher perceived saltiness of the NC46 stabilised emulsion in Test 2 demonstrates potential to reduce the sodium concentration in the emulsion to achieve similar saltiness to the PPI stabilised emulsion. This is confirmed by the results of Test 3 where the NC46 stabilised emulsion of the pair contained 18.2 % less salt in w_2 compared to the PPI stabilised emulsion. Overall, this equates to a salt reduction of 23.7 % without comprising saltiness perception. Not unexpectedly, if both of these emulsions were formulated with zero salt in the included water phase, the PPI emulsion was perceived as saltier than the NC46 stabilised emulsion because of the higher salt content in the former (Test 4).

Using commercially modified OSA starch, NC46, produced stable single, o/w and $w_1/o/w_2$ emulsions. Sensory testing demonstrated an overall perceptual difference in saltiness between the two systems. The $w_1/o/w_2$ emulsion with OSA modified starch as the external emulsifier released more sodium chloride during oral processing for subsequent perception, and indeed resulted in an enhanced perception when compared to the control, PPI.

Table 3.4 Saltiness perception using paired comparison tests: Emulsion composition, pairs and saltiness scores.

Test	Emulsifier	NaCl in internal (w_1) (mol/ L)	NaCl in external (w_2) (mol/ L)	Total NaCl (g/ 100 g emulsion)	No. of panellists selecting samples to be saltier	Result
1	PPI	0.171	0.171	0.650	62	similar[##]
	PPI	0	0.171	0.500	58	
2	PPI	0.171	0.171	0.650	41	
	NC46	0.171	0.171	0.650	79	saltier[#]
3	PPI	0.171	0.171	0.650	59	similar[##]
	NC46	0.100	0.140	0.496	61	
4	PPI	0	0.171	0.500	108	saltier[#]
	NC46	0	0.140	0.409	12	

[#] Samples perceived to be significantly saltier (p<0.05).
[##]Similarity concluded between the 2 samples (95 % confidence interval, p_d 30 %).

3.4 Conclusion

The chapter presents using both native and modified starches as an emulsifier. Native starches were concluded to be unsuitable for stabilising both oil-in-water (o/w) and water-in-oil-in-water ($w_1/o/w_2$) emulsions over long term storage. However, the initial concept of α-amylase induced emulsion instability was demonstrated in these starch stabilised emulsions.

Using a commercial modified starch (NC46) stable o/w and $w_1/o/w_2$ emulsions were produced. The $w_1/o/w_2$ emulsions were able to encapsulate up to 97.7 % sodium and the encapsulation efficiency (EE) was 96.5 % after 90 d. Sensory testing confirmed an enhancement in saltiness perception from NC46 stabilised $w_1/o/w_2$ emulsions comprising an encapsulated aqueous sodium phase which was partially released during oral processing.

Comparing sodium release and saltiness perception to $w_1/o/w_2$ emulsions formulated with a protein instead of starch, as well as quantifying the

breakdown of starch, clearly validated the hypothesis that a stabilising system susceptible to degradation in contact with salivary enzymes releases encapsulated tastant. A 23.7 % decrease in overall salt was achieved, without compromising saltiness perception when compared to a protein stabilised emulsion.

4 Optimising sodium delivery in double emulsions

4.1 Introduction

Chapter 3 highlighted the potential of entrapping sodium in a water-in-oil-in-water ($w_1/o/w_2$) emulsion to create a contrast in sodium concentration during oral processing to deliver an overall enhanced sodium perception. However, as the mechanism required starch to be partially hydrolysed by α-amylase, only a proportion of entrapped sodium was released and perceived. It is suggested that the rate of hydrolysis is affected by the degree of modification of starch (Wolf *et al.*, 1999, Wolf *et al.*, 2001). Therefore, this chapter investigates the use of different degrees of modified octinyl succinic anhydride (OSA) starch as external emulsifiers to stabilise double water-in-oil-in-water ($w_1/o/w_2$) emulsions and examines the *in vitro*, *in vivo* breakdown of the emulsion to deliver sodium for perception.

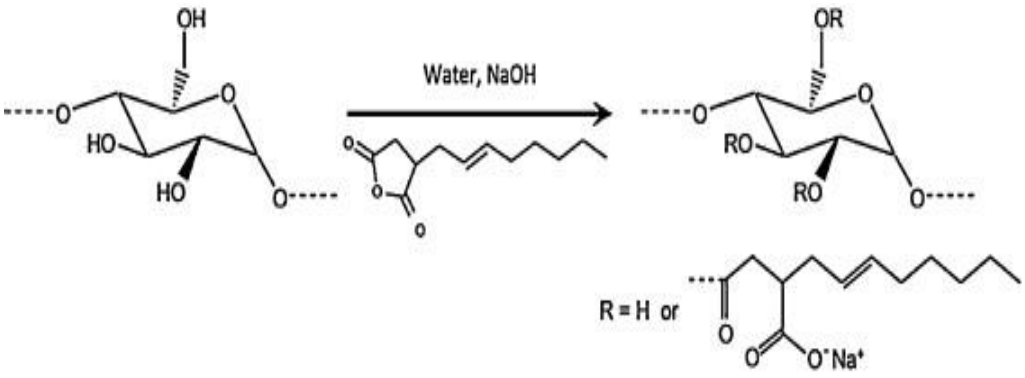

Figure 4.1 Structure of OSA modified starches (Sweedman *et al.*, 2013).

A starch with amphiphilic properties can be achieved by the esterification of starch and OSA as shown in Figure 4.1. The OSA provides hydrophobic regions and such modified starches have been shown to be surface-active and have improved emulsifying properties (Chanamai and Mcclements, 2002, Nilsson

and Bergenståhl, 2007, Prochaska *et al.*, 2007). Both starch molecules and particles have been demonstrated to stabilise emulsions (Cuevas *et al.*, 2010, Prochaska *et al.*, 2007). To stabilise emulsions, the octenyl succinate side chains in OSA starch molecules orientate towards the oil-water interface and the hydrophilic regions prevent droplet flocculation by steric stabilisation (Dickinson, 1992, Nilsson and Bergenståhl, 2006). Intact starch particles modified by OSA are mainly wetted by the aqueous phase to stabilise oil-in-water (o/w) emulsions and form Pickering emulsions (Yusoff and Murray, 2011).

Starch particles adsorbed at the interface forms a steric barrier that protects the emulsion droplets against coalescence. The particles are regarded as being irreversibly adsorbed, as long as the contact angle is not too high or low (Dickinson, 2012). The properties exhibited in Pickering emulsions consisting of OSA starches are regarded to be suitable for encapsulation of ingredients in both food and pharmaceutical products (Sweedman *et al.*, 2013).

It has been reported that both emulsion capacity (the mass of oil dispersed per gram of sample) and colloidal stability of the particles improved with increasing degree of substitution (DS) (Segura-Campos *et al.*, 2008, Segura-Campos *et al.*, 2010). The degree of modification of the starch alters the proportion of hydrophobic groups present in the starch and thus the stability of the emulsions formed from such starches.

Furthermore, studies have shown that the OSA modification of starch affected the extent of digestion of the starch (Wolf *et al.*, 2001). Starch modification with OSA has demonstrated an increase level of slowly digested starch and

resistant starch (Han and Bemiller, 2007), which results to the starches being more difficult to digest. The increase in DS resulted in less enzymatic hydrolysis of OSA modified waxy maize starch (Viswanathan, 1999). It was suggested that the OSA substitutions restrict the binding of α-amylase preventing hydrolysis (Wolf *et al.*, 2001).

To date, no published studies have been reported on the effect of α-amylase towards emulsions stability containing different degrees of modified starch, and how it may affect oral perception. The objectives of the study were to develop a range of OSA modified starches; test the stability of $w_1/o/w_2$ emulsions containing different degrees of modified OSA starch; to assess the loss of structural integrity during oral processing; and to understand the effects on sodium release and saltiness perception.

4.2 Materials and Methods

4.2.1 Materials

All materials used to modify the starch and prepare the emulsions were food grade. Sunflower oil (KTC, Wednesbury, UK), table salt (Saxa, London, UK), plain crackers (99 % Fat Free, Rakusen's, Leads, UK), mineral water (Evian, France) and Green apple (Granny Smith variety) were purchased from a local supermarket. Polyglycerol polyricinoleate (PGPR) was donated by Danisco (Dorset, UK). Orally inert pea protein isolate (PPI) was obtained from Myprotein (Manchester, UK). C*Gel 04201, a waxy maize starch containing approximately 95% amylopectin, was kindly donated by Cargill (Sas van Gent, Netherlands).

OSA was donated by Vertellus (Pennsylvania, USA). Sodium hydroxide (NaOH) was obtained from VWR International Ltd. (Lutterworth, UK). Calcium chloride ($CaCl_2$), ethanol, hydrochloric acid (HCl), 4-morpholinepropanesulfonic acid sodium salt (MOPS sodium salt), phenolphthalein, porcine α-amylase and sodium azide were obtained from Sigma-Aldrich (Gillingham, UK). Sodium azide was used as an antimicrobial agent and was only added to samples that were not intended for sensory analysis. The salivary α-amylase assay kit (1-1902) was purchased from Salimetrics LLC (State College, PA) which contained α-amylase substrate, α-amylase controls, α-amylase diluent and 96-well plate. Amyloglucosidase, D-glucose, standardised regular maize starch and thermostable α-amylase were provided as part of the Megazyme total starch assay kit purchased from Megazyme, Co. (Wicklow, Ireland). Deionised water, with a resistivity of 15MΩ/cm was used for the preparation of all solutions.

4.2.2 OSA starch modification

Waxy maize starch, C*Gel 04201 was hydrophobically modified by OSA treatment following published protocol (Bhosale and Singhal, 2006). C*Gel (125 g) was mixed with 475 mL deionised water using an overhead mixer with a four bladed propeller stirrer (EURO-ST D S2, IKA-WERKE, Staufen, Germany). The pH of the slurry was adjusted to pH 8.0 ± 0.2 by the addition of 2 % NaOH solution. OSA up to 3 % at 0.5 % increments, based on the weight of starch, was added drop-wise to the slurry over a 2 h period at room temperature. During the addition of OSA, the pH was maintained at 8.0 ± 0.2 by the addition of 2 % NaOH solution. The reaction was left to proceed for 24 h at 30 °C after which

the pH was adjusted to 6.5 using 2 % HCl. The slurry was then washed with water and centrifuged at 4193 x g. This process was repeated three times. The OSA starches were dried in an oven at 65 °C for 12 h and stored in a sealed container at room temperature until use. All steps of the modification procedure were repeated without the addition of OSA and the starch obtained from this process is considered as not hydrophobically modified. Although not corresponding to the original starch, C*Gel 04201 as obtained from the supplier, applied in this research, it is in the following referred to as 0 % OSA starch.

4.2.3 Determining degree of substitution of OSA starches

The degree of substitution (DS) is the average number of hydroxyl groups substituted per glucose unit and was determined by alkali saponification and back titration of excess alkali with HCl according to published protocol (Whistler and Paschall, 1967). A suspension of the OSA starch (5 g of starch in 50 mL water) was mixed with 25 mL of a 0.5 M aqueous NaOH solution and stirred for 24 h. Excess alkali was titrated with 0.5 M HCl, using phenolphthalein as an indicator. A blank titration of a suspension of unmodified starch (5 g unmodified starch in 50 mL water) was performed and the difference in HCl added to the modified and unmodified starch suspension was assumed to be due to chemically bound OSA. OSA substitution (%) was then calculated with Equation 4.

$$\%\ OSA = \frac{[(V_{Blank} - V_{Sample}) \times 0.1 \times M \times 100]}{W} \qquad \textbf{Equation 4}$$

where V_{Blank} is volume of HCl required for back titration; V_{Sample} is volume of HCl required for sample titration; M is Molarity of HCl; W is weight of sample taken (g) (Yusoff and Murray, 2011).

DS was determined from % OSA with Equation 5.

$$DS = \frac{Mw_g \times \%OSA}{Mw_{OSA} \times 100 - \left((Mw_{OSA} - 1) \times \%\ substitution\right)} \quad \textbf{Equation 5}$$

where Mw_g is molecular weight of glucose residue (162); Mw_{OSA} is molecular weight of *OSA* (210) (Yusoff and Murray, 2011).

4.2.4 Emulsification

4.2.4.1 Double $w_1/o/w_2$ emulsions

See Chapter 3, section 3.2.2.2, page 54.

4.2.5 Microstructure imaging

4.2.5.1 SEM

See Chapter 3, section 3.2.3.2, page 55.

4.2.5.2 Cryo-SEM

Cryo-SEM was performed on a FEI Quanta 3D 200 dual beam Focused Ion Beam Scanning Electron Microscope (FIB-SEM) to evaluate the structure at the interface of the emulsions. The sample was placed on the cryo-specimen holder and plunge-frozen in slush liquid nitrogen (-210 °C). The samples were then transferred to the cryo-unit, where it was fractured, sublimated (3 min and -95 °C) and sputter coated with platinum (2 min). Micrographs were acquired using secondary electron imaging at an accelerating voltage of 5–15 kV.

4.2.6 Particle size

4.2.6.1 Emulsion droplets

See Chapter 3, section 3.2.4.2, page 56.

4.2.7 Sodium release

See Chapter 3, section 3.2.5, page 56.

4.2.8 Encapsulation efficiency

See Chapter 3, section 3.2.6, page 57.

4.2.9 Total starch assay

See Chapter 3, section 3.2.7, page 58.

4.2.10 Comparing saltiness perception of double emulsions stabilised with different degrees of modified OSA starch

Overall perception of saltiness from the emulsions was evaluated using a series of paired comparison (PC) tests (BS ISO 5495:2007). Prior to testing, signed consent forms in agreement with ethical procedures were obtained from panellists. Panellists (aged 18-52; 58 female and 42 male) were recruited from the University of Nottingham and asked to attend two sessions.

In the first session, volunteers were presented with a series of 10 pairs of emulsion samples. Samples (10 mL) were presented in odourless, plastic pots labelled with three-digit codes; presentation order was randomised both within each pair and across the 10 PC tests. The tests were carried out following the protocol as described in section 3.2.9 and assessors were selected the sample they perceived to be saltier. Before and between testing samples,

assessors were required to cleanse their palate using green apple slices, unsalted crackers and mineral water. Within each PC, sample pairs had the same level of entrapped salt but were stabilised with starches of different levels of OSA modification including 1.5 %, 2 %, 2.5 % and 3 % as well as the unmodified starch. Only five different emulsions were selected to avoid carry over effects.

In the following session, samples presented consisted of a single o/w emulsion stabilised with PPI containing 0.65 g/100 g emulsion, which is compared to $w_1/o/w_2$ emulsions stabilised with different degrees of OSA starch, the same OSA starches used as the previous session, containing 15 % less overall salt in the emulsion compared to the single emulsion.

The test was used in forced-choice mode, so panellists were required to give an answer even if the perceived difference was negligible and panellists were given the opportunity to comment on the samples. Results were compared to tables A.2 and A.3 in BS EN ISO 5495:2007 to determine difference and similarity, respectively (British Standards Institution, 2007).

4.2.11 Time-intensity tests

Assessors (n=10; aged 44–72; nine female, one male) from the University of Nottingham external panel were recruited to take part in this study after signing consent forms consistent with ethical procedures. All trained assessors had extensive experience of sensory evaluation using Time-intensity methods and in addition attended 2 h training sessions to familiarise them with the samples. Following the sampling protocol detailed in Section 3.2.9, panellists

started rating their perception of saltiness immediately after the sample was placed in the mouth and continued rating for 30 s (swallowing the samples after 10 s according to the test protocol). The perceived intensity was recorded on a continuous line scale with data collected every 1 s using the computerised data acquisition system, FIZZ 2.46 (Biosystems, Couternon, France). Each panellist attended a total of five 2 h sessions to evaluate all samples. A total of seven emulsion samples were evaluated (five replicate assessments of each) , each sample containing the same concentration of 0.141 M salt in the internal phase and stabilised with unmodified or OSA modified starch (0.5 %, 1 %, 1.5 %, 2 %, 2.5 %, 3 % OSA).

Fizz Calculations normalisation and computation algorithm was used to extract key parameters from the Time-intensity curves: T_{max}=time to maximum intensity, I_{max}=maximum intensity, Area under the curve=total perceived intensity and I_{10}=intensity at 10 s.

Two-way ANOVA (panellist and sample) was performed using SPSS (SPSS, IBM, Version 22, USA) to identify any significant differences. Tukey's HSD multiple comparison tests were performed where appropriate to determine which samples were significantly different (p=0.05).

4.2.12 Expectorated samples

Panellists were instructed to follow the same protocol as the sensory evaluation of the emulsion samples but were instructed to expectorate the sample into a pre-labelled container after 10 s. After 10 s, 1 mL of 2 M HCl was

added to the sample to inactivate the enzyme and 0.02 % sodium azide was mixed into the sample to prevent microbial spoilage.

4.2.13 Salivary α-amylase

Individual's saliva α-amylase expression was measured to understand the impact of salivary α-amylase concentration on sodium release *in vivo* and subsequent time to maximum saltiness perception. Quintuplicate samples of stimulated saliva were collected from the external trained panellists at the start of each Time-intensity session. Panellists were instructed to expectorate saliva into a pot every 30 s for 5 min and the saliva collected was measured and the value was divided by the time the collection lasted to obtain salivary flow rate (mL/min). The salivary a-alyase assay kit was used to measure a-amylase activity. The microplate reader (LT-4000, Labtech, Sussex, UK) was incubated at 37 °C and absorbance was measured at 405 nm. The α-amylase substrate was heated to 37 °C. Saliva samples were diluted with the α-amylase diluent provided. Samples were initially diluted by pipetting 10 µL of saliva into 90 µL α-amylase diluent and mixed well. Further dilutions were carried out by pipetting 10 µL of the dilution into 190 µL α-amylase diluent. Controls and diluted saliva samples (8 µL) were in individual wells. The preheated α-amylase substrate (320 µL) was transferred into each well and was immediately placed in the reader for mixing at 400 rpm. The initial absorbance was measured after 1 min and the final absorbance was measured after 3 min. The amylase activity was calculated using Equation 6.

$$Amylase\ activity\ (U/mL) = \frac{\Delta\ Abs./min \times TAV \times DF}{MMA \times SV \times LP} \qquad \textbf{Equation 6}$$

where $\Delta Abs./mins$ is absorbance difference per min; TAV is total assay volume (0..328 mL); DF is dilution factor (200); MMA is milimolar absorptivity of 2-chloro-p-nitrophenol (12.9); SV is sample volume (0.008 mL); LP is light path (0.97).

The amylase activity was multiplied by the salivary flow rate and expressed as amylase expression (U/min).

4.3 Results and Discussion

4.3.1 Degree of modification of waxy maize starch

Previous studies have demonstrated a change in digestion after OSA modification (Han and Bemiller, 2007, He *et al.*, 2008, Heacock *et al.*, 2004, Wolf *et al.*, 2001, Zhang *et al.*, 2011). This may affect the release of encapsulated sodium in the mouth when applying OSA starch as an emulsifier. OSA modification (0 - 3 %) is permitted as a starch modifying agent in foods according to the USA FDA. Therefore, the effect of OSA addition to starch was studied within the acceptable range in foods and applied as an emulsifier for subsequent analysis.

Waxy maize starch was selected as it is the most commonly modified starch (Sweedman *et al.*, 2013). The degree of substitution (DS) of starch after OSA modification was measured, Figure 4.2. As the OSA concentration of the treatment increased the degree of substitution increased linearly (R^2=0.98), suggesting the treatment was effective and that OSA treatment was not limited by surface area or reactant availability. The DS at the maximum treatment

concentration of 3 % OSA was 0.216 ± 0.014, which is in close agreement with previously reported results (Bhosale and Singhal, 2006, Liu *et al.*, 2008).

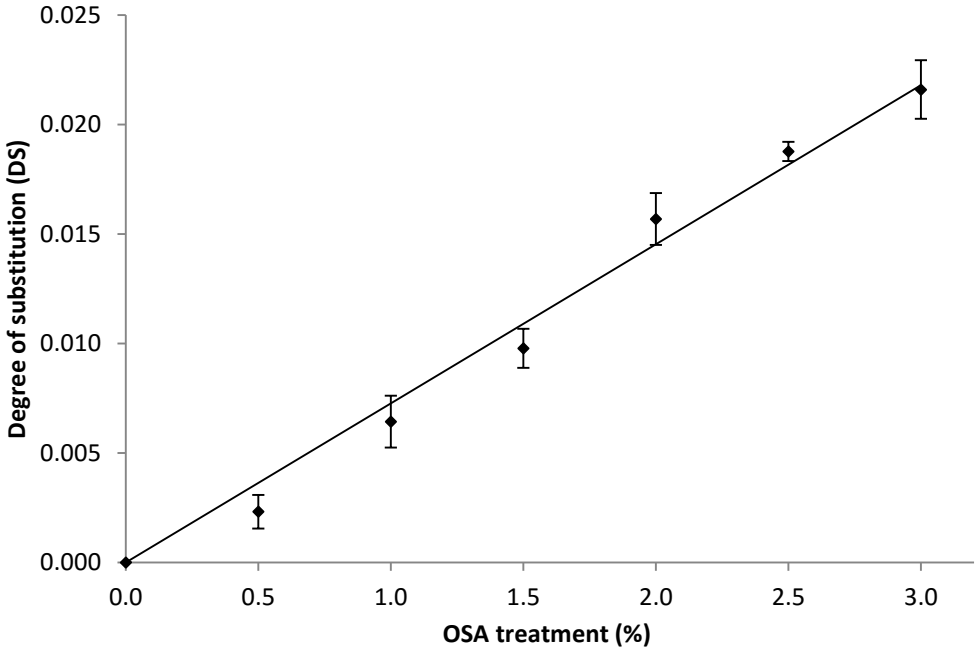

Figure 4.2 Degree of substitution of starch relative to concentration of OSA. Data are means of three replicates ± standard deviation.

The appearance of particles of the dry starch samples was investigated using scanning electron micrographs (SEM). The micrographs of the starch particles are presented in Figure 4.3.

Waxy maize starch (Figure 4.3A) displayed a range of spherical, angular, smooth and small indented distinct granules which were in agreement with other studies (Liu *et al.*, 2014, Sun *et al.*, 2014). As the concentration of OSA used to modify waxy maize starch increased, the surface roughness of the granules increased. Granules modified with more OSA had larger indented regions and this is especially evident at higher levels of modification (see Figure 4.3E, F and G). This suggests that esterification of starch increases hydrophobicity in addition to the surface roughness which together may play a role in the ability of the modified starch to stabilise the emulsion.

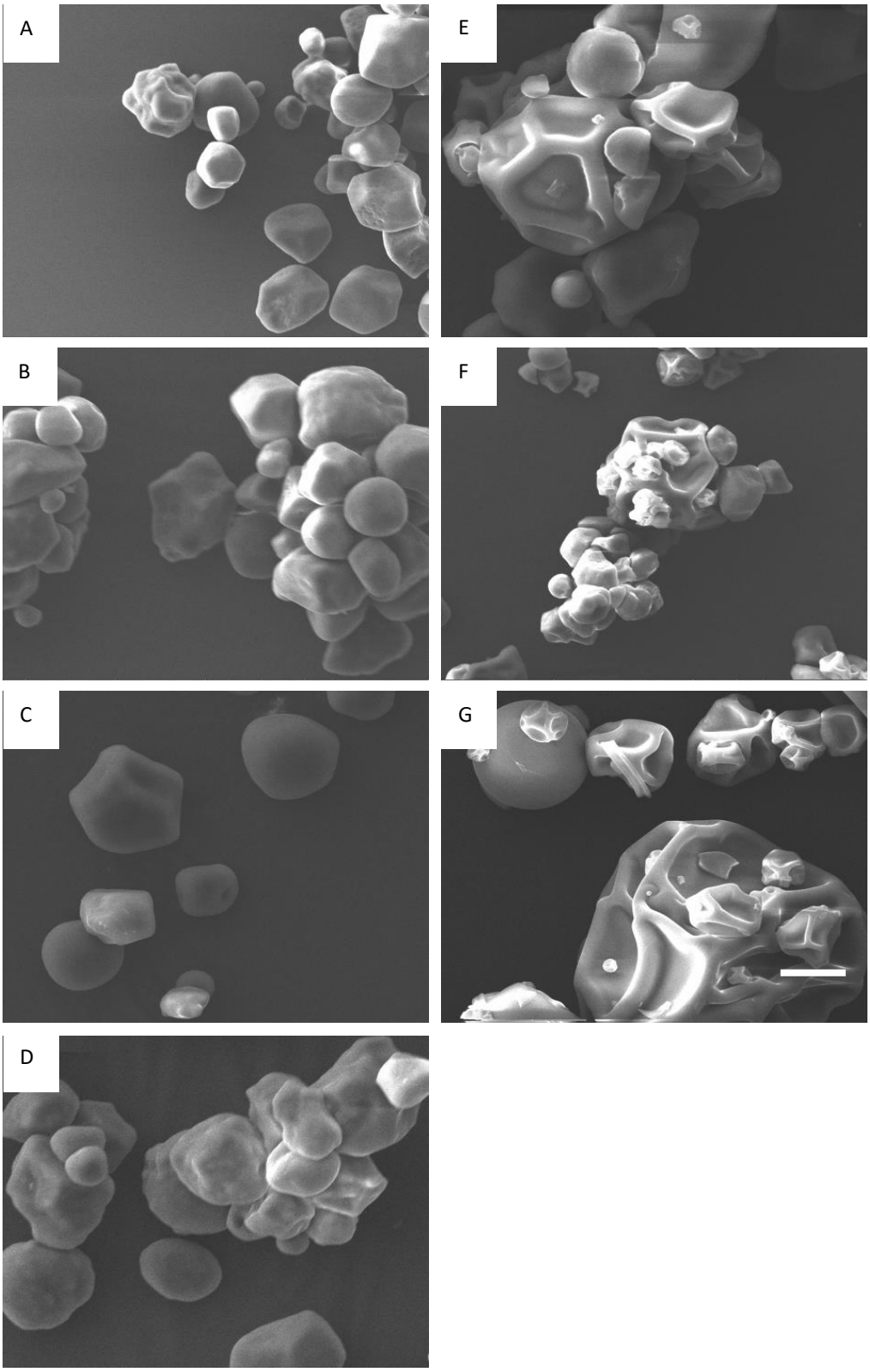

Figure 4.3 SEM micrographs of starches modified with 0 % (A), 0.5 % (B), 1 % (C), 1.5 % (D), 2 % (E), 2.5 % (F), 3 % (G) OSA based on weight of starch. Scale bar = 10 µm.

4.3.2 Double emulsion microstructure

Emulsion microstructure was characterised through acquisition of droplet size distributions and calculation of the surface based average droplet diameter utilising image analysis as well as cryo-SEM which was applied to visualise the structure of the oil droplet interfaces.

Increasing levels of OSA starch modification produced smaller (p<0.05) oil droplets (Figure 4.4) and the emulsions with higher OSA treatment levels were more stable over 90 d (p<0.05).

The initial droplet size of the emulsions, captured immediately after emulsification, ranged between 18 and 25 µm and was highest for the emulsion stabilised with the untreated starch and lowest for the emulsion stabilised with the 3 % OSA starch. None of the emulsions showed a significant change in mean droplet size after 1 d. However, with increasing time, emulsion stability depended on the level of OSA modification. Emulsions stabilised with unmodified starch and 0.5 % OSA starch showed high levels of coalescence after 3 d and were thus not further analysed. This is to be expected, as the control waxy maize starch (unmodified) is hydrophilic and therefore should not adsorb at oil/water interfaces (Shogren *et al.*, 2000). Although it was interesting to note that short term emulsion stability was observed here, this has previously been reported (Li *et al.*, 2013). At 1 % and 1.5 % OSA modification, emulsions were more stable and retained their characteristic microstructure for at least 30 d but the average droplet size increased and after 90 d the emulsion microstructure was no longer present. Emulsions stabilised with 2 %, 2.5 % and 3 % OSA starch retained their microstructure and were

stable for at least 90 d. Droplet diameter remained stable in 2.5 % and 3 % OSA starch stabilised emulsions. At 2 % OSA modification, a slow steady increase in droplet size over storage was noted although the absolute increase after 90 d was only ~4 µm.

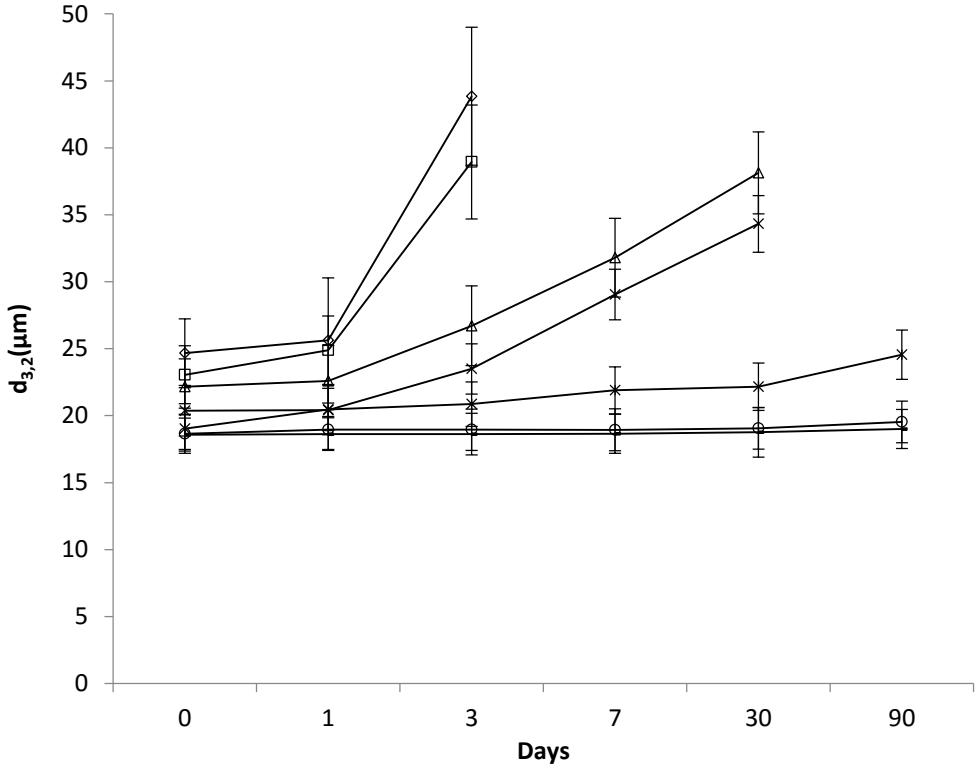

Figure 4.4 Mean droplet size ($d_{3,2}$) of $w_1/o/w_2$ emulsions over time prepared with 0 % OSA (◇); 0.5 % OSA (□); 1 % OSA (△); 1.5 % OSA (×); 2 % OSA (✳); 2.5 % OSA (+) and 3 % OSA (○). Emulsion droplet size measurements ceased once coalescence of the more unstable emulsions was observed.

OSA treatment increased surface hydrophobicity, resulting in the formation of an amphiphilic particle, this modified starch becomes surface active allowing it to act as particle stabiliser for o/w emulsions owing to its increased wettability and surface roughness (Sweedman *et al.*, 2013, Timgren *et al.*, 2013, Yusoff and Murray, 2011). The particle wettability of starch has been shown to enable the accumulation of particles at the o/w interface (Rayner *et al.*, 2012).

When used to stabilise emulsions, modified starches form Pickering emulsions, where the starch particles adsorb at the interface acting as a physical barrier

and are generally more stable against coalescence compared to those stabilised by surfactants (Binks, 2002). Cryo-SEM micrographs of the $w_1/o/w_2$ emulsions stabilised with different degrees of modified starch display the emulsion droplets and the interface between the oil and the continuous phase (Figure 4.5). The emulsion systems containing unmodified waxy maize starch had a smooth surface and with increasing levels of OSA modification of the starch the surface of the emulsions appear increasingly textured. The formation of a rough external surface was previously observed when cocoa particles were used to stabilise o/w emulsions (Gould *et al.*, 2013), suggesting that the starch is predominantly stabilising the emulsions and coating the emulsion surface.

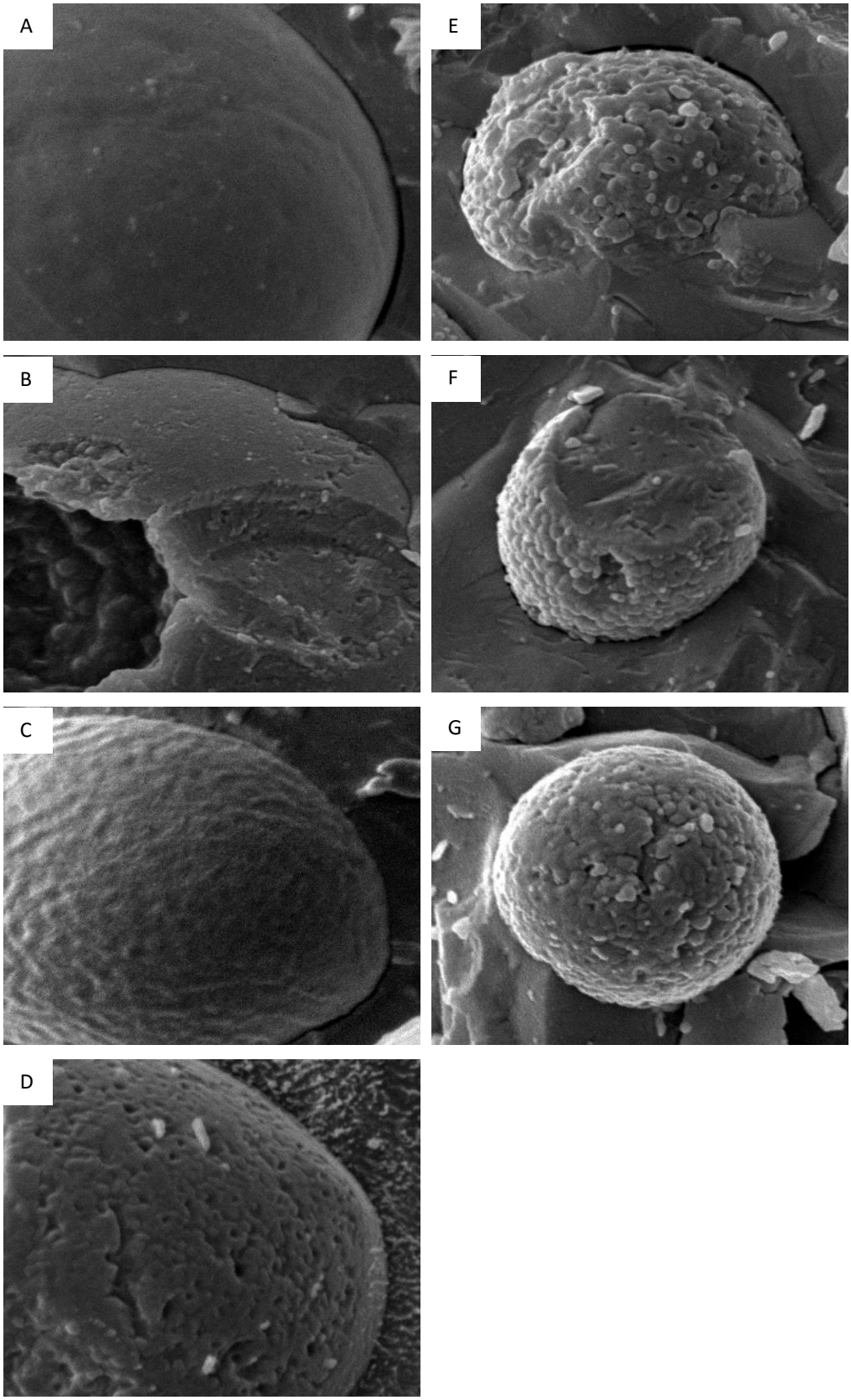

Figure 4.5 Cryo-SEM micrographs of $w_1/o/w_2$ emulsions stabilised with 0 % (A), 0.5 % (B), 1.% (C), 1.5 % (D), 2 % (E), 2.5 % (F), 3 % (G) OSA starch. Scale bar = 10 µm.

Encapsulation of sodium to the internal phase of the emulsion was determined and the encapsulation efficiency (EE) of the OSA starch samples was calculated, to help determine the stability of the $w_1/o/w_2$ emulsions. The EE of the emulsions improved with increasing levels of starch modification and the initial EE was highest for emulsions stabilised with 3 % OSA starch with 98 % and lowest for emulsions stabilised with unmodified waxy maize starch with 91.7 % (Figure 4.6).

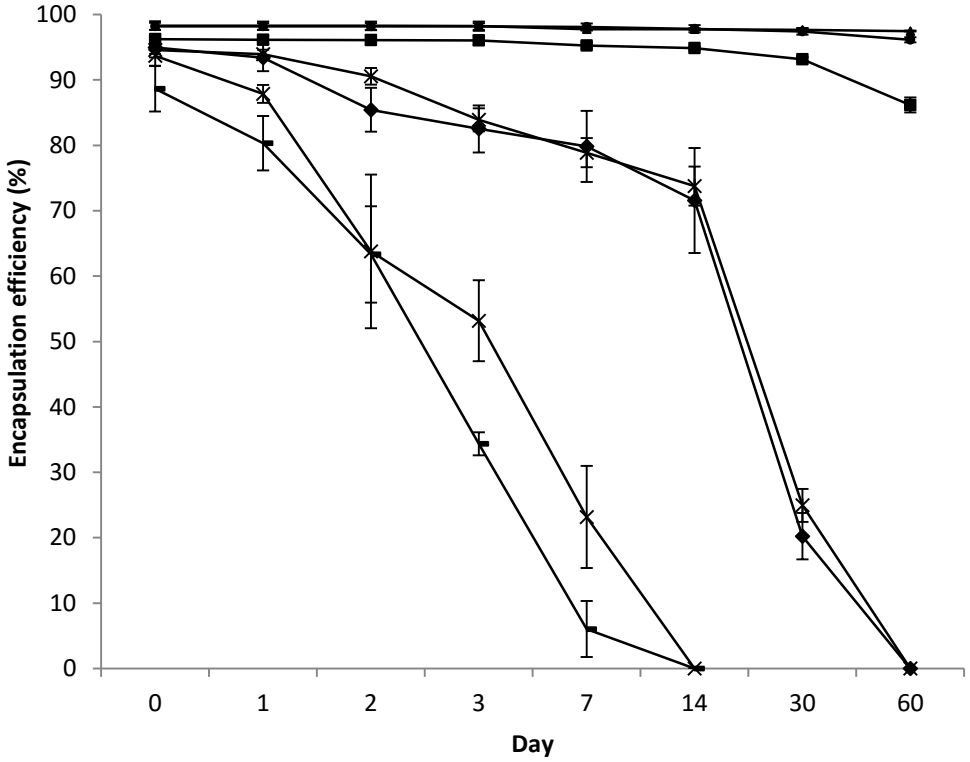

Figure 4.6 Encapsulation efficiency of $w_1/o/w_2$ emulsions stabilised with starches modified with: 0 % OSA (-); 0.5 % OSA (✕); 1 % OSA (◆); 1.5 % OSA (✳); 2 % OSA (■); 2.5 % OSA (●) and 3 % OSA (▲).

During storage, the EE of all the emulsions decreased and the EE of samples stabilised with starch containing 2 % or less OSA modification were significantly reduced. A low EE suggests that more encapsulated sodium is present in the continuous phase. Assuming that the sodium is evenly distributed within the internal aqueous phase of the emulsion, the sodium being detected would

reflect the internal aqueous phase released. Emulsions stabilised with a higher degree of modified starch produced emulsions that were more stable.

4.3.3 Remaining starch after *in vivo* and *in vitro*

The starch stabilised $w_1/o/w_2$ emulsions were exposed to α-amylase both in an *in vitro* assay and *in vivo* by oral processing, following which the percentage of non-digested starch was quantified. The percentage of remaining starch after digestion increased with increasing levels of OSA modification (Figure 4.7). OSA modification has previously been reported to affect the rate of starch digestion by rendering starch more resistant to digestion (Han and Bemiller, 2007). Starch digestion was greatest *in vivo* and a higher proportion of undigested starch remained in the *in vitro* emulsion, this may be due to the higher enzyme activity levels of some individuals *in vivo* compared to the *in vitro* digestion (50 units/mL), which is within human variability but is in the lower quartile. The variability of the remaining starch values measured was greater *in vivo* than *in vitro*, this is suggested to be due to individual variation in salivary flow rate and amylase activity, human salivary α-amylase activity has been shown to vary between individuals between 50 and 400 units/mL (Kivelä *et al.*, 1997, Mandel *et al.*, 2010).

Although the current study does not identify the spatial distribution of the OSA groups, it has been suggested that the distribution of these groups is more likely to occur at the external surface and within open channels of the starch particle due to the low solubility of OSA in water (Shogren *et al.*, 2000). However, the channels and cavities present in waxy maize granules aid the

distribution of OSA to penetrate to the interior of the granule (Shogren *et al.*, 2000, Wang *et al.*, 2013, Ye *et al.*, 2014). As enzyme hydrolysis is initiated from the exterior of the starch (Meireles *et al.*, 2009), the presence of alkenyl groups reduce enzyme hydrolysis and act as non-competitive inhibitors to α-amylase due to either physical hindrance or the hydrophobic environment imparted by the OSA groups (He *et al.*, 2008).

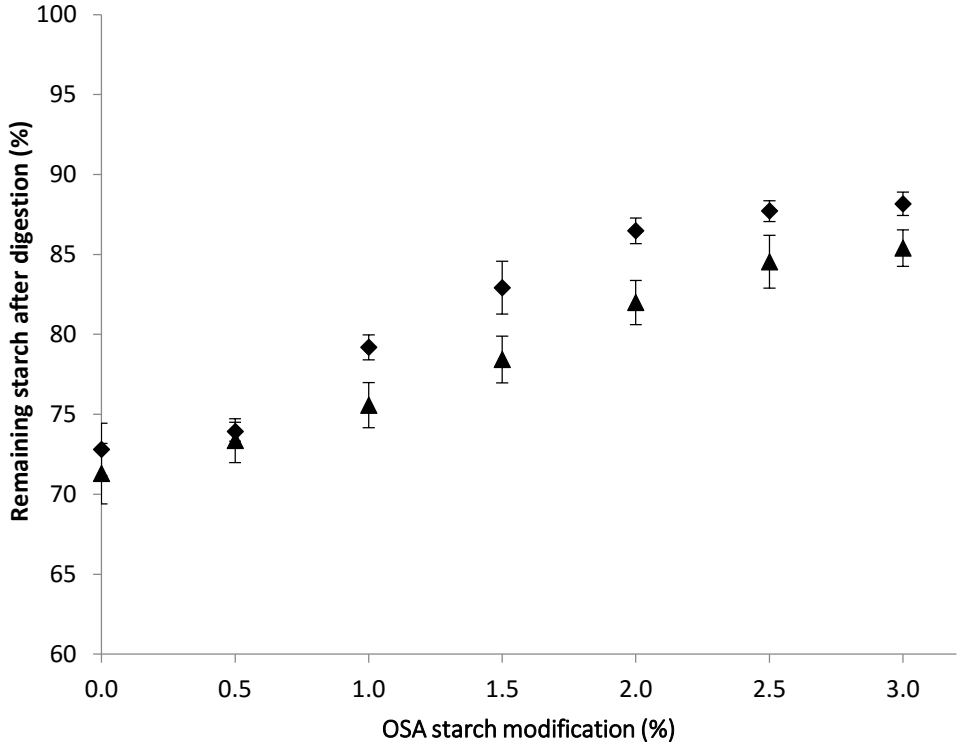

Figure 4.7 Remaining starch in emulsions stabilised with 0 - 3 % OSA starch after *in vitro* (◆) and *in vivo* (▲) digestion. Data are means of three replicates ± standard deviation.

4.3.4 Sodium release *in vitro*

In the presence of porcine α-amylase, salt release from the internal encapsulated aqueous phase to the continuous phase increased in all emulsions (Figure 4.8). Although a high proportion of starch remained associated with the emulsion, the hydrolysis of the starch appeared to have a direct effect on emulsion stability as the percentage of salt released was

greater as increasing amounts of starch are hydrolysed. The greatest increase was observed in emulsions stabilised without OSA starch modification (0 %). The level of salt release was reduced when the emulsions were stabilised with increasing degrees of OSA starch modification.

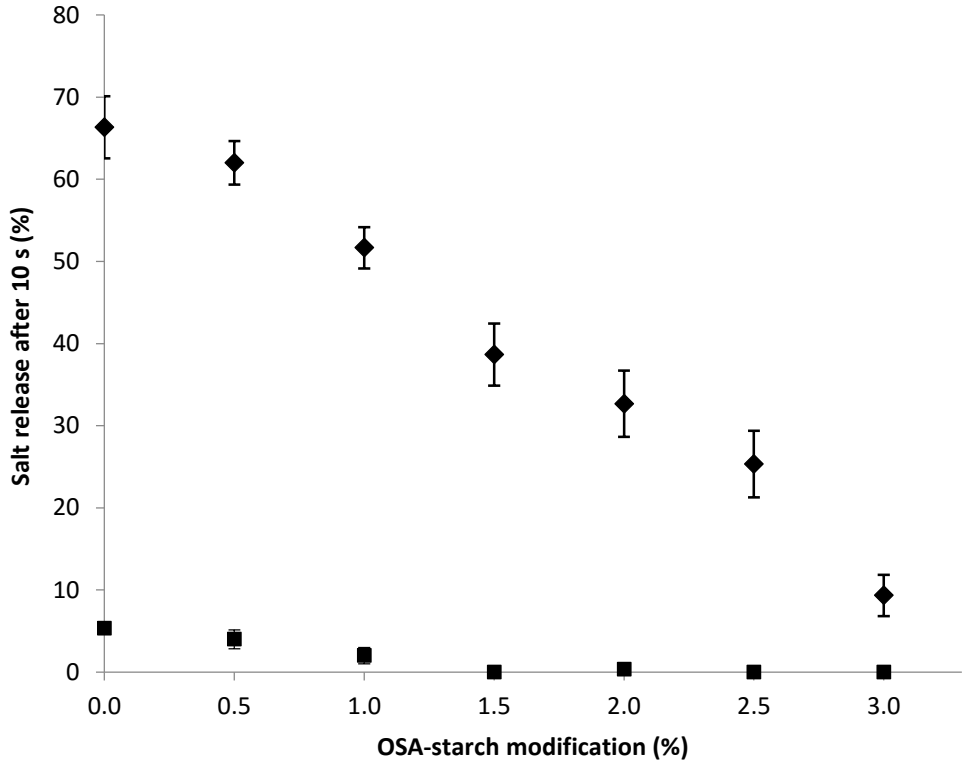

Figure 4.8.Total salt release after 10 s from emulsions stabilised with different degrees of modified OSA starch in presence of porcine α-amylase (◆) and in absence of α-amylase (■). Data are means of three replicates ± standard deviation.

In addition to the action of amylase, the release of salt may also be influenced by the initial instability of the emulsions. When lower degrees of modified OSA starch are used, fewer particles are present at the interface, therefore, less starch is needed to be removed from the interface to destabilise the emulsion. This supports the data shown previously that high levels of OSA treatment increased the stability of the starch to enzyme digestion. An optimum level of OSA modification may offer both physical emulsion stability and the ability to digest rapidly and release sodium when exposed to α-amylase.

4.3.5 Overall saltiness perception

4.3.5.1 Effect of the degree of OSA modified starch on overall saltiness perception

Naïve assessors were asked to evaluate saltiness intensity of emulsions using paired comparison (PC) tests. For each PC, the total number of assessors choosing each sample as saltiest is indicated (Table 4.1).

All tests, with the exception of test 5 and 8, showed a significant difference in saltiness intensity between samples. In general, results indicated that increasing the degree of starch modification decreased the intensity of saltiness and the greater the difference in the degree of modification between the two samples the greater the rejection of the more modified sample (test 4). The sample with 0 % modification was significantly saltier when compared to any OSA modified sample (tests 1 – 4). Although samples with 1.5 % OSA modification were significantly saltier than samples containing starch modified by 2.5 % and 3 % OSA, it did not significantly differ from sample with 2 % modification. The sample containing 2 % OSA was significantly saltier than 3 % but did not significantly differ from 2.5 %. The saltiness perception supports the instrumental measures of salt release recorded in Figure 4.8, indicating that the differences in the level of salt released within the *in vitro* assay corresponds to the ability of the assessors to discriminate between levels of perceived saltiness.

Table 4.1 Sample pairs presented in the paired comparison test of emulsions stabilised with five different degrees of OSA starch modification.

Test	Degree of modified starch used as an emulsifier (%)	Number of panellists selecting samples to be saltier	Result
1	0 1.5	85 15	saltier[#]
2	0 2	86 14	saltier[#]
3	0 2.5	94 6	saltier[#]
4	0 3	95 5	saltier[#]
5	1.5 2	55 45	similar[##]
6	1.5 2.5	71 29	saltier[#]
7	1.5 3	77 23	saltier[#]
8	2 2.5	53 47	similar[##]
9	2 3	62 38	saltier[#]
10	2.5 3	68 32	saltier[#]

[#] Samples perceived to be significantly saltier (p<0.05).
[##]Similarity concluded between the 2 samples (95 % confidence interval, p_d 30 %).

4.3.5.2 Comparing saltiness perception of sodium encapsulated emulsions stabilised with different levels of modified OSA starch and non-encapsulated emulsion

The aim of this experiment was to examine if the release of encapsulated sodium from OSA starch stabilised emulsions was able to produce a similar saltiness perception to an emulsion with more salt in the continuous phase. Using paired comparison (PC) tests, the overall saltiness of sodium

encapsulated $w_1/o/w_2$ emulsions was compared to single o/w emulsions, where all the sodium was present in the continuous phase (Table 4.2). In each pair, the total amount of salt in the $w_1/o/w_2$ emulsion was 15 % less than the single o/w emulsion. Results from the PC identified the $w_1/o/w_2$ emulsion, stabilised with 0 % starch modification, as the saltier sample despite more salt present in the single emulsion (Test 1). This may be due to the rapid breakdown of the $w_1/o/w_2$ emulsion in the mouth, resulting in sodium release to the continuous phase, creating a change in sodium available for perception. The perceived saltiness of the PPI stabilised single emulsion was similar to the sample containing 1.5 % OSA modified starch (Test 2) and 2 % OSA modified starch (Test 3). When higher degrees of modified OSA starch, 2.5 % and 3 %, was used to stabilise the $w_1/o/w_2$ emulsions, the sample with all salt at the continuous phase was saltier (Test 4 and 5, respectively). This suggests that less sodium was available and a perceptual difference of saltiness present in the continuous phase was detectable.

As sodium ions must transfer from the emulsion to the taste buds for perception, only sodium in the continuous aqueous phase is freely available. In the o/w emulsions all the sodium is available in the continuous aqueous phase and in the $w_1/o/w_2$ emulsions the sodium is divided between the internal (w_1) and continuous (w_2) aqueous phase, so only sodium present in w_2 is available for perception. The results from these tests suggest that the sodium available for perception in the $w_1/o/w_2$ emulsions was dependent on the emulsifier, as emulsions containing emulsifiers with more OSA modification were perceived to be less salty when compared to the same o/w emulsions, despite all $w_1/o/w_2$

having the same distribution of sodium between the two phases. Furthermore, the contrasting effect provided by the difference in sodium concentration in $w_1/o/w_2$ emulsions resulted in less overall salt being used to achieve a saltier ($w_1/o/w_2$ emulsion containing 0 % modified starch) and similar salty perception ($w_1/o/w_2$ emulsion containing 1.5 % modified starch or 2 % modified starch).

Table 4.2 Sample pairs presented in the paired comparison test of emulsions stabilised with five different degrees of OSA starch modification or PPI.

Test	Sample	o/w emulsifier	NaCl in w_1 (mol/ L)	NaCl in w_2 (mol/ L)	Total NaCl (g/ 100g emulsion)	No. of panellists selecting samples to be saltier	Result
1	o/w	PPI	-	0.171	0.65	40	
	$w_1/o/w_2$	0 % OSA starch	0.297	0.09	0.55	60	saltier[#]
2	o/w	PPI	-	0.171	0.65	43	
	$w_1/o/w_2$	1.5 % OSA starch	0.297	0.09	0.55	57	similar[##]
3	o/w	PPI	-	0.171	0.65	46	
	$w_1/o/w_2$	2 % OSA starch	0.297	0.09	0.55	54	similar[##]
4	o/w	PPI	-	0.171	0.65	66	saltier[#]
	$w_1/o/w_2$	2.5 % OSA starch	0.297	0.09	0.55	34	
5	o/w	PPI	-	0.171	0.65	91	saltier[#]
	$w_1/o/w_2$	3 % OSA starch	0.297	0.09	0.55	9	

[#] Samples perceived to be significantly saltier (p<0.05).
[##]Similarity concluded between the 2 samples (95 % confidence interval, p_d 30 %).

4.3.6 Temporal perception of saltiness

Time-intensity curves of saltiness perception over the time course of consumption of the emulsions were obtained from the 10 panellists. The average rating curves of the emulsions stabilised with the lowest (0 %) and highest (3 %) modified OSA starch were rated differently despite having the same amount of salt present in the emulsion (Figure 4.9). From the time-intensity curves, three parameters were extracted; maximum saltiness intensity (I_{max}), time to reach I_{max} (T_{max}) and the area under the saltiness curve (Table 4.3).

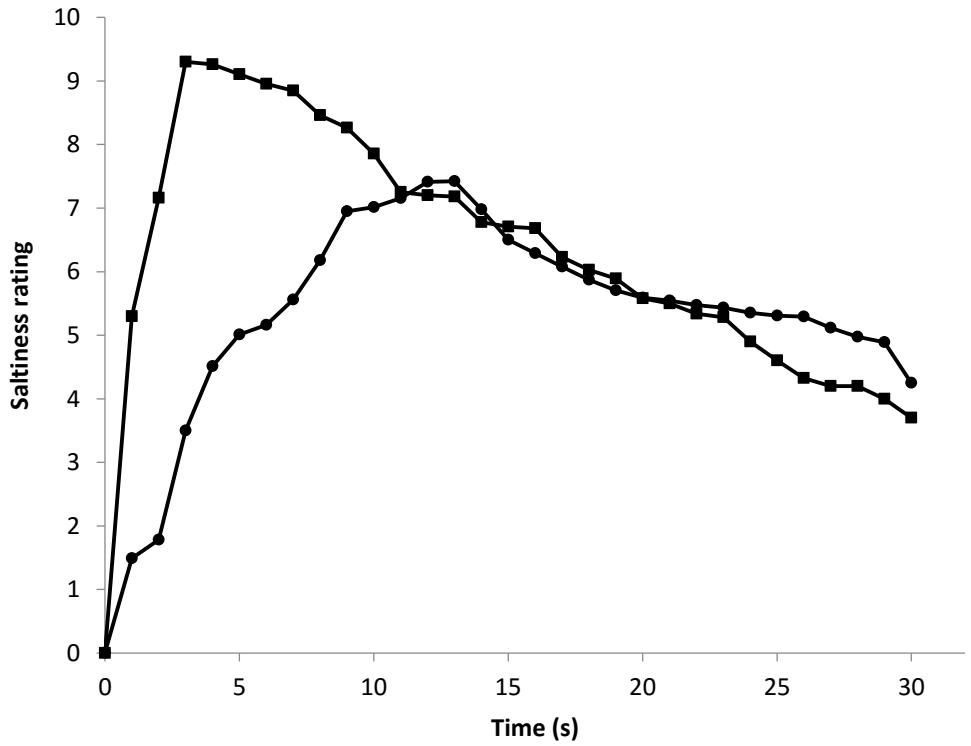

Figure 4.9 Average TI saltiness rating of the emulsions stabilised with 0 % OSA modified starch (■) and 3 % OSA modified starch (●).

The total area under the curve reflects the cumulative saltiness perceived during each test. Results suggested more saltiness was perceived when the emulsifier was less modified, suggesting that more sodium was being released

from the internal phase into the continuous phase, where it is available to interact with taste receptors. I_{max} varied significantly among samples (p<0.01). In general, the intensity of saltiness increased as the degree of modified OSA starch decreased. The sample with no (0 %) OSA modification had the highest I_{max} and the sample containing starch with 3 % OSA modification showed the lowest I_{max} value. These results were in agreement with the results of the *in vitro study* with porcine α-amylase, where the level of sodium in the continuous phase was higher in emulsions stabilised with lower degrees of modification and demonstrated that the extent of starch modification indeed had an effect on sodium release and affected the intensity of saltiness perception.

Table 4.3 ANOVA of extracted parameters for six different emulsion stabilised with different degree of modified OSA starch, with salt encapsulated in the internal phase. Samples with the same letter code in each row are not significantly different (p<0.05).

	OSA modification (%)					
	0	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**
T_{max} **(s)**	4.12 ^{a}	8.72 ^{b}	8.64 ^{b}	9.98 ^{bc}	9.42 ^{bc}	11.16 ^{c}
I_{max}	9.262 ^{d}	9.012 ^{cd}	9.116 ^{cd}	8.488 ^{ab}	8.206 ^{b}	7.196 ^{a}
Area under the curve	240.2 ^{d}	223.3 ^{cd}	225.9 ^{cd}	204.9 ^{ab}	194.2 ^{b}	162.3 ^{a}

The time to maximum intensity (T_{max}) also varied significantly among samples (p<0.01). In general, T_{max} of saltiness was reached prior to swallowing at 10 s, with the exception of the emulsion with 3 % OSA starch modification where maximum intensity occurred at 11.16 s. T_{max} varied depending on the degree of OSA starch modification, with emulsion samples stabilised with 0 % OSA modification starch having the fastest release (lowest T_{max}).

The total area under the curve reflects the cumulative saltiness perceived during each test. Total area was greatest for the emulsions with 0 % OSA starch modification and low levels of OSA modification (1 % and 1.5 %), and the total rated sodium was lowest for the more modified samples. This further contributes towards the justification for an optimum OSA modification level.

4.3.6.1 Effect of amylase on saltiness perception

It was noted that T_{max} varied among the panellists (Figure 4.10). The presence of salivary α-amylase is important for the hydrolysis of starch prior to swallowing (De Wijk *et al.*, 2004, Evans *et al.*, 1986). The release of salt from the internal phase of the emulsion to the continuous phase for perception is influenced by the presence of α-amylase, salivary flow rates and the amylase activity of panellists, it was therefore important to take these into account.

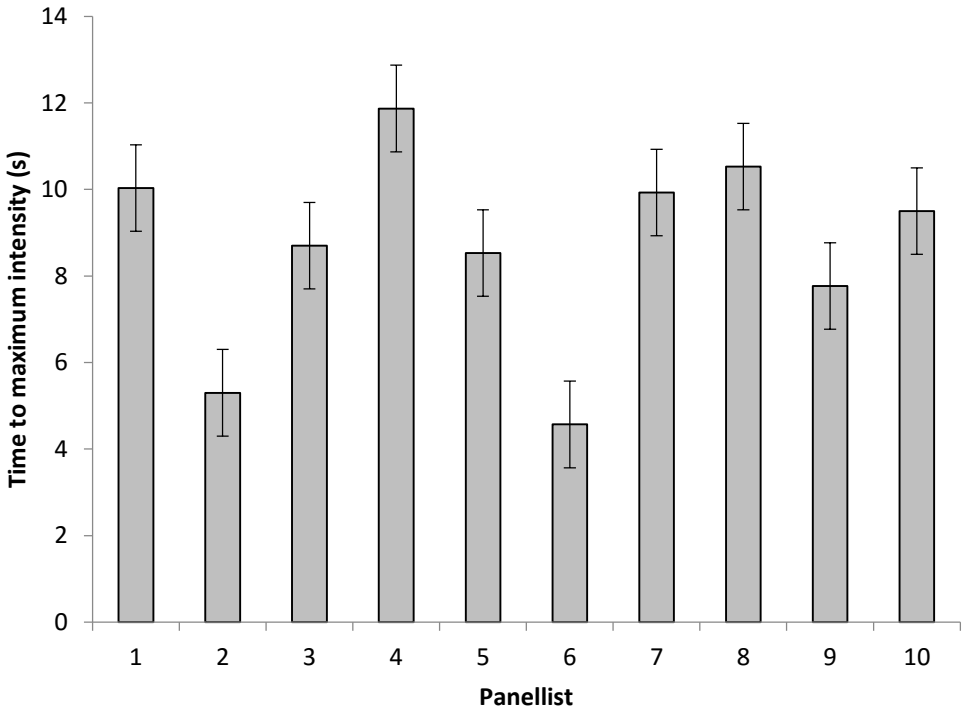

Figure 4.10 Average T_{max} of all samples from each panellist.

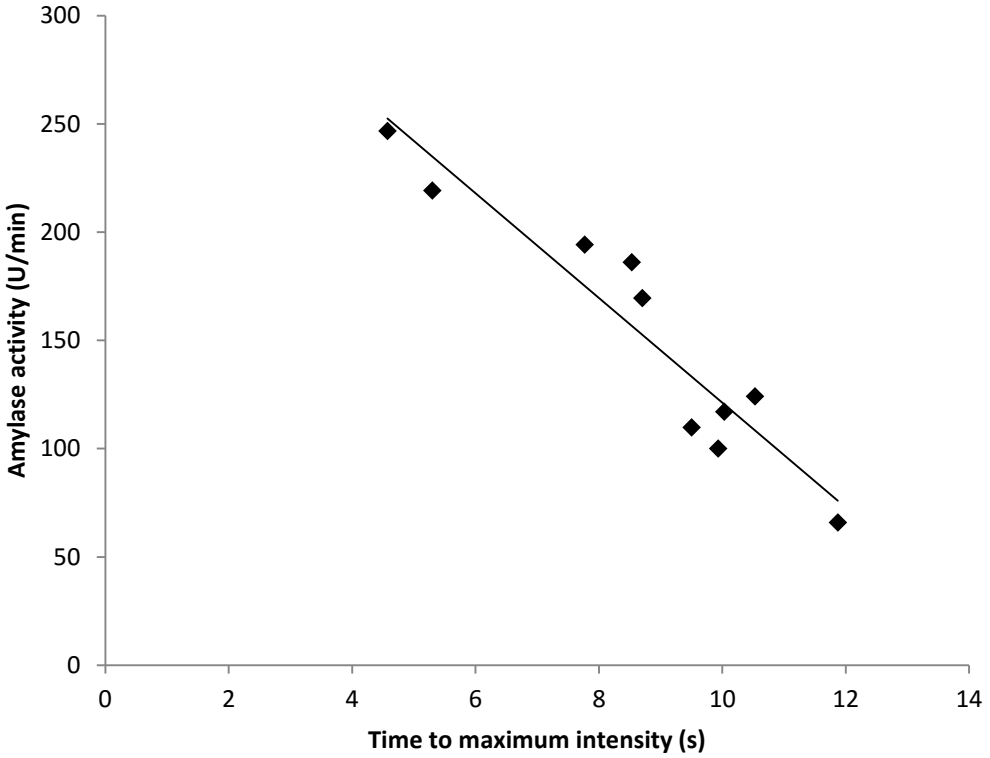

Figure 4.11 T_{max} and amylase expression of individual panellist.

Salivary α-amylase activities were highly correlated with T_{max} values (ρ=-0.95, p<0.001), panellists with the highest levels of α-amylase expression indicated they reached maximum perceived saltiness much faster compared to those with lower levels of enzyme expression (Figure 4.11). Amylase expression levels were between 65.84 and 246.84 U/min and was in agreement with previous studies (Mandel *et al.*, 2010). It was interesting to note that the time to maximum saltiness perception varied from ~12 s for the assessors with the lowest salivary α-amylase expression levels to ~4 s for the assessors with the greatest salivary α-amylase expression levels. This may have a significant impact on taste perception not only in the product studied herein, but also in other food systems.

4.4 Conclusion

The results presented in the chapter have demonstrated the possibility of using different degree of OSA starch as an oil-in-water emulsifier.

To understand if different levels of OSA modification would affect stability and sodium release for $w_1/o/w_2$ emulsions, different degrees of OSA modified waxy maize starch were used to form double emulsions. As OSA starch modification increased, the stability of the emulsions improved and droplet size reduced. In addition, the OSA esterification of waxy maize starch reduced the α-amylase digestibility and subsequent release of the encapsulated salt in the mouth for perception. The *in vitro* results correlated with sensory results, where emulsions stabilised with no or low levels of OSA were perceived as saltier compared to higher degrees of modification. Individual amylase activity was shown to influence the time of reaching maximum saltiness intensity. However, further studies on the effect of sodium perception of encapsulated $w_1/o/w_2$ emulsions are required to understand the impact of amylase activity.

Paired comparison testing also showed a $w_1/o/w_2$ emulsion containing 15 % less salt was perceived to be saltier (non-modified OSA starch) or similar (1.5 % and 2 % OSA modified starch) when compared to an o/w emulsion where all the sodium is at the continuous phase.

An optimum OSA starch modification level may achieve emulsion stability and maximise sodium release during oral processing for perception. The release of entrapped sodium in the mouth was able to provide a sodium contrast to bring about an enhanced perceived saltiness. In conclusion, $w_1/o/w_2$ emulsions

stabilised with modified starch may be applied as a novel tool in liquid and semi-solid systems as a sodium reduction approach.

5 Conclusions and Future Work

The main aim of this project was to develop the tools and knowledge to reduce dietary sodium by mitigating restrictions to flavour delivery and enhancing saltiness perception through sodium contrast effects in the mouth. This is achieved by restructuring semi-solid and liquid model food systems to achieve maximum flavour delivery for enhanced perception. Two different microstructures, foams and double emulsions, were examined in this thesis. A summary of the key finding are discussed below.

The first approach examined in this thesis was the use of air as an inert filler to replace the aqueous phase in a semi-solid system. This was effective in enhancing overall saltiness perception of the sample. Paired comparison tests revealed that increasing the level of air inclusion increased the saltiness perception of the sample. The displacement of water with air inclusions enhanced the perception of saltiness of a fixed volume of sample; it is hypothesised that this is due to an effective increase in concentration of salt in the aqueous phase of the sample.

The presence of air in the sample was also able to improve the delivery of a congruent aroma. This is proposed to be due to an increased surface area, thus enhancing aroma delivery. Sensory tests showed that the addition of a congruent aroma to a salted foamed food further enhanced the overall flavour perception. In general, the examination of aroma compounds of different physicochemical properties showed that introducing air to the samples

increased aroma release and that the efficiency of the increase was dependent on the physical properties of the aroma compound.

The second approach used structured water-in-oil-in-water ($w_1/o/w_2$) emulsions stabilised with starch as a vehicle to deliver entrapped sodium to the mouth during processing. This required the emulsion to remain stable during storage and destabilise in mouth, releasing the encapsulated sodium, thereby delivering a contrast of saltiness between the encapsulated phase and the continuous phase.

Emulsions stabilised with native starch demonstrated that the emulsions were partially stable during storage and could be destabilised in mouth when exposed to α-amylase. However, the inherently hydrophilic native starches were not suitable for stabilising emulsions over extended periods. An alternative commercially modified octenyl succinic anhydride (OSA) starch, NC46, produced stable single (o/w) and double ($w_1/o/w_2$) emulsions. The emulsions remained stable up to 90 d with no significant change in droplet size (p>0.05). The $w_1/o/w_2$ emulsions were able to retain 96.5 % of encapsulated sodium for up to 90 d.

NC46 stabilised $w_1/o/w_2$ emulsions were more salty when compared to emulsions stabilised with pea protein isolate (PPI), an amylase insensitive stabiliser. Compared to the PPI stabilised emulsion, an overall reduction of 23.7 % of salt was achieved in the NC46 stabilised $w_1/o/w_2$ emulsion without compromising perception. Encapsulated sodium was released from the NC46 stabilised emulsion through partial amylase hydrolysis, resulting in the enhanced perception of saltiness.

To identify an optimum level of starch modification for both emulsion stability and encapsulated sodium release during amylase exposure, waxy maize starch was modified to different levels of OSA treatment up to 3 % OSA.

Whilst stability of the emulsions improved with greater OSA modification, OSA modification reduced amylase hydrolysis rates which subsequently reduced sodium release during consumption and perceived saltiness. $W_1/o/w_2$ emulsions stabilised with low levels of OSA starch were saltier than emulsions stabilised with higher degrees of OSA modification.

Furthermore, Time-intensity tests revealed that samples containing lower degrees of OSA modification resulted in a higher maximum saltiness perception which was reached faster and the total saltiness (area under the saltiness time-intensity curve) was greater.

An optimised level of OSA treatment was defined as 1.5 % and 2 % depending on process stability requirements. With 15 % less salt, $w_1/o/w_2$ emulsion stabilised with the 1.5 % and 2 % OSA starch were able to deliver a comparable saltiness to a sample with salt homogeneously distributed in the aqueous phase.

In addition, individual amylase activity was associated to the time when maximum saltiness was reached, where an increase in amylase activity produced faster maximum saltiness perceptions.

The present findings provide two possible new approaches to reduce sodium in semi-solid and liquid systems, where one approach focuses on mitigate restrictions to flavour delivery and the other on enhancing saltiness perception by delivering sodium contrast.

Although the results were positive in demonstrating the capabilities of the model systems as sodium reduction strategies, there is still considerable need for further work for a better understanding of both these systems for future application.

The inclusion of air into an aqueous system demonstrated that the application was able to enhance saltiness perception. However, other sensorial attributes of air inclusions were not determined, including surface texture which can greatly impact on acceptance, preference and expectation (Chen, 2007). According to Goh *et al.* (2010) the inclusion of air did not affect taste perception, although it may have a considerable impact on preference and the extent of air incorporation.

It was interesting to find that different aroma volatiles when incorporated into the model foam system, delivered different levels of volatile release depending on the level of air inclusion and the partition coefficient (Log P). It would be interesting to examine more aromas where a resulting model could be determined and could be valuable for optimising product composition (i.e. reducing the concentration of aroma if air inclusion is increased).

Although the method developed to measure emulsion droplets through image analysis is relatively simple and requires only a light microscope and free image processing software, this method has limitations that should be highlighted for

further development. The method is time-consuming therefore only a small proportion of the emulsion droplets are captured to represent the droplet size distribution within each sample. Therefore, validation of the method and measuring emulsions droplets using other measuring techniques including 3D cross-correlation light scattering (CCLS) is required (Mezzenga *et al.*, 2004).

The use of OSA modified starches was able to bring about saltiness enhancement during consumption. Further research towards OSA modified starches may improve our understanding of OSA starch stabilised emulsions and how the hydrolysis of the starch renders the starch as an insufficient stabiliser. In particular, the distribution of OSA groups on the starch may provide insight into how the starch orientates at the interface to form Pickering emulsions. Furthermore, other types of modifications, including physical and other chemical modifications, as well as other starches should be considered as alternative options to stabilise and deliver encapsulated sodium using a $w_1/o/w_2$ emulsion.

In addition, the internal emulsifier, polyglycerol polyricinoleate (PGPR) could be replaced to promote a faster release of encapsulated sodium; in particular, the use of fat crystals, which have been previously shown to be able to stabilise w/o interface (Frasch-Melnik *et al.*, 2010a).

As sensory testing was conducted using the model systems, it would be beneficial to trial the $w_1/o/w_2$ emulsion in an existing food product (e.g. soups, sauces) for a better understanding of the saltiness perception as well as acceptance in a real food product.

The knowledge from these two systems regarding salt reduction may also be viable for adapting towards other tastants such as sugar, which also requires substantial reduction within human diets.

Although there are still more questions that need to be answered, the results from this PhD research have provided new knowledge and insights to effectively deliver sodium in semi-solid and liquid systems for peception without compromising taste, whilst enabling a significant sodium reduction.

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