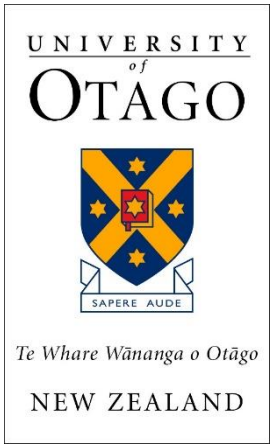

MONOGLYCERIDE-BASED EMULSIFIER TECHNOLOGY TO ENHANCE EMULSION STABILITY IN MILK COFFEE BEVERAGES

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Abstract

Milk coffee beverages are a protein-stabilised emulsion and consist of dispersed oil droplets in the aqueous phase. Casein, whey proteins and mono- and diglycerides (MDGs) are surface-active agents that adsorb at the oil-water interface to form and stabilise emulsions. Protein is an important natural emulsifying agent in emulsion systems and interact with MDGs at the oil-water interface to modify oil droplet size, zeta potential, flow behaviour, physical stability and oxidative stability. Many studies have investigated the interaction between milk protein and MDG and their effect on droplet properties, flow behaviour and food structure in whippable emulsions. However, there is very little knowledge around the interaction of MDG and milk protein in beverage emulsions. MDG is an oil-soluble emulsifier and has low solubility in water, making it not suitable for direct application in many food formulations. Encapsulation of MDG could be a potential solution to convert it into a stable water-dispersible powder; however, there is very little information published about the encapsulation of emulsifiers as a functional ingredient.

This project had two main objectives. The first objective aimed to gain fundamental understandings on the effect of MDG and milk protein compositions in a protein-stabilised oil-in-water emulsion system. The second objective investigated the preparation and characterisation of an encapsulated emulsifier system, including its physicochemical properties, stability after reconstitution and functionality in model emulsions. Five experimental studies were carried out to meet these objectives.

A model emulsion prepared by microfluidisation to mimic the characteristics of a beverage emulsion was used to investigate the effect of the composition of mono- and diglycerides (unsaturation of fatty acids; monoglyceride content) and co-emulsifier (sodium stearate) content on the physical properties and stability against creaming in protein-stabilised emulsions. The experimental results demonstrated that emulsions with 0.2% MDGs produced 15-30% smaller oil droplets and 17-27% lower polydispersity indices compared to the control (no MDG). Sodium stearate (6% w/w of MDG) increased the negativity of zeta potential by 12.6-17.3 mV in emulsions containing saturated MDGs and 1.8-5.0 mV in unsaturated MDGs. Unsaturated MDGs showed better creaming stability than the control after 28 days of ageing with no improvement observed for

saturated MDGs. Glycerol monooleate (GMO) demonstrated the best creaming stability among the unsaturated MDGs.

The next study investigated the effect of milk protein compositions (different ratio of sodium caseinate to whey protein concentrate) on physicochemical properties, creaming stability and oxidative stability of protein-stabilised emulsions containing GMO. The experimental results showed that the emulsion with only sodium caseinate produced smaller droplets (174.7 nm), a more negative zeta potential (-50.8 mV) and a more viscous emulsion (1.89 mPa s) compared to the emulsion with only WPC (191.4 nm; -38.8 mV; 1.65 mPa s). Protein composition had no significant effect on creaming stability. Eleven volatile compounds were identified as lipid oxidation markers, and six compounds (2-pentylfuran, octanal, nonanal, 3-octen-2-one, 2,4-heptadienal, 3,5-octadien-2-one isomers) demonstrated that emulsions with mixed protein types (sodium caseinate and WPC) had better oxidative stability than emulsions with a single protein type, i.e. either caseinate or WPC alone.

GMO demonstrated excellent creaming stability in protein-stabilised emulsions but was unsuitable for direct aqueous applications due to its low solubility in water. In order to use GMO as a functional ingredient in beverage emulsions, the feasibility of spray-drying to encapsulate oil-soluble MDG using appropriate wall materials to produce a stable water-dispersible powder with good reconstitution properties and extended shelf life was assessed. In addition, the effect of emulsion formulation (GMO concentration (33.6%, 47.0%); dextrose equivalent (DE) values of maltodextrin (DE 10, 18)) on emulsion properties, powder properties and oxidative stability was investigated. Results showed that all homogenised emulsions were suitable for spray-drying due to their high emulsion stability against phase separation, monomodal droplet size distributions (150-180 nm) and low viscosity (20-65 mPa s). All instantised powders exhibited good dispersibility (65-90%) in water and greater oxidative stability than bulk GMO. The instantised powder with low GMO and maltodextrin DE 10 showed both good dispersibility and low lipid oxidation, demonstrating that spray-drying can successfully produce an instantised GMO powder with a longer shelf life for food applications.

The application of two selected instantised GMO powders (low GMO content with maltodextrin DE 10 or DE 18) were investigated in protein-stabilised emulsions in terms of physicochemical properties, creaming stability and oxidative stability. Model

emulsions with bulk GMO, two instantised GMO powders and two controls (contain either maltodextrin DE 10 or DE 18 with no GMO) were prepared using microfluidisation. The emulsion physicochemical properties were characterised by droplet size, zeta potential, viscosity and creaming index, while the oxidative stability was assessed by the formation of volatile secondary lipid oxidation products during storage (28 days at 45 °C) using gas chromatography-mass spectrometry (GC-MS). Experimental results revealed that all three emulsions with GMO had smaller average droplet sizes (180.0 nm) and narrower size distribution (polydispersity index of 0.161) compared to the two controls (197.6 nm, 0.194). All emulsions with GMO also had greater creaming stability than the control emulsions. Principal component analysis of the volatiles revealed that storage time had the greatest influence on lipid oxidation. Three lipid oxidation markers, 3-octen-2-one, 2,4-heptadienal isomer 2 and 3,5-octadien-2-one isomer 1, showed that controls had the same oxidative stability as instantised GMO, but were more stable than bulk GMO. Therefore, GMO powders can form stable protein-stabilised emulsions with good physicochemical properties and oxidative stability.

The different concentrations of bulk GMO and instantised GMO powder (low GMO with maltodextrin DE 10) was evaluated in an application of a model coffee beverage emulsion in terms of physicochemical properties, creaming stability and volatile profile at different storage time. The increasing GMO level formed fresh coffee emulsions with smaller droplet sizes and narrower size distribution that resulted in greater emulsion stability against creaming compared to the control (no GMO). However, emulsions prepared with 0.1% and 0.2% GMO were not stable at pH near to the isoelectric point of casein during storage and resulted in the growth of droplet size. These emulsions at 28 days of storage showed the presence of flocculated oil droplets due to protein aggregates when visualised using an optical microscope. The visible sediment particles in the emulsions with 0.2% GMO were associated with protein aggregate induced flocculation and could be explained by having a zeta potential below the critical level for stability (-30 mV). The emulsions with 0.03% GMO powder demonstrated greater creaming stability than the control emulsions and had stable droplet size, zeta potential and viscosity. The chemical stability was similar to the control emulsions demonstrated by the very similar volatile concentrations.

In conclusion, the results from this thesis provide new insights into the relationship between milk proteins and a MDG-based emulsifier system and their effects on emulsion properties and volatile profile in model protein-stabilised and coffee emulsions. The knowledge from this study is useful to formulate a ready-to-drink coffee beverage with the desired emulsion properties and shelf stability. This study also presents an innovative application of spray-drying to design an emulsifier system that is not only in the right format for beverage emulsions but achieves the same functionality in the products at a lower application rate. Further development may be required to determine optimum dose rates for various food and beverage applications; but the instantised GMO powder will give better control over the dosage to ensure the desired functionality is obtained.

List of Publications

Refereed Journal Articles

Loi, C. C., Eyres, G. T., & Birch, E. J. (2019). Effect of mono- and diglycerides on physical properties and stability of a protein-stabilised oil-in-water emulsion. *Journal of Food Engineering, 240,* 56-64. doi:10.1016/j.jfoodeng.2018.07.016

Loi, C. C., Eyres, G. T., & Birch, E. J. (2019). Effect of milk protein composition on physicochemical properties, creaming stability and volatile profile of a protein-stabilised oil-in-water emulsion. *Food Research International, 120,* 83-91. doi:10.1016/j.foodres.2019.02.026

Book Chapters

Loi, C. C., Eyres, G. T., & Birch, E. J. (2019). Protein-stabilised emulsions. In L. Melton, F. Shahidi, & P. Varelis (Eds.), *Encyclopedia of food chemistry* (pp. 404-409). Oxford: Academic Press.

Conference Presentations and Proceedings

Loi, C. C., Eyres, G. T., & Birch, E. J. (2018, July). *Role of mono- and diglycerides in protein-stabilised emulsions.* Paper presented at the New Zealand Institute of Food Science and Technology Conference, Hamilton, New Zealand (Oral presentation).

Loi, C. C., Eyres, G. T., & Birch, E. J. (2018, October). *Effect of milk protein composition on physicochemical properties and volatile profile of an oil-in-water nanoemulsion.* Paper presented at the 19th World Congress of Food Science and Technology, Navi Mumbai, India (Poster presentation).

Loi, C. C., Eyres, G. T., Silcock, P., & Birch, E. J. (2019, July). *Preparation of a novel emulsifier system based on glycerol monooleate by spray-drying.* Paper presented at the New Zealand Institute of Food Science and Technology Conference, Christchurch, New Zealand (Oral presentation).

Loi, C. C., Eyres, G. T., Silcock, P., & Birch, E. J. (2019, August). *Novel instantised glycerol monooleate by spray-drying: Effect on physicochemical properties and stability of a protein-stabilised emulsion.* Paper presented at the Otago Student Research Symposium, Dunedin, New Zealand (Oral presentation).

Loi, C. C., Eyres, G. T., Silcock, P., & Birch, E. J. (2020, February). *A novel microencapsulated emulsifier to improve the stability of protein-stabilised emulsions.* Paper presented at the World Congress on Oils & Fats, Sydney, Australia (Oral presentation).

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List of Abbreviations

ANOVA	Analysis of variance
bGMO	bulk glycerol monooleate
CAR	Carboxen
DE	Dextrose equivalent
DVB	Divinylbenzene
GC-MS	Gas chromatography-mass spectrometer
GMO	Glycerol monooleate
HLB	Hydrophilic-lipophilic balance
HS-SPME	Headspace solid-phase microextraction
i.d.	Internal diameter
iGMO	Instantised glycerol monooleate
MD	Maltodextrin
MDG	Mono- and diglyceride
MG	Monoglyceride
m/z	Mass to charge
n	Number of replicates
NaCas	Sodium caseinate
NIST	National Institute of Standards and Technology
o.d.	Outer diameter
O/W	Oil-in-water

p	Probability
PARADISe	PARAFAC2 based Deconvolution and Identification System
PC-1	Principal component 1
PC-2	Principal component 2
PCA	Principal component analysis
PDMS	Polydimethylsiloxane
R^2	Coefficient of determination
RI	Retention index
RTD	Ready-to-drink
SPME	Solid-phase microextraction
TGA	Thermogravimetric analysis
TIC	Total ion chromatogram
UHT	Ultra-high temperature
USA	United States of America
WPC	Whey protein concentrate
WPI	Whey protein isolate
Z-average	Intensity-weighted mean diameter

List of Symbols

°C	degree Celcius
% v/v	volume per volume percentage
% w/w	weight per weight percentage
µL	microliter
µm	micrometre
au	area units
cm	centimetre
cP	centipoise
cSt	centiStroke
g	gram
h	hour
kDa	kiloDalton
kPa	kiloPascal
L	litre
m	metre
min	minute
mm	millimetre
mL	millilitre
mV	millivolt
nm	nanometre

mPa	milliPascal
MPa	megaPascal
ppm	parts per million
s	second

Chapter 1

Introduction

1.1 Introduction

Milk coffee beverages are popular among consumers in many parts of the world. The choice of coffee ranges from a cup of freshly brewed coffee at a specialised coffee shop to a ready-to-drink (RTD) coffee off the supermarket shelf or from vending machines. The interest of this research is in the RTD coffee segment that is growing in the Asia-Pacific region. The global RTD coffee market was valued at US $19.05 billion in 2017, which was primarily dominated by iced coffee in the Asia-Pacific region and cold brew coffee in the USA (Fortune Business Insights, 2019). In 2016, there were 29% new launches in the RTD coffee segment in the Asia-Pacific region and 10% in Europe (Arthur, 2017). Convenience and ease of access are two essential factors that have attributed to the strong growth of RTD coffee. RTD coffee beverages are widely available in vending machines and convenience stores in Japan (Oakland, 2011), South Korea (C.-u. Cho, 2016), China and Taiwan. Lightly sweetened coffee with milk, both cold and hot, are common in convenience stores throughout Japan and South Korea.

Milk coffee is an emulsion system made up of water, coffee, milk, sugar and other food additives. Milk coffee, being an emulsion with 2-10% total fat, is thermodynamically unstable. Milk coffee beverages tend to exhibit creaming and sedimentation during long-term storage. Creaming is an upward movement of dispersed fat globules in the water phase due to the density difference between the two phases. Homogenisation is a common processing step in the dairy industry to reduce the droplet size of an emulsion and improve creaming stability (McClements, 2015). The utilisation of thermal treatments such as retorting and ultra-high temperature (UHT) sterilisation are effective in extending microbiological stability, but at the same time can physically destabilise the emulsion. Sünder, Scherze, and Muschiolik (2001) demonstrated that an increase in whey protein content reduces aggregation of oil droplets and improves emulsion stability in beverage products.

Low-molecular-weight emulsifiers are important food additives that can delay creaming and sedimentation in milk coffee beverages (S. R. Euston, 2008). Some of the common emulsifiers that can improve milk coffee stability are mono- and diglycerides (MDGs), polyglycerol esters, lactylates, polysorbates and sucrose esters. Mono- and diglycerides have been investigated in this thesis because they are efficient emulsifiers of oil and water systems and have a relatively low cost of production compared to other emulsifiers,

making them cost-effective emulsifiers in food systems. Despite the benefits of mono- and diglycerides, their low solubility in water, long dispersion time and tendency to form gels in hot water (Moonen & Bas, 2007) make them challenging to use in beverage applications. The addition of co-emulsifiers, such as sodium stearate, can improve the dispersion of mono- and diglycerides in water; however, sodium stearate cannot disperse uniformly in a low melting point emulsifier, causing potential dispersibility issues when using this emulsifier in food formulations (Krog & Vang Sparsø, 2003). Spray-drying is a potential technique to encapsulate and transform mono- and diglycerides into a suitable water-dispersible format (Augustin & Oliver, 2014).

Understanding the interaction between milk proteins and emulsifiers is important to optimise physical and chemical stabilities of milk coffee. It has been reported that emulsifiers such as Tween 20 displace casein from the fat globule membrane (Dickinson, Radford, & Golding, 2003; Dickinson, Ritzoulis, & Povey, 1999), thus increasing rate of creaming in the emulsion. This competitive adsorption at the interface leads to detrimental effects on emulsion stability (Wilde, Mackie, Husband, Gunning, & Morris, 2004). Oil-soluble emulsifiers displaced less protein than water-soluble emulsifiers in the lactoglobulin- and casein-stabilised emulsions (Cornec, Mackie, Wilde, & Clark, 1996). However, Munk, Larsen, van den Berg, Knudsen, and Andersen (2014) found that a high proportion of unsaturated mono- and diglycerides can be present at the fat globule interface without displacing casein, thus improving physical stability. Mono- and diglycerides could displace protein in the same emulsions when high shear is being applied, resulting in emulsion instability (Davies, Dickinson, & Bee, 2000).

Besides physical stability, the chemical stability of emulsion systems are important quality parameters for food products, particularly resistance to lipid oxidation and the stability of volatile compounds with regard to flavour. Milk proteins are known to have an antioxidant effect as they can chelate transition metal ions and scavenge free radicals (Donnelly, Decker, & McClements, 1998; Faraji, McClements, & Decker, 2004). Metal chelation by the unadsorbed casein in the aqueous phase has been shown to reduce lipid oxidation in emulsions (Berton, Ropers, Bertrand, Viau, & Genot, 2012), but a negative effect on lipid oxidation was observed when metal chelation occurred at the interface as a result of the proximity between pro-oxidants and oil droplets (Villiere, Viau, Bronnec, Moreau, & Genot, 2005). The adsorption of milk proteins at the oil-water interface form

a barrier separating oil droplets from pro-oxidants (Donnelly et al., 1998) that improve oxidative stability; however, this barrier may also interact with the volatile flavour compounds, altering the perceived flavour of coffee products (Kühn, Considine, & Singh, 2006).

The present literature explains the mechanism of protein displacement and destabilisation by emulsifiers that is a desired feature in whipped products. However, the interaction between mono- and diglycerides and milk proteins, and their impact on the physical and chemical properties of milk coffee beverages has not been well explored, thus leaving a gap in the current knowledge base. There is considerable research around the encapsulation of oils and bioactive compounds; however, there is very little research on the encapsulation of emulsifiers and their effect on the physicochemical properties and stability of emulsions. This thesis will address the feasibility of encapsulating mono- and diglycerides and assess the functionality of this emulsifier in different emulsion systems. The understanding developed in this thesis will address some of the issues faced by food manufacturers, especially regarding optimal emulsifier use to achieve maximum product stability and quality.

1.2 Research objectives

The main objectives of this study are twofold: (1) to gain fundamental understandings on the effect of mono- and diglycerides and milk protein compositions in protein-stabilised oil-in-water emulsion systems, and (2) to prepare and characterise an encapsulated emulsifier system, including its physicochemical properties and stability after reconstitution.

In order to meet the main objectives, this research has been conducted with the following five specific objectives and discussed in different chapters as outlined in **Figure 1.1**.

1) To investigate the effect of different mono- and diglyceride compositions on oil droplet size distribution, zeta potential, viscosity, pH and creaming stability of an emulsion (**Chapter 3**).

2) To investigate the effect of milk protein composition on the physicochemical properties, creaming stability and oxidative stability of an emulsion (**Chapter 4**).

3) To prepare and characterise a novel instantised emulsifier system based on glycerol monooleate (GMO) using spray-drying (**Chapter 5**).

4) To evaluate the physicochemical properties, creaming stability and oxidative stability of an emulsion as a function of the instantised emulsifier system (**Chapter 6**).

5) To investigate the effect of concentrations of bulk GMO and an instantised GMO emulsifier system on physicochemical properties, creaming stability, oxidative stability and flavour compounds in an application of a model milk coffee emulsion (**Chapter 7**).

This thesis consists of eight chapters, including introduction, literature review, five working chapters, and a general discussion, future outlook and conclusions as outlined in **Figure 1.1**. Each working chapter investigated one specific objective and each contain specific introduction, materials and methods, results and discussion, and conclusions. The general discussion, future outlook and conclusions are given in **Chapter 8** to address how this thesis meets the main objectives, the key findings and novelty of this study, limitations and future recommendations.

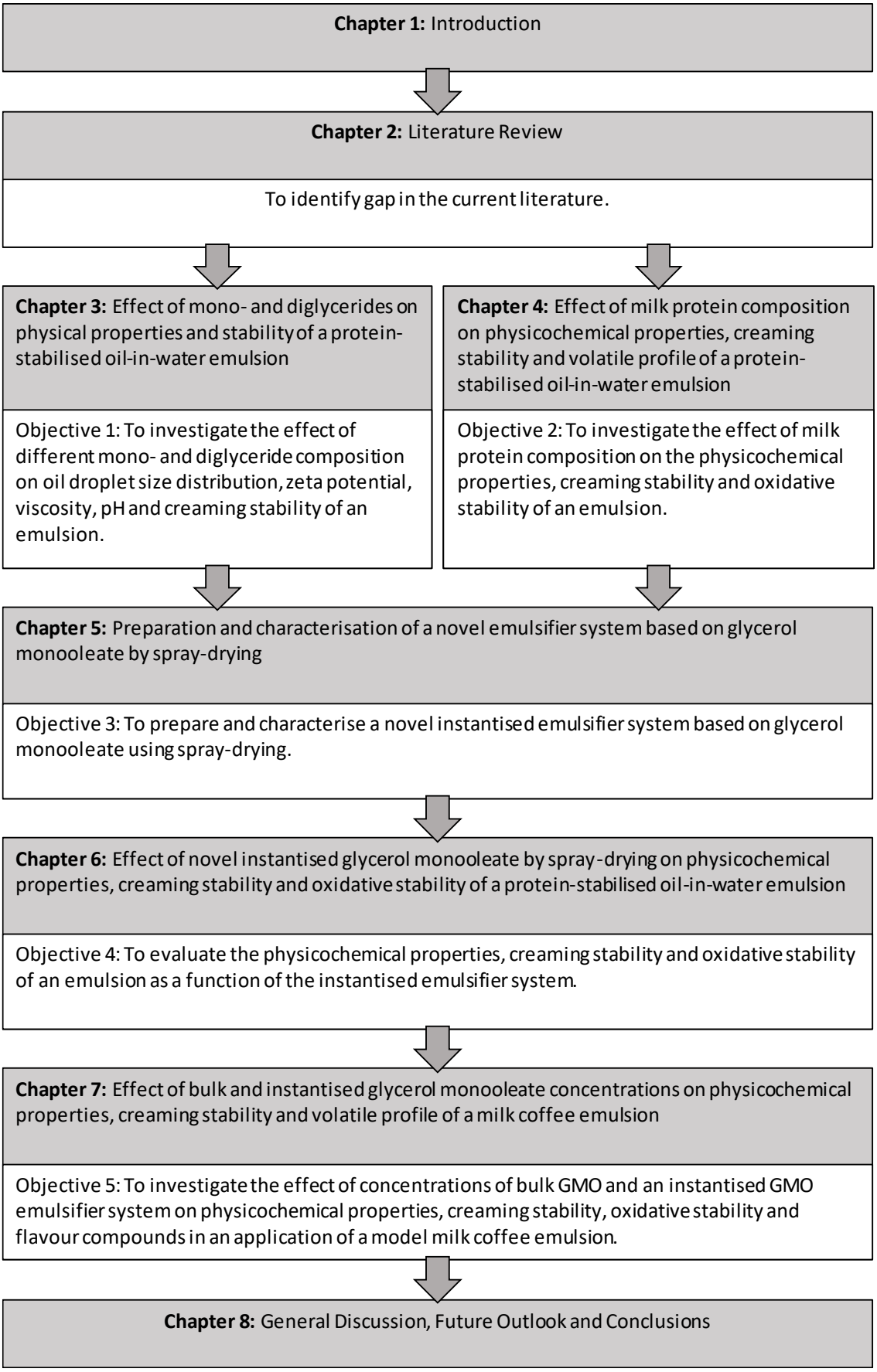


Figure 1.1 A schematic overview of the structure of the thesis.

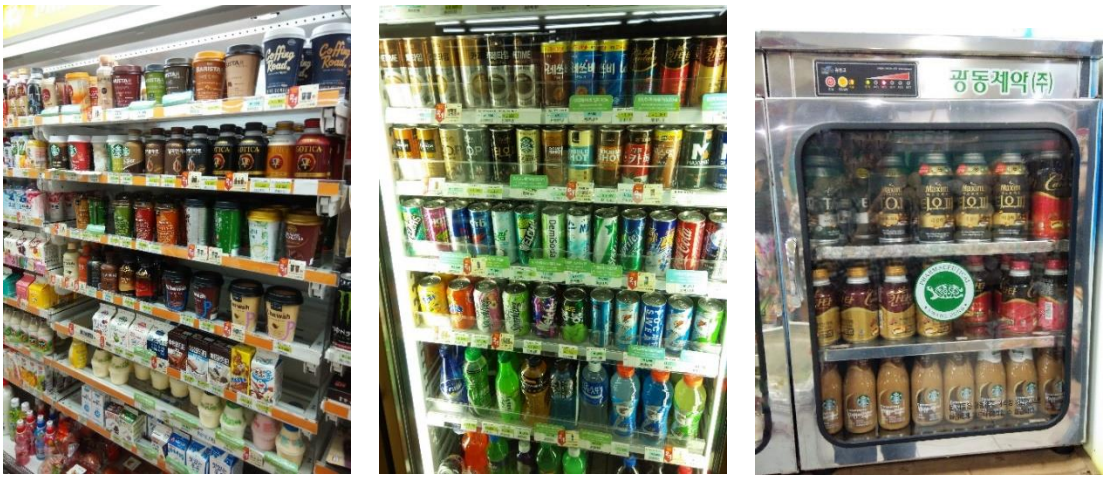
Chapter 2

Literature Review

Part of the contents presented in this chapter has been published previously as a book chapter: Loi, C. C., Eyres, G. T., & Birch, E. J. (2019). Protein-stabilised emulsions. In L. Melton, F. Shahidi, & P. Varelis (Eds.), *Encyclopedia of food chemistry* (pp. 404-409). Oxford: Academic Press. doi:10.1016/B978-0-08-100596-5.22490-6

2.1 Milk coffee

Milk coffee is becoming a popular beverage, especially among consumers in the Asian region, who have long been predominantly tea drinkers. The growth of coffee beverages differs from country to country and generally the increase in popularity is most evident for the Asia-Pacific region. The 2014-2019 historic growth data based on total volume of RTD coffee beverages from Euromonitor International (2019) revealed that the highest growth is in the Asia-Pacific region (26.2%), followed by Europe, Middle East and Africa (16.7%), Latin America (15.5%), and North America (6.9%). The growing number of people with more disposable income and exposure to western culture contributes to the increase in the number of coffee consumers. The rising demand for coffee in a convenient form led to the development of canned coffee in Japan in the 1970s (Oakland, 2011). This canned coffee quickly became an iconic beverage in the Japanese market. The ease and convenience of obtaining a canned coffee from vending machines and the development of vending machines that can provide self-heating and self-cooling coffee are behind the success of canned coffee in Japan (Anese & Nicoli, 2003). Canned coffee has spread to other Asian countries due to the increasing consumer demand for convenience beverages that suit their active lifestyles and fuel their busy lives. The preparation of coffee using a conventional brewing method or coffee machine is still the most important way to prepare a cup of coffee. A shift in the coffee consumption pattern has also been seen in the United States with the news of Coca-Cola teaming up with McDonald's and Dunkin Donuts to venture into the ready-to-drink market (Whitten, 2017). Therefore, ready-to-drink coffee beverages present an opportunity to meet new consumer demands for taste and flavour, size, shape and appearance with possibilities to incorporate nutrients and bioactive compounds (Guneser, Isleten Hosoglu, Aydeniz Guneser, & Karagul Yuceer, 2019). **Figure 2.1** shows the various distribution channels for ready-to-drink coffee.



(a) Convenience store (b) Cold vending machine (c) Hot vending machine

Figure 2.1 Examples of distribution channels for ready-to-drink coffee in South Korea.

Ready-to-drink milk coffee is composed of coffee extract and milk, with other minor components such as sugar, salt and stabilisers and typically has a pH range from 5 to 7 (Hayakawa, Shioya, Yamamoto, Ogura, & Kusaura, 2012). Coffee extract is a complicated mixture of thousands of volatile and non-volatile compounds that influence the flavour profile. Milk is important to deliver nutrients in the beverage and is comprised of protein, fat and phospholipid. Ready-to-drink coffee beverages can be categorised into flavoured milk or flavoured milk drinks owing to their nutrient compositions and the legal definition of a specific country (Guneser et al., 2019). The term "milk coffee beverages" used in this thesis is a generic term referring to any coffee drink with milk. The on-going discussion among the Codex committee to standardise this product category has been challenging owing to the varying product compositions from different geographical locations. This has allowed the food industry a lot of room for innovation, developing their own market segment based on consumer trends. The latest consumer trends are around the premium taste and flavour offered by cold brew coffee (Esposito, 2019).

While the formulation of milk coffee beverages evolves rapidly to meet consumer trends on taste and flavour, the beverage processing technologies are changing to cope with the new demand of container size, shape and appearance. A high-pressure homogeniser is typically used to ensure all the milk coffee ingredients mix well after reconstitution at

60-70 °C and improve stability of the emulsion droplets (Matsumiya, Takahashi, Inoue, & Matsumura, 2010). The beverages are then sterilised at 121 °C for 30-40 min using a retort steriliser in the case of metal cans or sterilised at 137 °C for 1 min using a ultra-high treated (UHT) pasteuriser in the case of plastic bottles (Ogawa & Cho, 2015). The heat sterilisation treatment applied to both canned and bottled milk coffee beverages eliminates pathogenic microorganisms and extends product shelf life. However, the severity and duration of the heat treatment may cause destabilisation of the emulsions (Liang, Patel, Matia-Merino, Ye, & Golding, 2013).

A milk coffee at quiescence will undergo physical separation. The most common phenomena are sedimentation of particles from the coffee extract and creaming of milk fat on top of the beverage. Another phenomenon is protein aggregation during storage due to the low pH of some coffee beverages (Ogawa & Cho, 2015; Scott, Duncan, Sumner, & Waterman, 2003). Although it is often easy to redisperse the separated layers by shaking, it is important to keep them at a minimum to prevent changes to taste and flavour. The separated layers have a different concentration of certain chemicals that could react during storage. One possible chemical reaction is lipid oxidation. The separated cream layer contains a higher level of lipid located near to the interface between liquid and air and makes them more susceptible to oxidation than the oil droplets far away from the contact with air (Berton-Carabin, Ropers, & Genot, 2014). Lipid oxidation forms volatile compounds that negatively affects taste and flavour of coffee beverages and decreases consumers' acceptance (Yesiltas, García-Moreno, Sørensen, Akoh, & Jacobsen, 2019). The application of heat treatment to the beverage also creates new chemical compounds through the Maillard reaction pathway, which may alter the flavour of the beverages. The microbiological aspect of the milk is not elaborated in detail here as it is not the area for investigation in this thesis.

2.2 Emulsions

An emulsion is a thermodynamically unstable mixture of two or more immiscible liquids. In an emulsion, one phase serves as the dispersing agent or continuous phase, whereas the second phase acts as the dispersed or inner phase. When the two immiscible phases, i.e. oil and water, are mechanically agitated, they form an emulsion. This emulsion is temporary and will separate into two layers when there is no further agitation. An emulsifier or surfactant is typically used to prepare emulsions with extended stability.

The biphasic nature of an emulsion means that it can disperse the droplets for a limited time only. The droplets tend to coalesce and form bigger droplets as time passes.

An emulsion can be categorised based on its dispersed phase and droplet size. Based on the classification of the dispersed phase, there are two types of simple emulsion, namely, oil-in-water (O/W) and water-in-oil (W/O) emulsions. An O/W emulsion consists of dispersed oil droplets in a continuous water phase while the W/O emulsion comprises of water droplets dispersed in the oil phase. Recent developments in emulsions have made it possible to have double emulsions such as W/O/W, where it has a W/O emulsion dispersed in a continuous water system. An O/W/O emulsion is the opposite of the former arrangement. **Figure 2.2** illustrates the simple and multiple emulsion systems.

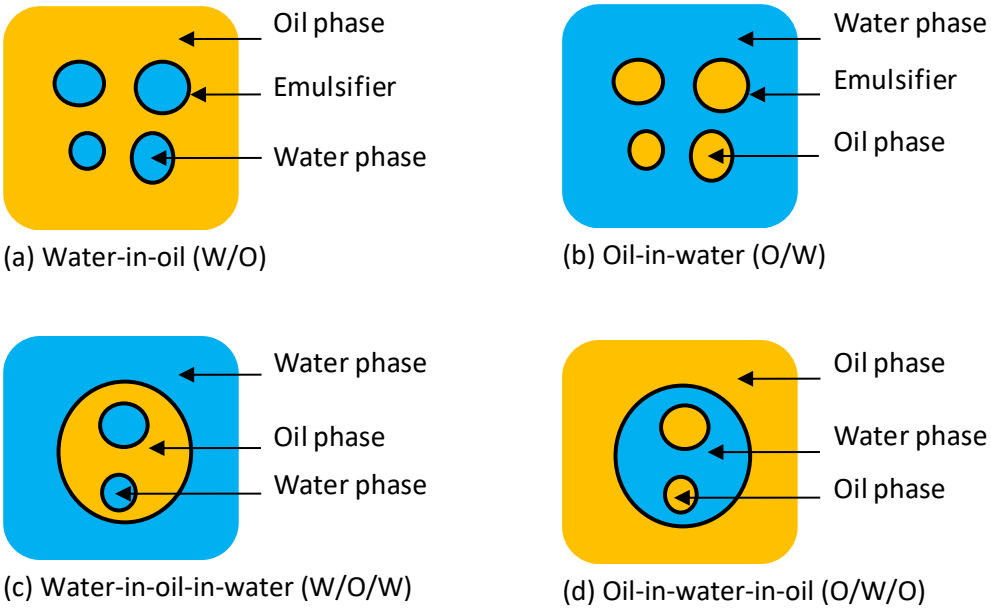
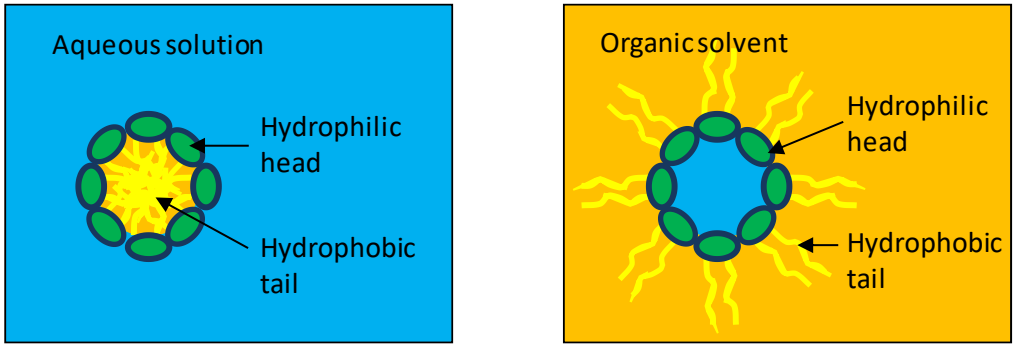


Figure 2.2 Phase arrangements for different types of emulsion.

When an emulsifier is added to the water that acts as a solvent, the hydrophilic head of the emulsifier will immerse in the water phase while the hydrophobic tails face inwards against each other, forming a micelle. A micelle has a hollow space in the centre and making it suitable to encapsulate a non-polar liquid such as oil. A micelle can form when an emulsifier is present in an oil continuous system, except that the hydrophobic tail will immerse in the oil while the hydrophilic heads will aggregate together making it suitable to hold a polar molecule such as water. **Figure 2.3**(a) and (b) show the micelle arrangement in aqueous and organic solvents. The micelle structure allows two

immiscible liquids to form an emulsion, and this becomes the basis of food processing such as meat, dairy and bakery products.



(a) Micelle of emulsifier in aqueous solution (b) Micelle of emulsifier in organic solvent

Figure 2.3 Formation of micelles and their configuration in (a) an aqueous solution and (b) an organic solvent.

The second classification is according to the droplet size of the emulsions. The three widely accepted categories are macroemulsions, microemulsions and nanoemulsions (Adjonu, Doran, Torley, & Agboola, 2014; McClements & Rao, 2011). **Table 2.1** shows the droplet size, shape, physical stability, appearance and energy required to form each emulsion.

Table 2.1 Comparison of macroemulsion, nanoemulsion and microemulsion (adapted from McClements and Rao (2011) and Adjonu et al. (2014)).

System	Macroemulsion	Nanoemulsion	Microemulsion
Average droplet diameter	200 nm - 100 μm	10 - 200 nm	4 - 200 nm
Shape	Spherical	Spherical	Spherical and bicontinuous structure
Emulsion stability	Thermodynamically unstable, kinetically stable	Thermodynamically unstable, kinetically stable	Thermodynamically stable
Appearance	Opaque	Transparent or translucent	Transparent or translucent
Formation	High and low energy methods	High and low energy methods	Low energy method

2.3 Protein-stabilised emulsions

Many proteins, in particular, milk proteins, are surface-active molecules that consist of both hydrophilic and hydrophobic functional groups (Dickinson, 1997; Wilde et al., 2004). Proteins have been used extensively as emulsifiers in foods (Rodríguez Niño, Sánchez, Fernández, & Rodríguez Patino, 2001; Sliwinski, Roubos, Zoet, van Boekel, & Wouters, 2003; Sünder et al., 2001) to form stable O/W emulsions such as in milk (including milk coffee beverages), cream, ice-cream, mayonnaise, salad dressing and gravies (McClements, 2015; Tornberg & Hermansson, 1977).

Milk proteins such as casein and whey proteins adsorb to the surface of oil droplets, reduce the surface tension between oil and water, and form a protective film surrounding the oil droplet to stabilise it (Walstra, 2002). **Figure 2.4** shows a schematic diagram of a protein-stabilised oil-in-water emulsion. The proteins at the interface provide repulsive forces such as steric and electrostatic forces, which stabilise the droplets for long-term stability (Bos & van Vliet, 2001; Tcholakova, Denkov, Sidzhakova, & Campbell, 2006; Wilde et al., 2004). The biphasic nature of an emulsion means that it can only keep droplets dispersed for a limited time, thus affecting the shelf life. Emulsions undergo physical destabilisation over time, according to several different mechanisms.

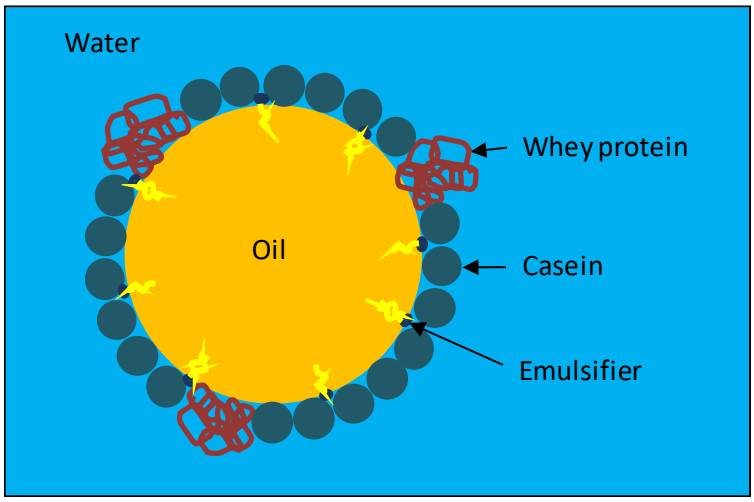


Figure 2.4 Schematic diagram of a protein-stabilised oil-in-water emulsion.

The destabilisation mechanisms of emulsions, extrinsic factors (homogenisation, thermal treatment) and intrinsic factors (pH, ionic strength, biopolymers, low-molecular-weight emulsifiers) affecting emulsion stability will be discussed later in **sections 2.4, 2.5** and

2.6, respectively. Chemical stability of the emulsions, i.e. lipid oxidation, including the failure mechanism and factors affecting the failure rate, will be discussed in **Section 2.7**.

2.4 Emulsion destabilisation mechanisms

Emulsion stability is the ability of the emulsion to remain unchanged in its physical properties over time (McClements, 2007). A protein-stabilised emulsion is a thermodynamically unstable mixture that can undergo destabilisation mechanisms such as flocculation, creaming, sedimentation and coalescence. **Figure 2.5** shows the common destabilisation mechanisms that occur in food emulsions. The destabilisation mechanisms are influenced by (1) the interfacial film around the droplets, (2) the particle size distribution of the droplets, and (3) viscosity, pH and ion concentration of the continuous phase.

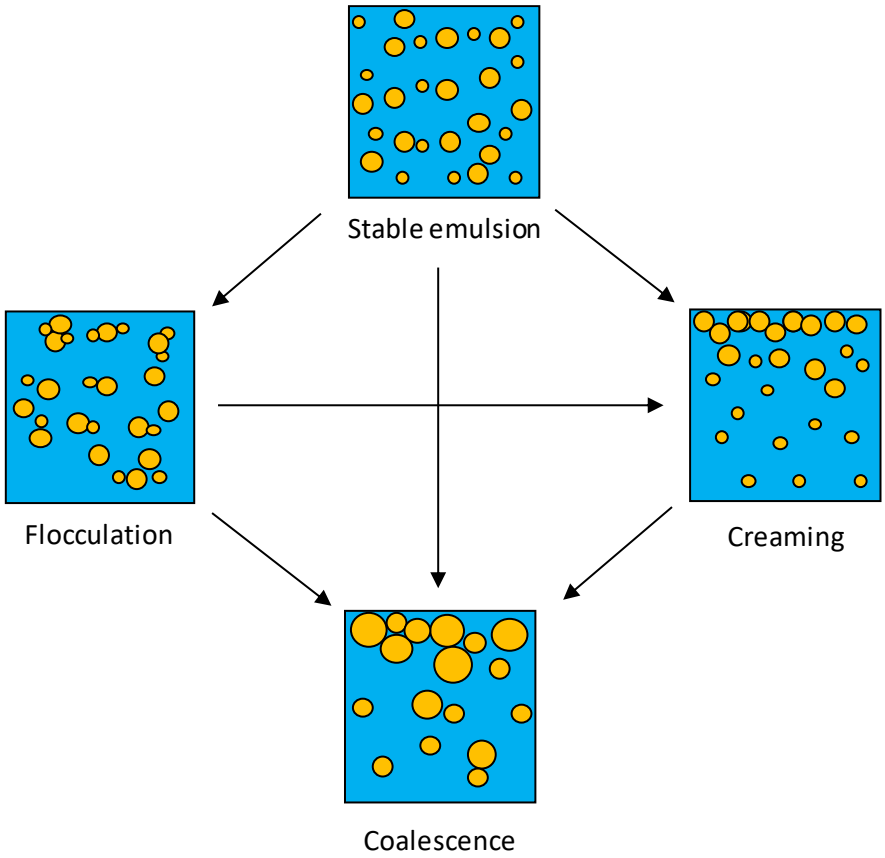


Figure 2.5 Physical changes to the oil droplets in a protein-stabilised emulsion (adapted from McClements (2007)).

2.4.1 Flocculation

Flocculation happens when neighbouring oil droplets come closer to each other and form colonies in the continuous phase (Dickinson, 2010). The aggregation is often not visible and may serve as a precursor to creaming and coalescence. Flocculation could also take place during or after creaming. It may be reversible or not depending on the strength of the intermolecular bond between droplets. However, flocculation may be a desired physical property in some food products such as whipping cream. The occurrence of flocculation could derive from the chemical nature of the emulsifier, phase volume ratio and concentration of electrolytes. The following steps can prevent flocculation from happening.

1) To make a uniform droplet size using an appropriate size reduction process.

2) To modify the charge surrounding the oil droplet so that it will exert repulsive forces with neighbouring droplets.

3) To increase the viscosity of the continuous phase and make the droplets relatively immobile.

Flocculation is common with protein-stabilised O/W emulsions. One classic example is the feathering of cream in hot coffee. The protein-stabilised oil droplets, when exposed to the hot and acidic pH in the coffee, will undergo conformation changes at the interface, thus causing the oil droplets to become more attracted to each other (Krog, 2011).

2.4.2 Creaming

Creaming is an upward movement of dispersed droplets in a continuous phase due to the density difference between the two phases. Creaming usually happens in an O/W emulsion due to the upward movement of the relatively lower density oil droplets in an aqueous phase (Dickinson, Golding, & Povey, 1997; McClements, 2015). Sometimes the movement of different globule sizes may result in a different colour shade of the emulsion layers. Creaming is a reversible process as the oil droplet does not merge with another droplet, and the micelle structure created by the emulsifier remains intact. In some instances, a little agitation such as shaking may revert the mixture to an evenly distributed emulsion. Creaming is different from flocculation as there are no attractive and repulsive forces among the oil droplets.

The creaming rate of an oil droplet in a dilute O/W emulsion system follows Stoke's law. It describes the movement of a small spherical droplet during the creaming process that freely moves without collision and has no physical or chemical attraction. According to Stoke's law, the creaming rate is given by **Equation 2.1**.

$$v = \frac{d^2(\rho_o - \rho_c)\, g}{18\, \eta} \qquad \textbf{Equation 2.1}$$

where v is the creaming rate, d is the diameter of droplet, ρ_o is the density of droplet, ρ_c is the density of continuous phase, g is the acceleration of gravity, and η is the viscosity of the continuous phase.

From the equation, the three main factors that affect creaming are droplet size, the viscosity of continuous phase and the difference in the densities between oil droplets and continuous phase. Theoretically, the creaming rate can be delayed by the following actions:

1) Reduce the particle size by homogenisation. For example, reducing the diameter of the oil droplet by half will increase creaming stability by a factor of four.

2) Increase the viscosity of the continuous phase by adding thickening agents such as xanthan gum or starch.

3) Reduce the difference in the densities between the dispersed phase and continuous phase.

2.4.3 Coalescence

Coalescence is a process where the oil droplets merge to form a larger droplet. In this irreversible process, the oil droplets come close to each other and cause breakage of the interfacial film in order to merge (Fredrick, Walstra, & Dewettinck, 2010). This will normally result in an increased droplet size and reduce the number of oil droplets. Eventually, coalescence will lead to *breaking* where all of the interfacial film is destroyed and a complete separation of oil and water phases occurs (Friberg, Larsson, & Sjöblom, 2004). A similar phenomenon is Ostwald ripening, where large droplets grow at the expense of the small droplets due to mass transport of dispersed phase through the continuous phase. However, it is considered to be negligible in O/W emulsions due to

the low solubility of triglycerides in the continuous phase (McClements, 2007). Some of the common factors for coalescence to occur include an insufficient amount of emulsifying agent, large droplets, and weak repulsion between droplets (McClements, 2007). One preventative action is to reinforce the mechanical strength of the interfacial film, which involves the selection of the right emulsifying agent and the right choice of the mixing process, such as high-shear dispersion and high-pressure homogenisation.

2.5 Extrinsic factors affecting emulsion stability

2.5.1 Homogenisation

Homogenisation is a process to mix two immiscible liquids so that they can be in the same phase. This process will turn one of the liquids into small droplets that disperse uniformly in the other liquid. In the milk processing industry, homogenisation is used to reduce oil droplets to about 1 μm in diameter and to lower the tendency of creaming and coalescence (Bylund, 2015). Small oil droplets are created when milk is forced through a narrow gap at high velocity. Homogenisation is usually carried out at elevated temperature because it is more effective to reduce droplet size where the fat is in a liquid state (Tobin, Heffernan, Mulvihill, Huppertz, & Kelly, 2015). Two events happen at that time, where the milk fat globule membrane is partially disrupted, and milk proteins adsorb at the interface to form a new oil droplet (Lopez, 2005). Homogenisation is also applied to emulsions before spray-drying to ensure uniform droplet size distribution and prepare it for encapsulation.

Protein-stabilised emulsions are usually formed using a two-step homogenisation process. A coarse emulsion is first created using a mechanical homogeniser, and then the emulsion is subjected to a high-pressure homogenisation to form fine emulsion droplets (Juttulapa, Piriyaprasarth, Takeuchi, & Sriamornsak, 2017). **Figure 2.6** shows a schematic diagram of mechanical and high-pressure homogenisation devices that can be used to produce food emulsions. A mechanical system, such as a colloid mill and high-shear homogeniser, can produce small oil droplets of several microns. High pressure instruments, such as a high-pressure valve homogeniser and microfluidiser, can reduce oil droplets to submicron sizes that improve emulsion stability. Atarés, Marshall, Akhtar, and Murray (2012) reported that homogenisation reduces the droplet size and size range distribution of O/W emulsions containing sunflower oil and whey proteins. High-pressure homogenisation has been shown to be more effective at reducing droplet

size compared to mechanical homogenisation (Perrier-Cornet, Marie, & Gervais, 2005). The type of homogenisation process will determine the emulsion droplet size and stability. A microfluidiser is a high-pressure homogeniser that is suitable to form the range of droplet sizes for the investigation of protein-stabilised emulsions mimicking a milk coffee beverage.

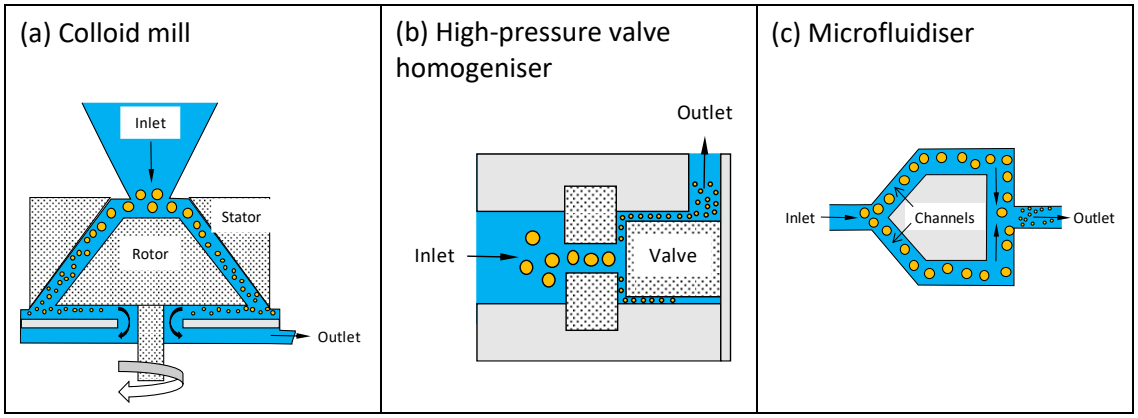


Figure 2.6 Schematic diagram of mechanical and high-pressure homogenisation devices that can be used to produce food emulsions (adapted from McClements and Rao (2011) and (Urban, Wagner, Schaffner, Röglin, & Ulrich, 2006)).

2.5.2 Heat treatment

Heat treatment has an important role in preserving the quality of food products by destroying any pathogenic microorganisms while retaining the nutritional benefits. Nicolas Appert in 1810 used heat treatment to preserve milk for the first time but was unsuccessful due to protein coagulation in milk during heating (O'Connell & Fox, 2016). In 1860, Louis Pasteur was successful in preserving wine and beer using a low-heat treatment, a process known as pasteurisation. Pasteurisation was found to destroy pathogenic microorganisms in milk without affecting its physical properties. Heat treatment has become a vital step in the dairy industry, but exposure to high temperatures can denature milk proteins and negatively affect emulsion stability during storage.

The types of milk proteins, namely casein and whey proteins, experience different effects during heat treatment. Casein is very stable at high temperature (Fox, Uniacke-Lowe, McSweeney, & O'Mahony, 2015a), where there is relatively little aggregation upon heating. Casein-stabilised emulsions do not aggregate at 120-130 °C (Guo, Fox, Flynn, & Mahammad, 1989), and it has been demonstrated that heated casein has better adsorption at the oil-water interface (Liang et al., 2017). In contrast, whey proteins undergo conformational changes such as unfolding, denaturation and aggregation when heated above 65 °C (Croguennec, O'Kennedy, & Mehra, 2004; Jang & Swaisgood, 1990). These conformational changes negatively affect the composition at the interface and resulted in aggregation of oil droplets (S. R. Euston, Finnigan, & Hirst, 2000), increased viscosity and increased the droplet size (Sliwinski et al., 2003). Whey protein-stabilised emulsions are reported to be highly sensitive to heat treatment (Liang et al., 2013).

2.6 Intrinsic factors affecting emulsion stability

2.6.1 pH

In a protein-stabilised emulsion, the proteins adsorb to the oil-water interface and form a thin protective membrane around the oil. The emulsions are stable against flocculation due to the strong electrostatic repulsion between the charged droplets (Dickinson, 1997; McClements, 2004; Tangsuphoom & Coupland, 2008). Protein-stabilised emulsions are sensitive to the pH of the continuous phase. For example, casein aggregates and flocculates as a non-soluble component near to its isoelectric point around pH 4.6.

Flocculation has been observed in emulsions near to their isoelectric point, where there is no net interface charge and thus the repulsion forces are minimised (Rampon et al., 2004; Tangsuphoom & Coupland, 2008). The change in pH also leads to the folding and unfolding of proteins, which occurs via an intermediate stage called the molten globule (Matsumura, Mitsui, Dickinson, & Mori, 1994). The molten globule state is the intermediate stage between the native and the completely unfolded state, and is characterised by the retained secondary structure and unfolded tertiary structure (Nylander, Arnebrant, Bos, & Wilde, 2008). Some proteins at the molten globule state adsorb at a greater degree to the interface than the native state. For example, Nylander et al. (2008) found that the adsorption of α-lactalbumin to the interface increased with the lowering of pH, where the protein is in the molten globule state.

2.6.2 Ionic strength

The presence of ions such as Na^{+}, Fe^{2+} and Ca^{2+} in protein-stabilised emulsions can interact with the charged droplets and affect emulsion stability. These ions can bind to the oppositely charged droplets and subsequently reduce the electrostatic repulsion between charged droplets. Multivalent ions have higher binding potential to the charged oil droplets than monovalent ones and ions of the same valence with bigger size have higher selectivity than the smaller ones (Bylund, 2015). The ionic strength of the emulsion has a significant effect on flocculation (Das & Kinsella, 1990; Dickinson, 1997; Tangsuphoom & Coupland, 2008; Tcholakova et al., 2006). Flocculation becomes rapid when ionic strength exceeds a certain limit.

2.6.3 Biopolymers

Protein-stabilised emulsions contain one or more biopolymers (McClements, 2004). Biopolymers are naturally occurring polymers found in living organisms, such as proteins and polysaccharides (including gums). Both proteins and polysaccharides can be used together in food formulations. Polysaccharides interact with protein-stabilised emulsions to modify the flow behaviours of the aqueous phase such as thickening, gelling and emulsifying capability (McClements, 2004; Ye, 2008). The thickening and gelling effect from polysaccharides increase emulsion stability through the formation of a three-dimensional network to trap and retard droplet movement, preventing droplet coalescence and creaming (McClements & Rao, 2011). For example, Ye and Singh (2006) reported that gum at high concentration increased viscosity, reduced collision

between the oil droplets and increased emulsion stability against coalescence and creaming. Polysaccharides may interact with the adsorbed protein to behave differently at the same emulsion conditions or during manufacturing processes (Dickinson, 2003). For example, protein-polysaccharide interactions have been shown to reduce droplet aggregation in emulsions with high salt concentrations (Harnsilawat, Pongsawatmanit, & McClements, 2006) and also modify interfacial rheology by forming a bulkier polymeric layer around emulsion droplets (Akhtar & Dickinson, 2007). Milk protein is the principle biopolymer in protein-stabilised emulsions and will be further elaborated in **Section 2.7**. Polysaccharides will not be discussed in detail as this is not the focus of this thesis.

2.6.4 Low-molecular-weight emulsifiers

Low-molecular-weight emulsifiers interact with proteins at the oil-water interface and can alter emulsion stability. The term low-molecular-weight emulsifiers and emulsifiers will be used interchangeably throughout this thesis, and they are different from proteins which are biopolymers exhibiting emulsifying properties. Emulsifiers can be categorised according to their ability to dissolve in water or oil. Examples of common oil-soluble emulsifiers are lecithin and mono- and diglycerides, while polysorbates are water-soluble emulsifiers. Alternatively, emulsifiers can be grouped according to their head group, either non-ionic (no charge) or ionic (anionic or cationic). For examples, mono- and diglycerides have no charge, while sodium stearoyl lactylate has a charged head. Various types of emulsifiers for food applications will be discussed in **Section 2.9**.

Emulsifiers can quickly adsorb to the oil-water interface and lower the interfacial tension to a greater extent than proteins or vice versa depending on the concentrations of the emulsifier (Bos & van Vliet, 2001). It is well known that emulsifiers compete with proteins at the interface and can displace protein from the interface, which may cause emulsion destabilisation (Dickinson, 1997). The displaced proteins in the water phase could interact with each other during food processes, and many studies have looked at the protein-protein interactions with respect to the effect of heating and foaming (Howell, 1992). The study around the effect of temperature (25 °C vs 50 °C) on emulsion surface chemistry by Richards, Golding, Wijesundera, and Lundin (2011) showed that emulsifiers crystallise at lower temperature to partially or fully displace protein from the interface while the emulsifiers remain in a molten state at higher temperature, thus

allowing readsorption of protein to the interface. One aspect of protein displacement is the ability of ionic emulsifiers to bind with protein, which alters the heat stability of proteins and adsorption strength to the interface (Nylander et al., 2008). Anionic emulsifiers, such as sodium dodecyl sulfate, improve the thermal stability of emulsions by enhancing the electrostatic repulsion between droplets and increasing the denaturation temperature of the adsorbed protein (Kelley & McClements, 2003). Anionic emulsifiers can act synergistically with protein to improve emulsion stability, particularly creaming (Dickinson & Ritzoulis, 2000).

Another factor affecting protein displacement is the solubility of emulsifiers in water or oil and their concentrations in the emulsion (Nylander et al., 2008). Water-soluble polysorbates displace protein to a greater extent than oil-soluble mono- and diglycerides (S. E. Euston, Singh, Munro, & Dalgleish, 1995). Oil-soluble emulsifiers such as mono- and diglycerides and sorbitan esters at low concentration do not displace protein (Dickinson & Hong, 1994), thus can enhance the robustness of emulsion droplets against destabilisation. Lecithins and monoglycerides may displace milk protein from the interface or work synergistically to enhance emulsion stability of the protein-stabilised emulsion (O'Regan & Mulvihill, 2010). The formation of smaller size emulsion droplets due to a thinner interface layer could form a highly stable emulsion towards creaming and sedimentation (Kentish et al., 2008; McSweeney, 2008). The knowledge of protein displacement is important to food technologists in order to select the right types of emulsifiers when formulating emulsions.

2.7 Lipid oxidation

Oxidation of lipids such as triglycerides, diglycerides, monoglycerides and free fatty acids is a reaction occurring in the presence of oxygen during processing and storage. Lipid oxidation leads to detrimental effects on the flavour of food products, destroys essential fatty acids and produces compounds potentially toxic to the human body (Berton-Carabin et al., 2014). Lipid oxidation mainly targets unsaturated fatty acids such as oleic (n-9), linoleic (n-6) and linolenic (n-3) acids, and forms compounds associated with off-flavours in foods and beverages. **Table 2.2** shows the typical fatty acid compositions of milk fat, hydrogenated coconut oil and canola oil while **Table 2.3** shows the secondary lipid oxidation products of unsaturated fatty acids, comprising of aldehydes, alcohols, ketones, furans and short-chain hydrocarbons. Lipid oxidation can

negatively affect food acceptability in term of sensory perception, nutritional quality and toxicity in food products (Choe & Min, 2006). The processing and storage of food products can lead to lipid degradation following either one or both oxidation mechanisms, namely autoxidation and photosensitised oxidation.

Table 2.2 Typical fatty acid composition of milk fat, hydrogenated coconut oil and canola oil (adapted from Gunstone (2008)).

Fatty acid	Anhydrous milk fat	Hydrogenated coconut oil	Canola oil
C4:0 Butyric acid	3		
C6:0 Caproic acid	2		
C8:0 Caprylic acid	1	8	
C10:0 Capric acid	3	7	
C12:0 Lauric acid	4	48	
C14:0 Myristic acid	12	16	
C16:0 Palmitic acid	26	9	3
C16:1 Palmitoleic acid	3		
C18:0 Stearic acid	11	11	3
C18:1 Oleic acid	28		78
C18:2 Linoleic acid	2		10
C18:3 Linolenic acid			3
Others	5	1	3

Table 2.3 Secondary lipid oxidation products of n-9, n-6 and n-3 fatty acids (adapted from Genot, Meynier, and Riaublanc (2003)).

Class	n-9 oleic acid	n-6 linoleic acid	n-3 linolenic acid
Aldehydes	Heptanal Octanal Nonanal Decanal 2-Decenal 2-Unedecenal	Pentanal Hexanal *trans*-2-Heptenal *trans*-2-Octenal *trans*,*trans*-2,4-Decadienal	Propanal 2-Propenal *trans*-2-Butenal *trans*-2-Pentenal *cis*-2-Pentenal *cis*-3-Hexenal *trans*-2-Hexenal 2,4-Hexadienal 2,4-Heptadienal *trans*,*cis*-2,6-Nonadienal 2,5,7-Decatrienal
Alcohols	Heptanol Octanol	1-Octen-3-ol	1-Penten-3-ol *trans*-2-Penten-1-ol *cis*-2-Penten-1-ol 1,5-Octadien-3-ol
Ketones		1-Octen-3-one	1-Penten-3-one 1,5-Octadien-3-one
Furans		2-Pentyl furan	2-Ethyl furan
Hydrocarbons		Pentane	

2.7.1 Autoxidation

The lipid breakdown mechanisms by autoxidation have been studied extensively (Kamal-Eldin, Mäkinen, & Lampi, 2003). Autoxidation is the result of triplet oxygen reacting with lipids following the free radical chain reaction, which involves initiation, propagation and termination steps. **Figure 2.7** shows the breakdown mechanisms of autoxidation.

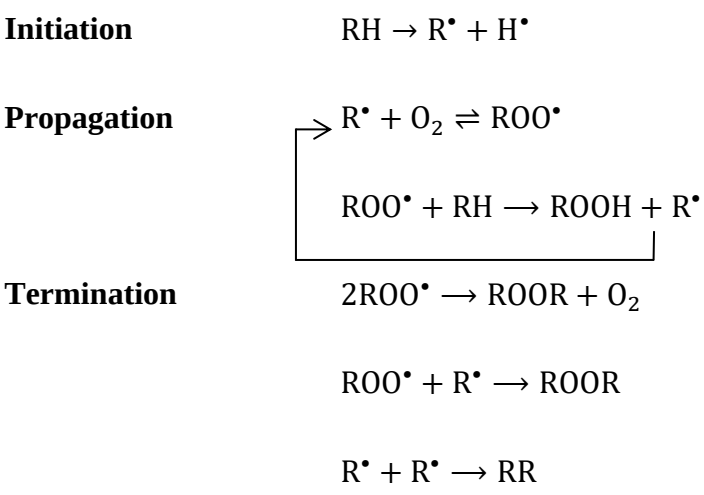


Figure 2.7 Schematic diagram of breakdown mechanisms of lipid by autoxidation (adapted from Kamal-Eldin et al. (2003)). R: lipid alkyl, R•: lipid alkyl radical, ROO•: lipid peroxy radical, ROOH: hydroperoxide

Lipid oxidation occurs in the presence of a favourable environment (heat, metal catalyst, UV and visible light), where a hydrogen atom in the conjugated fatty acids is removed to produce lipid alkyl radicals (R•). The lipid alkyl radicals will react with triplet oxygen to form lipid peroxy radicals (ROO•). Then lipid peroxy radicals take a hydrogen atom from the conjugated fatty acids to form hydroperoxides. All the free radicals continue to catalyse the oxidation process and initiate the free radical chain reaction (cyclic reaction). Lipid hydroperoxides are not stable at high temperature or in the presence of metal, and they are readily broken down to form aldehydes, ketones, acids, alcohols, esters and short-chain hydrocarbons, generating flavour defects.

2.7.2 Photosensitised oxidation

Photosensitised oxidation occurs in the presence of light and sensitisers, such as riboflavin in dairy products, chlorophyll in plant materials and myoglobin derivatives in meat products (Beauchamp, Zardin, Silcock, & Bremer, 2014; Min & Lee, 1999). The lipid breakdown mechanisms behind the photosensitised oxidation follow the singlet oxygen oxidation pathway, where singlet oxygen participates in the initiation step (E. C. Lee & Min, 1988; Rawls & Van Santen, 1970). Photosensitised oxidation produces nonconjugated hydroperoxides that are not formed by autoxidation (Choe & Min, 2006). The differences between the two types of lipid oxidation is also shown in the secondary oxidation products that are generated. For example, photosensitised oxidation of oleic acid produces more *cis*-2-decenal and octane than autoxidation (Frankel, 1985) while the autoxidised oleic acid had a higher content of octanal and 10-oxodecanoate.

2.7.3 Factors affecting lipid oxidation in protein-stabilised oil-in-water emulsions

Lipid oxidation in bulk oil is influenced by the fatty acid composition of the oil, oil processing, energy from heat or light, oxygen concentration, free radicals, oxidised compounds, free fatty acids, mono- and diglycerides, transition metals, pro-oxidants and antioxidants (Choe & Min, 2006). The lipids in the protein-stabilised oil-in-water emulsions are not directly in contact with air and consist of oil droplets encapsulated in an interface dispersed in the aqueous phase. Thus, the lipids of an emulsion are affected by the liquid partitioning and interfacial properties, in addition to the bulk oil.

Lipids with a higher level of unsaturated fatty acids are more susceptible to oxidation (Parker, Adams, Zhou, Harris, & Yu, 2003). The increase in unsaturation will increase the rate of lipid oxidation and form more volatile compounds at the end of the storage. The types of fatty acids also have an impact on lipid oxidation. For example, lipids containing polyunsaturated fatty acids have more double bonds and a higher oxidation rate compared to lipids with monounsaturated fatty acids (Choe & Min, 2006).

In the autoxidation, decomposition of hydroperoxides to secondary compounds increase as the temperature increases (Choe & Min, 2006). The availability of light is more important than heat for photosensitised oxidation, where singlet oxygen is formed and serves as an initiator for the reaction.

Oxygen is an important factor determining the rate of oxidation. All the three precursors, lipid, oxygen and catalyst, must be present and able to interact at the contact point for lipid oxidation to occur. The headspace of packaged oil usually has a low concentration of oxygen that is available to interact with the lipid. Lipid oxidation is usually higher in liquid oil than solid fat due to the higher unsaturation in fatty acids (Choe & Min, 2006). Oxygen is more soluble in oil than water, thus oxidation rate increases when oil droplets have a large ratio of surface to volume (Genot et al., 2003).

Pro-oxidants are compounds that increase the rate of oxidation in lipid, while antioxidants are compounds that can donate hydrogen to a free radical and convert them into more stable non-radical products. Pro-oxidants such as free fatty acids (Min & Lee, 1999) and transition metals such as iron and copper (Choe & Min, 2006) reduce the activation energy of the initiation step in the autoxidation, and hence increase the rate of lipid oxidation. Antioxidants can occur naturally in oil or can be added intentionally to improve oxidative stability. Examples of natural antioxidants are tocopherol, carotenoids, phenolics and sterols. There are compounds such as phospholipids, monoglycerides and diglycerides that can act as either pro-oxidants or antioxidants, depending on their concentration and the presence of metal ions (Choe & Min, 2006; Gomes, Caponio, Bruno, Summo, & Paradiso, 2010; Min & Lee, 1999; Mistry & Min, 1988). Riboflavin or vitamin B_2 is naturally present in milk and can act as both a pro-oxidant and antioxidant. Riboflavin acts as a strong sensitiser in the presence of light to produce singlet oxygen (Min & Lee, 1999) and form volatile lipid oxidation compounds associated with *sunlight* and *metallic* flavours in bovine milk (Beauchamp et al., 2014; Zardin, Silcock, Siefarth, Bremer, & Beauchamp, 2016). Y.-H. Lee, Lee, Min, and Pascall (2014) demonstrated that riboflavin acts as an antioxidant to protect the vegetable oil in salad dressing when stored under light at 25 °C for 5 days by donating hydrogen to free radicals (Min & Lee, 1999).

Mei, Decker, and McClements (1998) showed that Fe^{3+} and Fe^{2+} are strongly bound to sodium dodecyl sulphate (anionic) but not with dodecyltrimethylammonium bromide (cationic) and polyoxyethylene 10 lauryl ether (nonionic) stabilised emulsions. The strong association of pro-oxidants (iron) with anionic stabilised emulsion droplets is due to opposite charges of the pro-oxidants and oil droplets and consequently lead to a higher rate of oxidation (Berton-Carabin et al., 2014; McClements & Decker, 2000). If the

emulsion has the same charge as the pro-oxidants, a repulsive electrostatic interaction will occur and push pro-oxidants away from the oil droplets. Therefore, a low rate of oxidation is expected for this emulsion. Metal chelators such as phosphoric acid, citric acid, ascorbic acid and EDTA (ethylenediaminetetraacetic acid) can also decrease lipid oxidation by converting iron or copper ions into insoluble complexes or preventing the formation of complexes between the metals and lipid hydroperoxides (Choe & Min, 2006).

Where the emulsion droplets and antioxidants having different charges, they are attracted to one another, and the proximity between the two increases the effectiveness of antioxidants reacting with the oxygen. If they are far away, the efficacy will drop due to a higher chance of oxygen reaching the lipid core instead of reaction with the antioxidants (Mei, McClements, & Decker, 1999). The pH is associated with the charged and ionic environment of the emulsion and can affect the location of ionic antioxidants (Mei et al., 1999). In protein-stabilised emulsions, the unadsorbed casein or whey proteins will act as an antioxidant by chelating metals or scavenging free radicals (Berton-Carabin et al., 2014). When the pH becomes the same as the isoelectric point of casein, casein will have zero net charge and lose its antioxidant properties (Jacobsen, 2016). However, it is a challenging decision to decide which food components will interact with the pro-oxidants or antioxidants that may be inherent to the food ingredients. The complexity often results in different results between a simple model system and complex food matrices (Harper, 2008).

2.8 Milk protein

Milk is the first form of food that supplies a balanced diet to a mammalian newborn. Due to the physiology uniqueness of each species, the milk produced by the female mammal has a different composition to serve the nutritional requirement of its newborn. Among the 4000 species of mammal, bovine milk is one of the most extensively studied. Bovine milk contains principally water, approximately 3-4% fat, 3-4% protein, and 4-5% lactose (Fox, Uniacke-Lowe, McSweeney, & O'Mahony, 2015b; Pereira, 2014).

Milk protein has many unique properties that affect the properties of milk, and due to this importance, it has been studied and characterised in depth. Protein composition is influenced by the lactation stage, species, breed, feeding regime and health status of the

lactating animal (Fox et al., 2015b). Milk protein consists of two major protein groups, casein and whey protein. The important development work and findings on milk protein since the 19th century have been summarised by McMeekin (1970) and O'Mahony and Fox (2014). The word *casein* was first introduced by H. Braconnot in 1830 in a published paper (McMeekin, 1970). Olav Hammarsten improved the acid (isoelectric) precipitation of casein method in 1883-1885 and suggested that the isoelectric casein (also known as Hammersten casein) was homogeneous. The isoelectric point of casein is at pH 4.6, where casein carries no net charge and becomes insoluble in solution, resulting in casein precipitation. In 1918, T. B. Osborne and A. J. Wakeman using differential solubility in ethanol-hydrochloric acid solutions to show that isoelectric casein is heterogeneous. Confirmation on the heterogeneity of casein was obtained by K. O. Pederson in 1936 using analytical ultracentrifugation and by O. Mellander in 1939 using the free-boundary electrophoresis method (McMeekin, 1970).

The liquid remaining after isoelectric precipitation of casein is known as whey. Whey is a dilute solution of serum proteins, lactose, organic and inorganic salts, vitamins and other minor components. In 1885 J. Sebelein fractionated whey proteins into soluble albumin and insoluble globulin via a salting-out method with magnesium sulphate. According to McMeekin (1970), in 1899 Wichmann crystallised a protein from the albumin fraction with the addition of ammonium sulphate and acidification. Researchers in the late 19th century found that whey proteins are similar to the corresponding protein fractions of blood.

Milk protein is widely used in the food industry to provide many functional applications. **Table 2.4** shows the applications and functional roles of milk protein in a variety of food products. Milk proteins have little flavour on their own but interact with flavour compounds to alter the perceived flavour of food as they bind or release flavour compounds in food matrices (Dury-Brun, Chalier, Desobry, & Voilley, 2007; Guichard & Langourieux, 2000; Kühn et al., 2006; O'Neill, 1996). Increased protein concentrations modify the retention of volatiles depending on the relative affinity of the flavour compounds to the protein matrices (Andriot, Harrison, Fournier, & Guichard, 2000). β-Lactoglobulin is the most studied milk protein in terms of its interaction with flavour compounds such as aldehydes, ketones and esters (Guichard, 2002; van Willige, Linssen, & Voragen, 2000). Guichard and Langourieux (2000) reported that β-lactoglobulin

decreases the volatility of hydrophobic flavour compounds, which leads to a lower perception of flavour in food.

Table 2.4 Application of milk proteins (caseins and whey proteins) in food products (adapted from Fox et al. (2015a); Mulvihill (1992)).

Product Category	Where use	Function
Bakery	Bread, biscuits/cookies, breakfast cereals, cakes, pastries, muffins, croissants	Nutritional, sensory, emulsifier, dough consistency, egg replacer, texture, volume/yield
Dairy	Imitation cheese (vegetable oil, caseins, salts and water)	Fat and water binding, texture enhancing, melting properties, stringiness and shredding properties
	Coffee creamers (vegetable fat, carbohydrate, caseins, stabilisers and emulsifiers)	Emulsifier, whitener, gives body and texture, promote resistance to feathering, sensory properties
	Yoghurt, Quarg, ricotta cheese	Yield, nutritional, increased gel firmness, reduced syneresis
	Milk beverages, imitation milk, liquid milk fortification, milk shakes	Nutritional, emulsifier, foaming properties
	High-fat powder, shortening, whipped toppings and butter-like spread	Emulsifier, texture enhancing, sensory properties
	Cream cheese, cream cheese spread, sliceable/squeezable cheese, cheese filling and dip	Emulsifier, gelling, sensory properties
Beverages	Drinking chocolate, fizzy drink, fruit beverage	Stabiliser, whipping and foaming properties
	Cream liqueur, wine aperitif	Emulsifier
	Wine and beer industry	Fines removal, clarification, reduce colour and astringency
	Soft drink, fruit juice, powdered or frozen orange beverage	Nutritional
	Milk based flavoured beverage	Viscosity, colloidal stability

Product Category	Where use	Function
Dessert products	Ice-cream, frozen dessert, frozen juice bar	Whipping properties, skim-milk solid replacement, emulsifier, body/texture
	Mousses, instant puddings, whipped topping	Whipping properties, film former, emulsifier, impart body and flavour
Confectionery	Toffee, caramel, fudge	Confer firm, chewy, resilient texture, water binding, emulsifier
	Marshmallow, nougat	Foaming, high temperature stability, improve flavour and brown colour
	Aerated candy mixes, meringues, sponge cake	Whipping properties, emulsifier
Pasta product	Macaroni, pasta and imitation pasta	Nutritional, texture, freeze-thaw stability, microwaveable
Meat products	Comminuted meat products	Emulsifier, water binding, improve consistency, release meat protein for gel formation and water binding
	Frankfurter, luncheon meat	Pre-emulsion, gelation
	Injection brine for fortification of whole meat products	Gelation, yield
Convenience food	Gravy mix, soup mix, sauce, canned cream soup and sauce, dehydrated cream soup and sauce, salad dressing, microwaveable food, low lipid convenience food	Whitener, dairy flavour, flavour enhancer, emulsifier, stabiliser, viscosity controller, freeze-thaw stability, egg yolk replacer, lipid replacer, water biding ability, acid solubility
Textured product	Puffed snack food, protein enriched snack. Meat extender	Structure, texture, nutritional
Infant food	Pre-term formula, term formula, follow-on formula	Nutritional
Special dietary preparation	Slimming food, special diet for ill and convalescent patient, special food for patient with pancreatic disorder of anaemia	Nutritional
Clinical food	Intraveneous feed	Nutritional

2.8.1 Casein

Casein is a common protein found in milk, comprising approximately 80% of the protein in cow's milk. Casein content in cow's milk ranges from 24-29 g/L with high phosphorus content (0.7-0.9%) (Southward, 1998). The caseins are small and relatively hydrophobic molecules that have a molecular weight of 20-25 kDa. The caseins contain very little sulphur type amino acids (O'Mahony & Fox, 2014) and are rich in non-polar amino acids (35-45%) such as valine, leucine, isoleucine, phenylalanine, tyrosine and proline (Southward, 1998). The caseins contain all the essential amino acids for the human diet except cysteine. The caseins have a heterogeneous configuration due to the arrangement of amino acids in the protein molecule. There are four major structural configurations in the caseins known as α_{s1}-, α_{s2}-, β-, and κ-casein (Hazlett, Schmidmeier, & O'Mahony, 2018). The caseins contribute to the white opaque appearance of milk (along with milk fat globules) and forms quaternary colloid particles called micelles when combined with calcium and phosphorus. The caseins were used in technical and non-food applications until the 1960s before it was used in food to enhance food properties such as foaming, emulsification, water binding, texture and nutrition (Southward, 1998).

According to Fox and Brodkorb (2008), the caseins can be purified through several mechanisms including isoelectric precipitation, ultracentrifugation, salting out, ultrafiltration, microfiltration, gel filtration, alcohol-induced precipitation, cryoprecipitation, rennet-induced proteolysis and the addition of alkalis (pH 6.7). The caseins can be prepared from whole or skim milk, and the latter is the preferred starting material in the dairy industry. Skim milk produced from the centrifugal separation of whole milk to remove the fat fraction will avoid blockage of fat during isoelectric precipitation and further interference from the fat during protein characterisation (Fox et al., 2015a). Isoelectric precipitation is used to precipitate casein from the non-casein fraction by acidifying the skim milk to its isoelectric point at pH 4.6 at 20 °C to produce acid casein, of which the casein curd is separated from the whey via centrifugation, and subsequently washed and dried.

Besides isoelectric precipitation, the caseins can be separated by adding rennet enzyme to skim milk to produce rennet casein. Since the skim milk does not require acidification, the pH remains at 6.6 for the whole process. Normally calf rennet or microbial rennet will be added to the skim milk at around 29 °C. The enzyme will cleave one of the bonds

in κ-casein to release part of the protein referred to as macropeptides (Fox et al., 2015a). This action will destabilise the casein micelle causing it to coagulate with the calcium ions present in the milk. The coagulated casein is separated from the whey, then washed and dried. Rennet casein has very different properties compared to acid casein, i.e. rennet casein remains insoluble in water at nearly neutral pH and insoluble in alkali. Rennet casein will become soluble after treatment with calcium-binding salts such as citrate and phosphate, making it very suitable for cheese analogue applications (Fox et al., 2015a). **Figure 2.8** shows the industrial processes for the manufacture of acid and rennet casein.

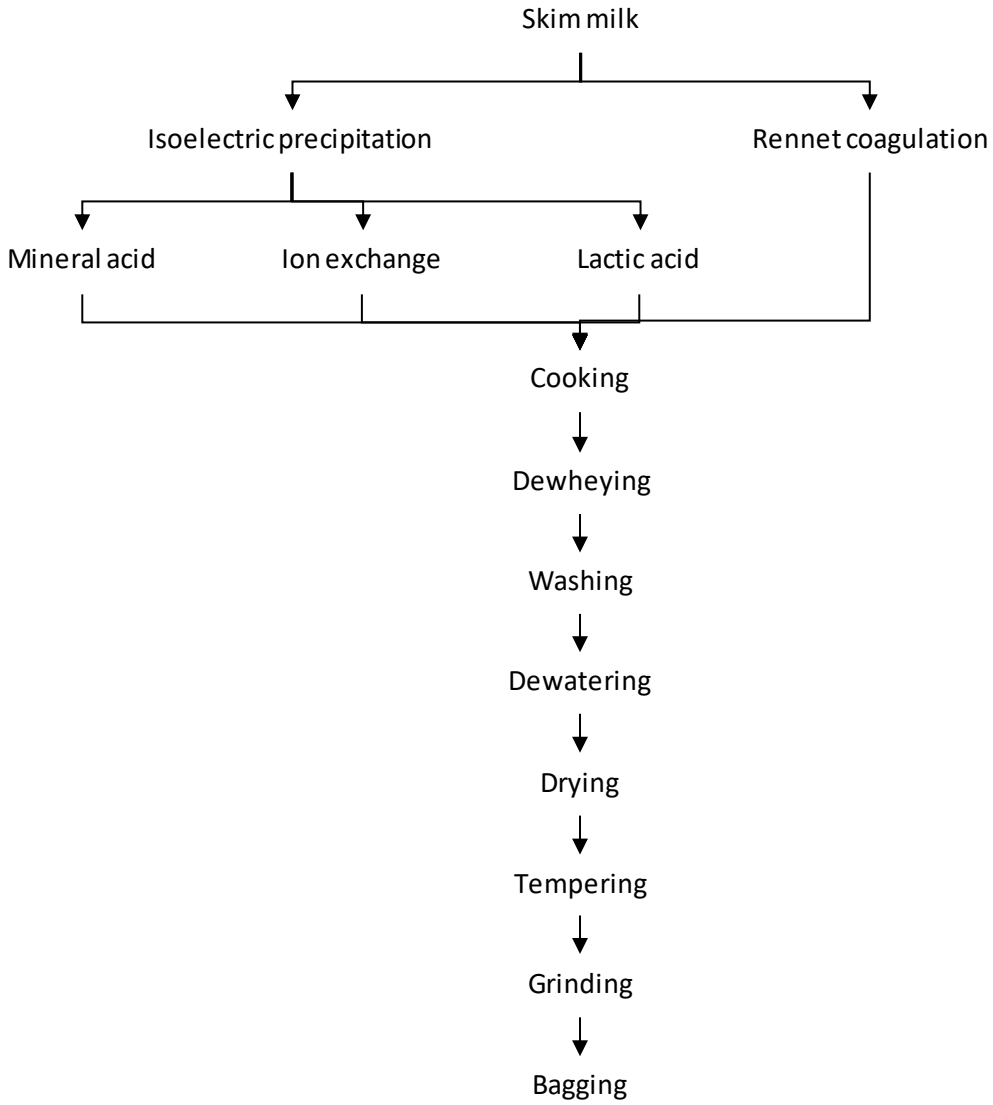


Figure 2.8 Flow chart of industrial process for the manufacture of acid and rennet casein (adapted from Fox et al. (2015a).

Pure acid casein is insoluble in water, but it can further react with alkalis to around pH 6.6 to produce water-soluble caseinate. **Figure 2.9** shows a simplified equation for the neutralisation of acid casein using sodium hydroxide. Other alkalis such as potassium hydroxide, ammonium hydroxide and calcium hydroxide can also be used to form the corresponding caseinates (O'Mahony & Fox, 2014). Sodium caseinate is the most common type of caseinate available as an ingredient and is typically converted into a powder form by spray-drying. Sodium caseinate has superior emulsification and water binding properties compared to other caseinate forms, which make it an important ingredient for coffee whitener, whipped toppings and meat products.

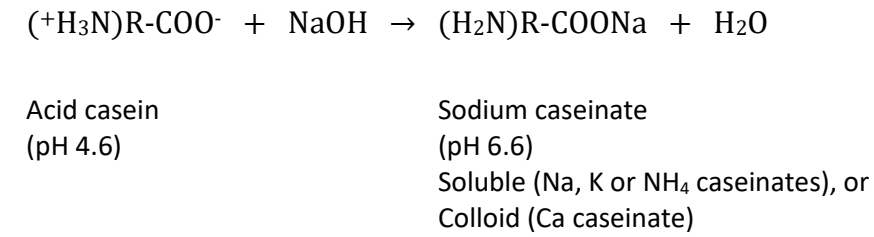


Figure 2.9 Neutralisation of acid casein with alkali where R represents casein protein.

2.8.2 Whey protein

Whey has had relatively little importance in the past and was often regarded as waste in the dairy industry. With growth in the dairy industry, whey became a serious pollutant for the water supply. In every 100 litres of milk used for cheese or casein production, 90 litres of whey is produced as waste. With the advancements in science and technology, whey has been transformed from dairy waste to be a highly valuable dairy ingredient. Today, whey is used to enhance the nutrition and functional properties in many foods.

Whey is the soluble fraction of milk from the acid precipitation or rennet coagulation of casein, or after cheese is made. Whey comprises about 20% of the total protein in bovine milk. A typical whey contains about 93.8% water, 4.9% lactose, 0.7% whey protein, 0.5% ash and 0.1% fat (Bansal & Bhandari, 2016; Bylund, 2015).

Whey protein can have different composition depending on the casein separation or cheese making method used. Whey from both acid precipitation and rennet coagulation of casein contains proteose peptones and non-protein nitrogen, while rennet coagulated whey from cheese making contains another fraction called macropeptides due to the

cleaving of κ-casein by rennet. **Table 2.5** summarises the typical protein composition of whey.

Table 2.5 Typical protein composition of bovine whey (adapted from Hahn, Schulz, Schaupp, and Jungbauer (1998)).

Type of protein	Concentration in whey, g/L
β-Lactoglobulin	3.5
α-Lactalbumin	1.5
Serum albumin	0.5
Immunoglobulins	0.8
Serotransferrin	0.06
Lactoferrin	0.05
Proteose peptone	0.5

Whey protein is produced commercially into whey protein concentrate (WPC) and whey protein isolate (WPI). WPC is produced by removing lactose, minerals and water from whey to a concentrated protein of at least 25%. Ultrafiltration and diafiltration are the two common membrane separation techniques used to concentrate protein (Fox et al., 2015a). Ultrafiltration separates molecules according to their molecular size. Ultrafiltration will separate whey into insoluble material and compounds larger than 20 kDa (retentate) and a permeate phase, which consists of mostly lactose, minerals and water. Diafiltration is employed as an additional washing step to remove lactose and minerals in the retentate to produce WPC with more than 50% protein. The retentate is spray dried to a powder containing between 30-85% protein (Bansal & Bhandari, 2016). The nutritional composition of common commercial grades of WPC and WPI is shown in **Table 2.6**. WPC is a common ingredient in the bakery products, confectionery, desserts, sauces, ready-meals, ham, infant formulae, dairy beverages, soft drinks, and sports nutrition products. The potential use of whey protein as emulsifiers for food applications has been shown by various studies (Adjonu et al., 2014; S. J. Lee & McClements, 2010; Relkin, Shukat, Bourgaux, & Meneau, 2011).

Table 2.6 Typical composition of whey protein concentrate (WPC) and whey protein isolate (WPI) powders (adapted from Smith (2008)).

Component	WPC 34	WPC 55	WPC 80	WPI
Protein (dry basis), %	34	55	80	89
Lactose, %	52	31	9	2
Ash, %	7	6	4	3
Fat, %	4	6	6	1
Moisture, %	4	4	4	5

Most WPC still contains about 4-7% milk fat, and it is possible to make a higher grade of protein that is almost fat-free. The higher-grade WPI is an important ingredient to make clear acid sports beverages (Archer, 1998). WPI is prepared by using ion-exchange chromatography, where whey proteins are adsorbed in the solid phase by changing the pH of the solution while lactose and salts are being eluted from the system. Proteins adsorbed on the solid phase can be recovered by using a suitable pH modifier. The recovered protein will be desalted and then spray dried into a powder. WPI powder contains more than 90% protein, 0.2-2.0% lactose, 0.3-4.5% ash, 0.2-1.5% fat and 4-6% moisture (Morr & Ha, 1993). WPI is a high purity protein used in medical nutrition, health food, infant nutrition and sports nutrition products. **Figure 2.10** shows a typical flow chart to produce WPC and WPI.

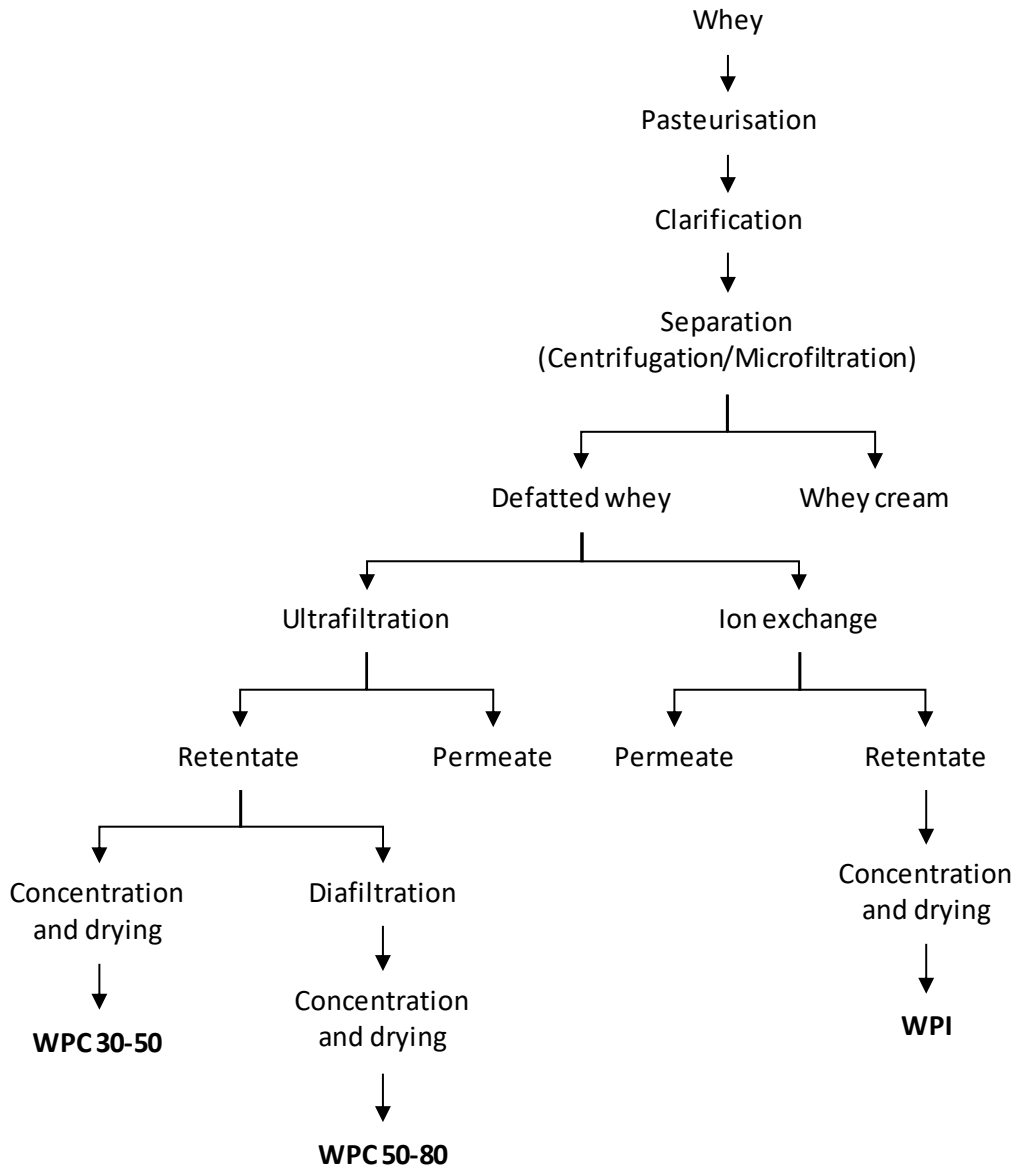


Figure 2.10 Typical production steps of whey protein concentrate and whey protein isolate (adapted from (Bansal & Bhandari, 2016)).

2.9 Low-molecular-weight emulsifiers

This subsection on low-molecular-weight emulsifiers is an elaboration of **Section 2.6.4** and discusses the various types of emulsifiers for food applications. An emulsifier is a common minor component in everyday products such as food, pharmaceuticals, personal care, lubricants, plastics and many more. Some of the common food items containing emulsifiers are margarine, chocolate, mayonnaise, ice-cream and coffee whitener. A food emulsifier is generally recognised as safe (GRAS status), and there are many publications on its application, safety and chemical properties. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is the global organisation who evaluates emulsifiers as a food additive and publishes information on safety, chemical and analytical properties on each of them. Although JECFA evaluates food emulsifiers at a global level, some countries have expertise and funds to carry out further risk assessment to establish their own monographs to regulate application of emulsifiers in food products. This means that some emulsifiers can be used in one country but not another. For example, succinylated monoglyceride is approved for use in the USA, while it is not permitted in Europe, Australia and New Zealand.

Emulsifiers play a significant role in the emulsification during margarine preparation, and it was not until the 1930s that more applications of emulsifiers became known. Today we know that apart from emulsification, an emulsifier has a major role in starch complexing, crystal modification, anti-bacterial functions, as cleaning agents, for protein interactions, lubrication and many more (Krog & Vang Sparsø, 2003). **Table 2.7** summarises the functions of emulsifiers, with specific examples in food applications.

Table 2.7 Functions of emulsifiers and their application in food.

Functions of emulsifiers	Food application
Emulsification	Margarine, mayonnaise, dairy drink, sausage
Dispersion	Chocolate, peanut butter
Aeration	Whipped cream, cake
Anti-foaming	Sorbet, jellies, puddings
Solubilisation	Flavour
Cleaning	Food grade cleaning agent
Starch complexing	Bread, pasta
Crystal modification	Margarine, shortening, chocolate
Creaming ability	Margarine, shortening
Gluten modification	Dough conditioner
Anti-bacterial	Canned coffee
Antioxidant	Margarine
Plasticising	Chewing gum
Viscosity modification	Chocolate
Lubrication	Pasta, noodle, pet food
Processing aids	Pharmaceutical excipient

An emulsifier is an amphiphilic molecule made up of hydrophilic and hydrophobic functional groups. The amphiphilic properties of emulsifiers make it possible to blend two or more immiscible liquids to form an emulsion. In the case of water and oil, stirring the mixture can turn them into an emulsion but they will soon separate into two immiscible layers once stirring is stopped. Separation of water and oil is due to the high surface tension between them that acts as an expelling force. Emulsifiers, when added to the water and oil interface, will lower the surface tension and thus allow the mixture to form emulsions more easily. Emulsification will also decrease the size of the dispersed particles, reduce the interfacial surface tension and protect the surface of the droplets. Emulsifiers can act both as foaming and anti-foaming agents by changing their concentration in a food system and depending on their behaviour. Emulsifiers, being fat-like molecules, can interact with fat to alter the crystallisation behaviour and morphology, thus, changing the way a fat crystal is dispersed in a food system. Ionic emulsifiers can also interact with gluten to improve crumb structure and bread volume (Young, 2014).

The world production of emulsifiers stands around 500,000 metric tons (Hasenhuettl, 2008) and comprises of 20 different types of emulsifiers. The biggest contribution comes from glycerol fatty acid esters and its derivatives, which account for about 70% of global food emulsifiers (Moonen & Bas, 2007). **Figure 2.11** shows how each component reacts with triglycerides and fatty acids to derive the different types of emulsifier. Principal raw materials used to make emulsifiers are derived from vegetable oils such as palm oil, soya bean oil, sunflower oil and rapeseed oil, and animal fats (Krog, 2011). These oils undergo hydrolysis to form fatty acids and glycerol, where the hydrolysed components can be used to make emulsifiers. Hydrophilic-like raw materials such as glycerol, lactic acid, sucrose, polyglycerol, propylene glycol and sorbitol can react with triglycerides or fatty acids to form emulsifiers with specific characteristics (Krog, 2011). Emulsifiers such as mono- and diglycerides can be modified by reacting with organic acids like lactic acid, citric acid, tartaric or succinic acid to improve their solubility in water (Krog, 1977). Sorbitan esters can be reacted with ethylene oxide to become a water-soluble emulsifier (Cottrell & Peij, 2007). For the context of this thesis, mono- and diglycerides will discussed in **Section 2.9.1** and its effect on emulsion stability will be discussed in **Section 2.9.2**.

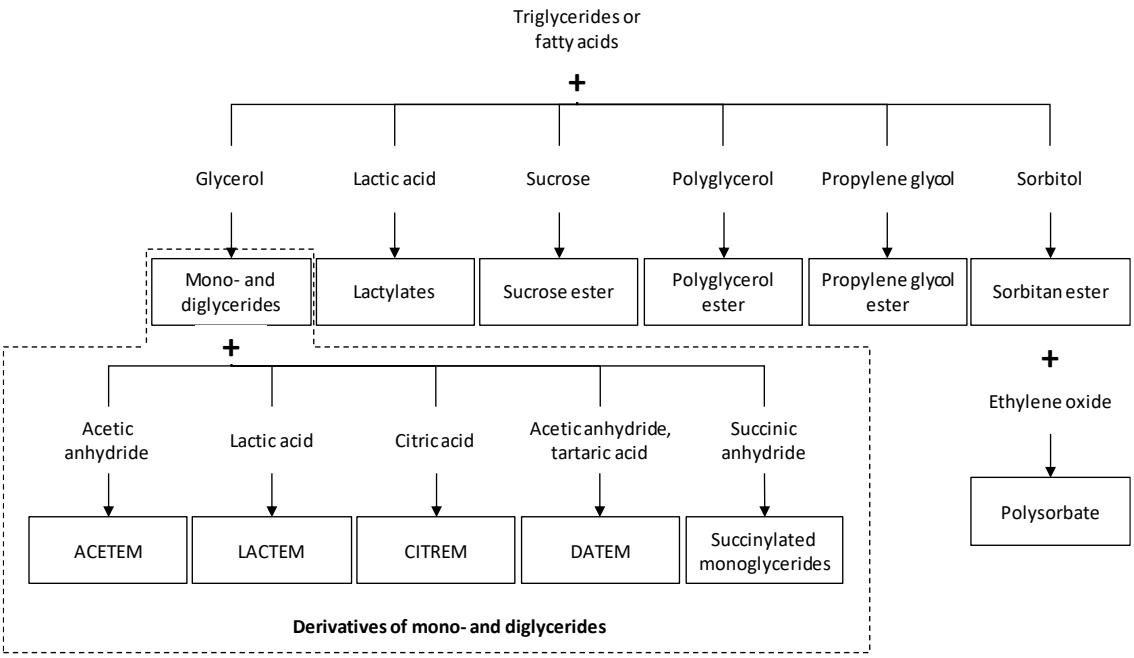


Figure 2.11 Emulsifiers derived from fats, oils and fatty acids (Krog & Vang Sparsø, 2003).

2.9.1 Mono- and diglycerides

Mono- and diglycerides are a mixture of mono-, di- and triesters of fatty acids together with a small amount of glycerol and free fatty acids. Mono- and diglycerides are produced through heating of oils and glycerol or by direct esterification of glycerol and fatty acids at a high temperature above 200 °C in the presence of a suitable food-grade catalyst (Moonen & Bas, 2007). Mono- and diglycerides may undergo purification steps such as molecular distillation to produce distilled monoglycerides with more than 90% monoglyceride fraction. The term 'mono- and diglycerides' is used in this thesis to refer to mixed mono- and diglyceride esters of fatty acids whereas 'monoglycerides' refers to a single class of ester consisting of a glycerol molecule linked to a fatty acid chain.

A typical yield of a randomised chemical reaction is about 35-45% monoglycerides, 40-50% diglycerides, and the balance made up of unreacted material such as glycerol, triglycerides and free fatty acids (Moonen & Bas, 2007). In direct esterification of glycerol and fatty acids, the removal of water continuously from the reaction flask by inert gas sparging or pulling of vacuum is crucial to shift the reaction equilibrium towards producing monoglycerides. It is also important not to keep the mixture of mono- and diglycerides in the molten stage for an extended period as the composition in the mixture will try to reach another equilibrium and normally result in lower monoglyceride content as the temperature becomes lower (Lauridsen, 1976; Woods, 1959). Woods (1959) patented a rapid cooling process to prevent the mixture from shifting into another reaction equilibrium. His study showed that direct addition of glycerine into the reaction mixture coupled with glycerine distillation is possible to get higher monoglyceride content. A review by Márquez-Alvarez, Sastre, and Pérez-Pariente (2004) showed that there are strong interests in the development of a solid catalyst to improve efficiency and economy of the synthesis process. Khan and Rathod (2015) reviewed the enzymes used as biocatalysts and their limitations in the synthesis of esters and emulsifiers, while Bornscheuer (1995) discussed the development of the enzymatic synthesis of mono- and diglycerides.

Mono- and diglycerides are the most commonly used emulsifiers in the food and pharmaceutical industries (Bornscheuer, 1995). Mono- and diglycerides meet the international food additive standard outlined by the Joint FAO/WHO Expert Committee on Food Additives and was granted GRAS status by the U.S. Food and Drug

Administration (21 U.S. Code of Federal Regulations (CFR) §184.1505), while the European Union regulates it under food additive number E471. Most countries further regulate the permissible level due to the wide application of food additives into foods that are native to that country, e.g. Food Standards Australia New Zealand (FSANZ) Code under Standard 1.3.1 regulates the types of food product that can use mono- and diglycerides and their permissible level in food products.

The wide range of fatty acids either from vegetable or animal origin that can react with glycerol allows specific functional mono- and diglycerides to be produced. The growing demand for Halal, Kosher and vegan diets has caused a shift from traditionally animal-based fatty acids to plant-based alternatives. The types of fatty acid chains affect the physicochemical properties of mono- and diglycerides. Mono- and diglycerides with predominantly saturated fatty acids, such as stearic acid and palmitic acid, are solid with a high melting point and high oxidative stability. On the other hand, mono- and diglycerides with unsaturated fatty acids, such as oleic acid and linoleic acid, is soft and more prone to oxidation with a melting point close to room temperature. Mono- and diglycerides with saturated and unsaturated chains may also be blended to achieve some specific functionalities. Glycerol monostearate is a better fat crystallisation initiator than glycerol monooleate (Davies, Dickinson, & Bee, 2001), and thus their combination can be used to control crystallisation profile for an ice-cream emulsion (S. R. Euston, 2008).

The direct application of mono- and diglycerides in food applications without the addition of oil is challenging due to its poor dispersibility in water. Industry has adopted two major directions to overcome these functionality and dispersion issues. First is the addition of co-emulsifiers such as sodium stearate to aid dispersion of mono- and diglycerides in water (Krog & Vang Sparsø, 2003). According to the mono- and diglycerides monograph outlined by Joint FAO/WHO Expert Committee on Food Additives, mono- and diglycerides can contain up to 6% sodium stearate. The presence of sodium stearate as a co-emulsifier has possible advantages when used in low-fat systems such as milk and sauces, as it simplifies the processing sequence during manufacturing without having to melt mono- and diglycerides in oil prior mixing with other water-based ingredients.

Another direction is the modification of the physical structure of mono- and diglycerides to a ready-to-use and active form. Hansen (1965) patented a process to prepare a hydrated

gel of mono- and diglycerides to allow easier incorporation and complete dispersion of mono- and diglycerides in the product, thus enhancing the quality of bakery products. Hansen (1967) improved the hydrated gel formulation with the incorporation of pectin, which resolved the issue of white powder forming on the gel without compromising the performance. Rusch, France, Davis, and Tenney (1982) developed a preparation method to have a high content of distilled monoglyceride (about 40% to 60%) in the hydrated form.

2.9.2 Effects of mono- and diglycerides on physicochemical properties and emulsion stability

Mono- and diglycerides can alter the physicochemical properties and emulsion stability when added to a protein-stabilised emulsion (McClements, 2004). Mono- and diglycerides are more surface active than proteins and thus adsorb rapidly at the interface (Fredrick et al., 2013). At low concentrations of mono- and diglycerides, protein adsorbed at the interface is high (Dickinson & Hong, 1994; S. R. Euston & Hirst, 2000). At this stage, mono- and diglycerides fill the small gaps at the interface without displacing protein and form a compact interface (S. R. Euston & Hirst, 2000; Munk et al., 2014). However, a higher protein displacement is seen when increasing the shear or lowering the temperature of the emulsions containing mono- and diglycerides (Carrera Sánchez & Rodríguez Patino, 2004; Davies et al., 2001; Dickinson & Tanai, 1992). Glycerol monooleate (GMO) has a lower surface activity than glycerol monopalmitate (GMP) at high emulsifier concentration, which explains the lower protein displacement in whey protein isolate-stabilised emulsions with GMO. However, GMO at low concentration produces a more expanded interface and displaces more protein than GMP at the interface when shear is applied (Carrera Sánchez & Rodríguez Patino, 2004). The lowering of temperature will cause monoglycerides to crystallise, which can also lead to protein displacement at the interface (Davies et al., 2001; Krog, 1997).

Creaming is the most common emulsion destabilisation mechanism amongst dairy-based emulsions. According to Stoke's Law, large droplets have a faster rate of creaming compared to smaller ones and are more likely to separate due to gravity (McClements, 2007). Mono- and diglycerides can effectively reduce droplet size and size range distribution of protein-stabilised emulsions (Dickinson & Hong, 1994; Krog, 2011; Liang, Wong, Pham, & Tan, 2016; Matsumiya et al., 2010). Thus, reducing the number

of large droplets should delay the occurrence of creaming. The cream layer rich in oil droplets is in proximity with oxygen in the headspace of the emulsions and is therefore more likely to experience a higher rate of lipid oxidation (Sun, Gunasekaran, & Richards, 2007; Yesiltas et al., 2019).

2.10 Encapsulation

Encapsulation is a common technique in the food industry to enclose sensitive food components such as lipids, flavours and bioactive compounds (antioxidants and vitamins), materials or functional ingredients within a stable shell (Shahidi & Han, 1993), whereby an emulsion is first formed and then dried using techniques such as spray- and freeze-drying to form a powder. The purpose of encapsulation is to protect or control the release of the core material (Fuchs et al., 2006), and improve stability of functional ingredients (McClements, 2015). Encapsulation may protect the core material against oxygen, water, temperature, light, chemical interaction with other ingredients and evaporation. The encapsulated powder must have good handling and storage properties, be easily dispersed in water and exhibit controlled release of the materials at specific conditions (Augustin & Oliver, 2014). Generally, the encapsulated material is referred to as the core material, internal phase, or active material, and the wall is known as the wall material, shell, coating, or membrane. The encapsulated powder is also known as powder particles, dried particles, dried powders or capsules. These terms will be used interchangeably throughout the thesis.

An encapsulated powder can be categorised based on the size of the capsule. The three common categories are macro- (<5000 μm), micro- (1-5000 μm) and nano-capsules (<1 μm) (Jafari, Assadpoor, He, & Bhandari, 2008). The powders can differ in morphology due to the encapsulation method and physicochemical properties of both core and wall materials (Bakry et al., 2016). There are many morphologies of the encapsulated capsule (**Figure 2.12**), with the most common morphologies being single-core capsules and matrix particles (Fang & Bhandari, 2010). A single-core capsule consists of a core material that is surrounded by a shell, whereas a matrix particle has multiple droplets of core material within a continuous network of the shell. Several distinct core materials can also be incorporated within the same capsule, named multiple core capsules. Single-core multiple wall capsules can be prepared by coating an existing single-core capsule with another layer of wall material, e.g. with a lipid. It is also possible

to assemble several single-core capsules within a secondary wall or matrix to form an assembly structure.

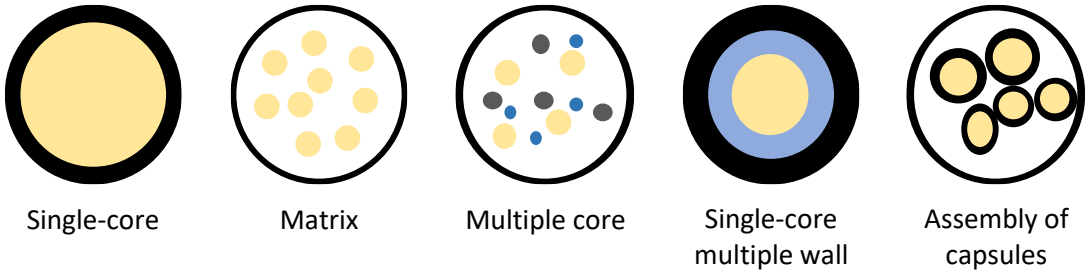


Figure 2.12 Morphologies of encapsulated powders.

The ability to manipulate the morphology of the encapsulated powders allows food formulators to design protection and release properties for sensitive food components. A single core encapsulated powder tends to quickly release the core material, whereas multiple wall capsules can achieve designed release behaviour at specific time and conditions. The wall material influences the protection and release profile of the core material (Augustin & Hemar, 2009).

2.10.1 Encapsulation method

Many encapsulation methods can be used for food applications. The selection of a method depends on economics, existing facilities, the physicochemical properties of the core and wall materials, the sensitivity of the core material, the release mechanism, desired powder properties and the final application of the powder (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007; Shahidi & Han, 1993). A list of the encapsulation methods is summarised in **Table 2.8**. For the context of this thesis, only the encapsulation method via spray-drying will be discussed in detail (**Section 2.10.2**).

Table 2.8 Examples of encapsulation methods (adapted from Shahidi and Han (1993)).

Encapsulation method	Examples
Physical	**Spray-drying**, spray-cooling/congealing, freeze-drying, fluidised bed coating, extrusion (centrifugal, pressure and hot melt), spinning disk coating and co-crystallisation
Chemical	Molecular inclusion (including complexation) and chemical polymerisation
Physicochemical	Coacervation (aqueous phase separation), organic phase separation, liposome entrapment

2.10.2 Spray-drying

Spray-drying is the most common encapsulation technique in the food industry (Fuchs et al., 2006; Shahidi & Han, 1993) to encapsulate functional ingredients and flavour. Spray-drying can be arranged with drying air flowing either co- or counter-current (Turchiuli, Fuchs, et al., 2005). **Figure 2.13** shows a schematic diagram of a co-current spray-drying process. The mixture of core and wall materials is atomised into a stream of hot air in the drying chamber which results in rapid evaporation of the solvent to form a capsule around the core material with a matrix particle arrangement (Fang & Bhandari, 2010). The final spray-dried powders generally have low moisture contents below 3%. This process is mainly controlled by product composition and conditions in the drying chamber (air flow, temperature). Many reviews on spray-drying have been published around encapsulation of food ingredients (Gharsallaoui et al., 2007), including oils (Bakry et al., 2016; Sagiri, Anis, & Pal, 2016), polyphenols (Fang & Bhandari, 2010), probiotics (Haffner, Diab, & Pasc, 2016) and flavours (Reineccius & Yan, 2016). Vega and Roos (2006) reviewed the effect of spray-drying and storage on the properties of reconstituted emulsions.

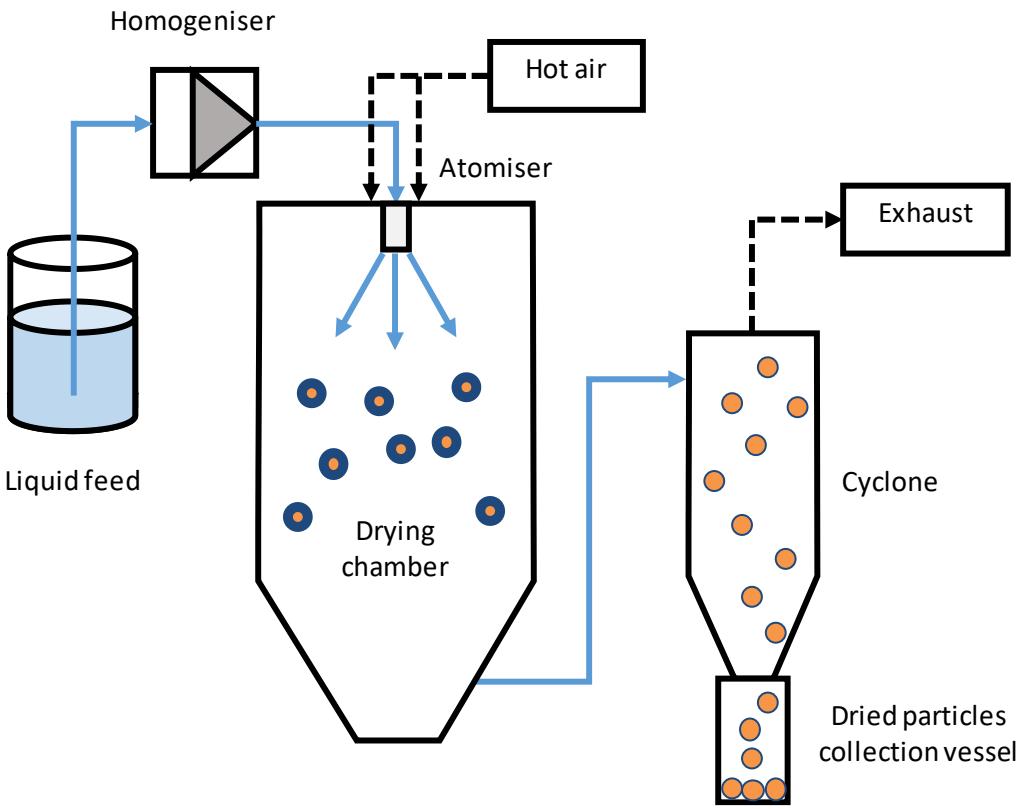


Figure 2.13 Schematic diagram of a co-current spray-drying process.

The main advantages of spray-drying are: consistent product quality when drying conditions are held constant (e.g. structure and appearance, low moisture content, low water activity, good flow properties, high bulk density and small particle size); continuous and easy drying operations; and that it can be used for a wide range of materials (Augustin & Hemar, 2009; Bae & Lee, 2008; Fang & Bhandari, 2010; Shahidi & Han, 1993; Vega & Roos, 2006). However, this technique has disadvantages around the choice of wall materials that require high solubility in water, low viscosity at high solids, the ability to form stable emulsions (Fang & Bhandari, 2010), and the high yield loss due to powder sticking to the internal drying chamber (O'Callaghan & Hogan, 2013). The rapid, reproducible results and the scalability are the key attributes that are responsible for the high adoption of this technology by the food industry (Bakry et al., 2016).

2.10.3 Wall materials

The selection of wall materials depends on the core material and the application, and is the most challenging part as it can impact on the drying procedure and particle characteristics after drying (Augustin & Hemar, 2009). An ideal wall material should have good solubility, emulsifying properties, form low viscosity at high solids and be able to protect the core material from chemical degradation, i.e. oxidation (Gharsallaoui et al., 2007). The pre-drying emulsion properties have a significant effect on the spray-drying process, for example, emulsions with high viscosity interfere with the atomisation process and form large particles that may result in incomplete drying (Gharsallaoui et al., 2007).

Maltodextrin is commonly used as a wall material in spray-drying due to its relatively low cost, neutral odour, flavour and taste, minimal effect on viscosity, and good protection against undesired reactions (Carneiro, Tonon, Grosso, & Hubinger, 2013; Fernandes et al., 2016; Vega & Roos, 2006). However, maltodextrin has poor emulsifying properties and are used together with emulsifiers and biopolymers to improve the emulsifying and interface properties to encapsulate lipid-like core materials (Hogan, McNamee, O'Riordan, & O'Sullivan, 2001).

2.11 Methods used to assess emulsion properties and stability (techniques of analysis)

2.11.1 Particle size distribution

A particle is a small discrete matter ranging from macroscopic to microscopic and subatomic size ranges (Walstra, 2002). Macroscopic and microscopic particles, either in solid, liquid or gaseous form, are the most common particles found in food products. Particle size characterisation of food is important because it influences food stability, texture, viscosity, optical properties and sensory attributes (McClements, 2007). Particle size also influences material properties such as emulsion stability of milk, the texture of peanut butter, the dissolution rate of sugar and flowability of milk powder. Better knowledge of particle size could lead to better control on product quality and improve understanding of product behaviour, ingredients and processes (Bremond & Bibette, 2012; McClements, 2015).

Particles exist as three-dimensional matter, and they cannot be completely described by a single dimension such as diameter. The equivalent sphere model is used to describe particle size with the assumption that any particle being measured is a perfect sphere, and thus it can be described as using the diameter of an equivalent sphere (McHale & Newton, 2011). The equivalent sphere model works well for regular-shaped particles but could be deceptive with irregularly shaped particles such as needles. Over the years, there are many analytical techniques developed based on different equivalent sphere models to describe the particle size of matter. Some of the common techniques are sieving, sedimentation, static light scattering, dynamic light scattering, electrozone sensing and automatic imaging (McClements, 2007).

The particles in a food product are not the same size and are distributed in a range of different sizes. The polydispersity of particle sizes is better described by particle size distribution, which reports the particles according to the different size classes. **Table 2.9** shows the types of particle size distribution and the analytical techniques used to measure the particle size. **Figure 2.14** illustrates a sample having an equal number of 10 and 100 nm particles and its comparison in different types of distribution. The number weighted distribution based upon the absolute number of particles will show similar peak heights in both the size classes. A 100 nm particle has 1000 times the volume of a 10 nm particle, and this is reflected in the volume-weighted distribution. A large particle has a bigger surface to scatter light compared to a small particle and it is the principle used in an intensity-weighted distribution. It is important to select an appropriate particle size distribution parameter to gain insights into the possible origin or instability in an emulsion system (McClements, 2007). Particle size is reported as mean and standard deviation for the convenience of data analysis (Hunter, 1986).

Table 2.9 Types of particle size distribution and their measurement technique.

Type of particle size distribution	Description	Particle size measurement technique
Weighted distribution	Particle size distribution based on the weight of particle retained on a particular sieve.	Sieving
Number-weighted distribution	Particle size distribution based on the absolute number of particles.	Automated imaging
Volume-weighted distribution	Particle size distribution based on the volume of each particle.	Static light scattering
Intensity-weighted distribution	Particle size distribution based on the intensity of light scattered by each particle.	Dynamic light scattering

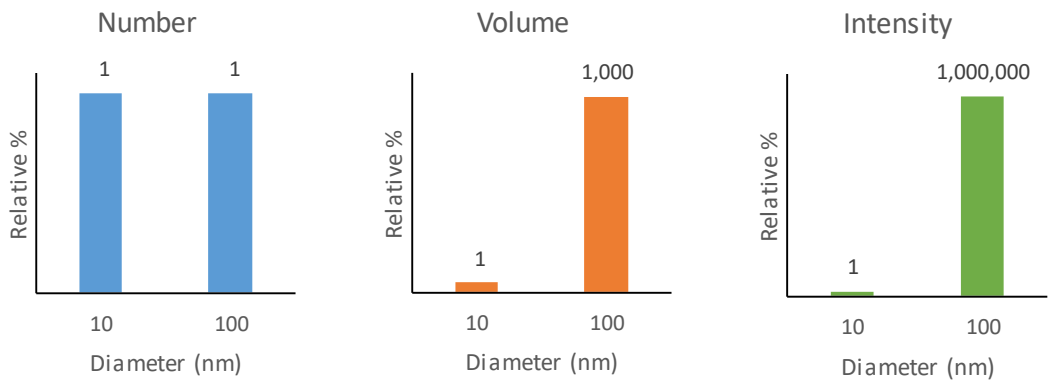


Figure 2.14 Example of number, volume and intensity weighted particle size distributions for the same sample.

Most of the particle size characterisation techniques require a sample to be in a dispersed form where each particle is separated. Two common approaches are wet and dry dispersions. Wet dispersion requires particles to be dispersed in a liquid medium, while dry dispersion utilises gas or air as the dispersant. Wetting of the particles in wet dispersion will lower their surface energy and reduce the force of attraction between neighbouring particles (McClements, 2007). However, it is important to use a suitable dispersant so that the particles in the sample will not swell, dissolve or react with the dispersant. Stirring or agitation could also break any agglomerate, but sometimes ultrasonic irradiation is required to break strongly bound agglomeration of very fine

material. In dry dispersion, particles are suspended in a gas stream. Due to the nature of the dry dispersion, it often required higher energy to break up agglomeration compared to wet dispersion (Malvern Instruments Limited, 2015).

Dynamic light scattering for particle size analysis will be discussed in further detail, as it will be the technique used in this thesis.

2.11.1.1 Dynamic light scattering

Dynamic light scattering, also known as photon correlation spectroscopy, is a non-invasive technique to measure particle size (McClements, 2015). The particles suspended in a liquid undergo Brownian motion due to the interactions between particles and molecules of the liquid. When a laser beam passes through the particle, the intensity of the scattered light varies according to the size of the particles (Dalgleish, Srinivasan, & Singh, 1995). Small particles move more rapidly in a Brownian motion compared to larger particles. The intensity of the scattered light by these particles provides information on the particle size based on the Stokes-Einstein relationship (Malvern Instruments Limited, 2015). Particles in a diluted system are assumed to have no interaction with one another and obey the particle translational diffusion coefficient, as described by the Stokes-Einstein equation:

$$F_d = 6\pi\,\eta\,r\,\upsilon \qquad \textbf{Equation 2.2}$$

where F_d is the frictional force between the particle and the dispersant, η is the dynamic viscosity, r is the radius of the spherical particle, and υ is the flow velocity relative to the particle.

The particle size measured by this technique includes the particle core and any adsorbed material on the surface structure or ions close to the surface structure. **Figure 2.15** illustrates the particle size based on translational diffusion coefficient. This means that particle size based on translational diffusion coefficient is generally larger than electron microscopy. The measured particle size distribution is expressed as an intensity weighted distribution.

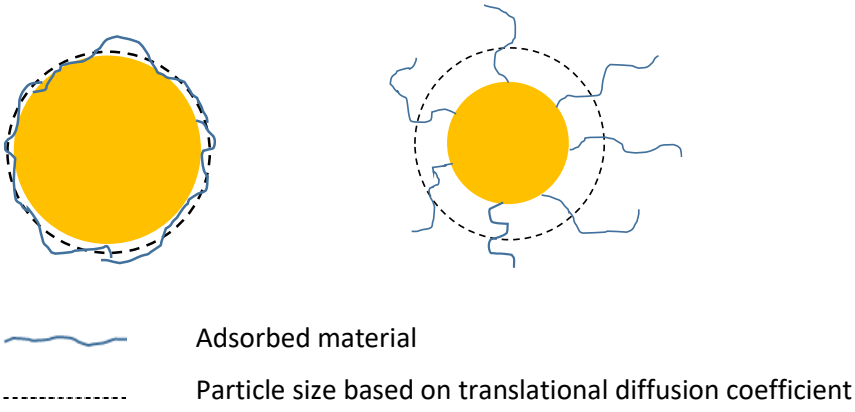


Figure 2.15 Illustrations of particle size based on translation diffusion coefficient (adapted from Malvern Instruments Limited (2015)).

2.11.2 Zeta potential

Zeta potential is a measure of the repulsive and attractive forces between particles in a liquid suspension as a function of electrostatic potential (Hunter, 1981). **Figure 2.16** shows a schematic representation of zeta potential. In principle, two liquid layers surround the particle surface, namely the inner region (Stern layer) and outer region. The Stern layer consists of ions in the opposite charge that are attracted to the surface layer. Within the outer region, there is a boundary called the slipping plane where the ions and particles are stable. The ions beyond the slipping plane stay in in the bulk dispersant. Zeta potential is the electrical potential (measured in mV) at the slipping plane.

This magnitude of zeta potential indicates the stability of an emulsion system in which the particles with large positive or negatives charges retain strong electrostatic repulsive forces. An emulsion system having more than ±30 mV is deemed to be stable (Ross & Morrison, 1988) where the dispersed particles do not tend to coagulate and/or merge with another particle. Zeta potential can be used to optimise the formulation of a suspension and predict long-term stability. pH, conductivity and concentration of a component in the formulation are the main factors affecting zeta potential in solution (Hunter, 2013). For example, the pH of a protein-stabilised emulsion at which the zeta potential equals to zero is the isoelectric point of the adsorbed protein (Hunter, 2013), and yields an unstable emulsion.

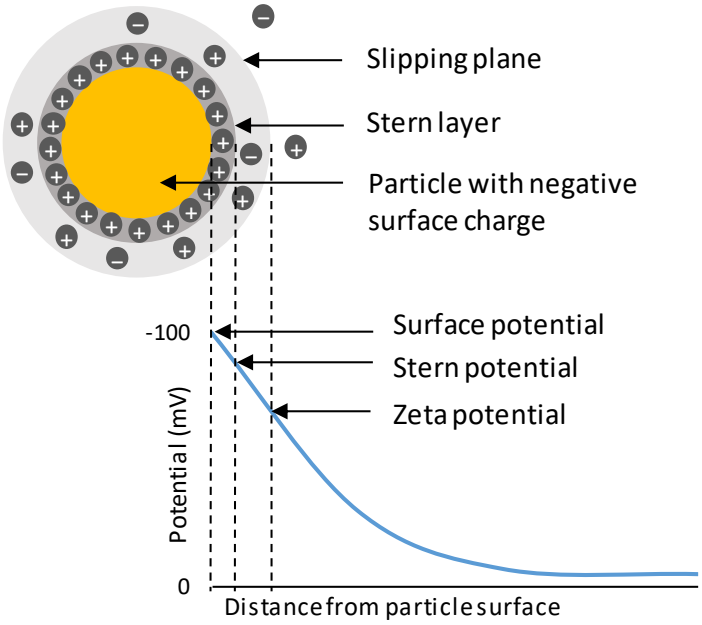


Figure 2.16 Schematic representation of zeta potential.

Measurement of zeta potential utilises the concept of Doppler velocimetry combined with a dynamic light scattering method. Electrical energy is provided to the particles by a pair of electrodes. The charged particles are attracted to the oppositely charged electrode, and the velocity of a particle in a unit of electric field strength is calculated as electrophoretic mobility. The electrophoretic mobility can derive zeta potential using Henry's equation as follows (Hunter, 2013).

$$U_E = \frac{2\,\varepsilon\,z\,f(\kappa a)}{3\,\eta} \qquad \textbf{Equation 2.3}$$

Where U_E is the electrophoretic mobility, z is zeta potential, ε is the dielectric constant, η is viscosity and $f(\kappa a)$ is Henry's function.

2.11.3 Viscosity

Viscosity is the measurement of internal flow resistance of a liquid substance, or in simplified terms, the measurement of the thickness of a liquid. There are two types of measurement for viscosity, namely kinematic viscosity and dynamic viscosity. The two viscosity measures are inter-related and their relationships are shown in **Equation 2.4**. Kinematic viscosity is reported as $mm^2\ s^{-1}$ or centiStroke (cSt) while dynamic viscosity is reported in mPa s or centipoise (cP).

$$\text{Dynamic viscosity} = \text{Kinematic viscosity x Density} \qquad \textbf{Equation 2.4}$$

In physical chemistry, viscosity is defined as the force required to move the layers of a liquid at different speed against shear stress (Bergmann Tiest, 2015). Shear stress is the force applied to the liquid by the measuring sensor divided by the surface area of the sensor and is shown in the equation below.

$$\tau = \frac{F}{A} \qquad \textbf{Equation 2.5}$$

τ is shear stress, F is force and A is the area of upper plate.

Shear rate, or rate of shear strain, indicates the velocity of the liquid as the position changes perpendicular to the movement direction of the measurement sensor. The equation below shows the derivation of shear rate.

$$\dot{\gamma} = \frac{v}{h} \qquad \textbf{Equation 2.6}$$

$\dot{\gamma}$ is shear rate, v is velocity of the upper plate, h *i*s the distance between the two plates.

According to Newton's law, viscosity is the ratio of shear stress over shear rate for a given temperature and pressure (McClements, 2015). There are two types of flow behaviour in liquids, either Newtonian or non-Newtonian. Newtonian liquids obey Newton's law of viscosity where its viscosity is independent of shear rate. Non-Newtonian liquids exhibit changes in viscosity depending on the shear rate. Two common behaviours of non-Newtonian liquids are shear thinning and shear thickening. Shear thinning (or pseudoplastic) flow behaviour shows a decrease in the viscosity with

increasing shear rate, while shear thickening (dilatant) flow behaviour demonstrates increasing viscosity with shear rate.

2.11.4 Emulsion stability

Protein-stabilised emulsions tend to be quite stable and unlikely to have complete separation of oil and aqueous phases (Dickinson et al., 1997; Srinivasan, Singh, & Munro, 2001). Creaming is a common cause of physical destabilisation in protein-stabilised emulsions due to gravitational phase separation into cream and serum. A creaming index is calculated as the ratio of cream height divided by the total height of the emulsion sample up on standing for a defined length of time (Dickinson et al., 2003). The simplest and cheapest way to observe creaming is by the naked eye (McClements, 2007) and the rate of creaming is determined by the changes over time.

The observation of creaming can be complemented with other imaging techniques or analytical measurements to investigate emulsion instability. Mengual, Meunier, Cayre, Puech, and Snabre (1999) developed an optical technique, Turbiscan, to evaluate creaming during storage as a function of changing light scattering behaviour at different height of the emulsion in a tube. Another approach is to assess emulsion stability at accelerated conditions by measuring the depth of cream layer and the percentage of the fat collected in the cream or aqueous layer (Srinivasan et al., 2001; Tornberg & Hermansson, 1977). Mild centrifugation is one way to accelerate creaming rate of oil droplets, taking into account of storage time and temperature of the emulsions. Matsumiya et al. (2014) developed an accelerated method called shaking-redispersion method or vibration-redispersion method that successfully predicted the long-term stability of milk-based emulsions. Lumifuge is an analytical instrument employing centrifugal force that accelerates the occurrence of instability in emulsions such as sedimentation, creaming and flocculation. For example, this instrument has been used to predict emulsion stability in walnut emulsion (S. Liu, Liu, Xue, & Gao, 2016), β-carotene emulsion (D. Xu, Wang, Jiang, Yuan, & Gao, 2012) and nanosized silica dispersion (Petzold, Goltzsche, Mende, Schwarz, & Jaeger, 2009). The measurement of particle size distribution over time provides more information on the changes in droplet properties during storage, which can explain the destabilisation mechanism of the emulsion (Mita, Yamada, Matsumoto, & Yonezawa, 1973).

2.11.5 Oxidation

The determination of lipid oxidation can be carried out using chemical methods, thermal methods and by volatile analysis. No single method is able to provide a complete measure of lipid oxidation. Determination of hydroperoxides (primary oxidation products) content can explain the early development of lipid oxidation and report as peroxide value. Hydroperoxides break down over time to form secondary lipid oxidation products such as aldehydes, which can be determined as p-anisidine value. However, these two methods are only applicable to bulk lipid and cannot use for encapsulated lipid and emulsified oil droplets.

Thermal methods such as thermogravimetric analysis (TGA) utilise heat and airflow to measure the mass change over time to explain the rate of oxidation. An increase in the mass of the sample indicates the formation of hydroperoxides. The main advantage of this technique is a small sample size, but it is only suitable for dried samples.

Alternatively, measurement of volatile compounds during storage can explain oxidative stability in protein-stabilised emulsions according to the secondary oxidation products generated. The use of headspace solid-phase microextraction (HS-SPME) has become a popular method to study secondary products of lipid oxidation such as aldehydes, ketones and alcohols (Damerau, Kamlang-ek, Moisio, Lampi, & Piironen, 2014). This method requires adsorption of volatile compounds to the SPME fibre during an extraction step at a defined incubation temperature. Desorption of compounds from the fibre occurs in the hot injector port of a gas chromatograph (GC) system for separation of the extracted volatiles. A mass spectrometer (MS) hyphenated to the GC system is used to identify and quantify the volatile compounds of interest. Mass spectrometry can detect chemical compounds by its mass and estimate its amount based on the signal intensity. This method allows the analysis of a large variety of volatile compounds and can provide more in-depth knowledge of the change in volatile compounds. HS-SPME have been used to monitor oxidative stability in a wide range of food products such as oil-in-water emulsions (Berton et al., 2012; Villiere et al., 2005), human milk (Elisia & Kitts, 2011) and microencapsulated oil (Damerau et al., 2014; Jónsdóttir, Bragadóttir, & Arnarson, 2005). Graciano-Verdugo et al. (2010) has used this method to study the effect of antioxidants on oxidative stability of corn oil.

2.12 Research gap

After conducting the literature review, it was found that there are very few publications on the interaction between commercial mono- and diglyceride with milk proteins in a protein-stabilised beverage emulsion. The majority of research investigating the interaction between emulsifiers and milk proteins were carried out in emulsions with 10-30% fat levels (Chung et al., 2019; Dalgleish et al., 1995; Dickinson et al., 2003; Munk et al., 2014). As milk coffee beverages are emulsions with a low-fat content (below 10% fat), the interaction between emulsifiers and milk proteins at the oil-water interface could behave differently from high fat emulsions. There are very few studies investigating the interaction between emulsifiers and milk proteins in milk coffee emulsions (Matsumiya et al., 2010; Ogawa & Cho, 2015), however, water-soluble and water-dispersible emulsifiers such as sucrose fatty acid ester, diglycerol ester and self-emulsifying mono- and diglycerides are being used. Water-soluble and water-dispersible emulsifiers may not present the same behaviour as oil-soluble mono- and diglycerides. Therefore, this presents a gap in the current knowledge for a systematic investigation on the interaction between commercial mono- and diglyceride compositions, which is relevant for ready-to-drink beverages and important for food technologists to better utilise emulsifiers in a more effective way to achieve better product quality at optimum ingredient costs.

There are several reviews on the encapsulation of lipophilic functional ingredients such as marine, vegetable and essential oils (Bakry et al., 2016), flavour and aroma compounds (Castro et al., 2016), bioactive compounds and food ingredients (Đorđević et al., 2015; Fang & Bhandari, 2010; McClements, Decker, & Weiss, 2007), but none have reported on the successful encapsulation of mono- and diglycerides as a functional ingredient. Encapsulation of mono- and diglycerides is another research gap that can be extended from the current research in encapsulation of food ingredients and presents an opportunity to understand the properties of a new emulsifying ingredient and its application in food matrices after reconstitution. This knowledge is crucial to the food industry for innovation around emulsifier systems to achieve better product quality.

Chapter 3

Effect of Mono- and Diglycerides on Physical Properties and Stability of a Protein-Stabilised Oil-in-Water Emulsion

Part of the contents presented in this chapter has been published previously as a peer-reviewed journal article: Loi, C. C., Eyres, G. T., and Birch, E. J. (2019). Effect of mono- and diglycerides on physical properties and stability of a protein-stabilised oil-in-water emulsion. *Journal of Food Engineering, 240*, 56-64. doi:10.1016/j.jfoodeng.2018.07.016

3.1 Introduction

Oil-in-water emulsions are the basis of many food products such as mayonnaise, whipped cream, salad dressings, sauces, soup, milk, infant formulae and beverages (McClements, 2015). Emulsions are important in food systems to deliver nutrients, and enhance sensory characteristics (Hu, Ting, Hu, & Hsieh, 2017). Oil-in-water emulsions can contain fat levels as high as 70-80% in mayonnaise and as low as 0.2% fat in lemon juice. Homogenised whole milk contains about 3.3% milk fat and some flavoured milk-based beverages have as high as 10% fat. Fat plays an important role in modulating the sensory experiences in food such as taste, flavour, mouthfeel and creaminess.

An oil-in-water emulsion consists of small oil droplets dispersed in an aqueous phase in the presence of at least an emulsifier. In the milk and beverage industry, homogenisation is used to overcome the energy barrier of forming an emulsion (Van der Meeren, El-Bakry, Neirynck, & Noppe, 2005) and/or to reduce the size of fat droplets into smaller ones (Bylund, 2015). Emulsifiers adsorb to the oil-water interface created during the homogenisation process and lower the interfacial tension, which allows the formation of stable oil droplets.

An emulsifier is a type of surface-active agent that can alter the surface tension of a fluid (Hernández Sánchez, Cuvelier, & Turchiuli, 2015; Krog & Vang Sparsø, 2003). Emulsifiers contain both the hydrophilic and hydrophobic functional groups in the same molecule. Milk protein and low-molecular weight emulsifiers such as mono- and diglycerides are common emulsifying agents in milk-based beverages. Milk is a naturally occurring emulsion, where phospholipids, casein and whey proteins have surface-active structures that facilitate emulsion formations in dairy products (Adjonu et al., 2014; Genot et al., 2003; McClements, 2004).

Commercial mono- and diglycerides are generally mixtures and denote the partially hydrolysed structures of triglycerides (Krog & Vang Sparsø, 2003). Monoglycerides have one hydrophobic fatty acid esterified to the hydrophilic glycerol molecule, while diglycerides have two fatty acids. Commercial mono- and diglycerides have a wide range of compositions in term of monoglyceride content, fatty acid types and presence of a co-emulsifier, such as sodium stearate. Molecular distillation is the typical manufacturing process to increase the monoglyceride content in the emulsifier preparation. The

molecular weight and degree of unsaturation of the fatty acid present in the mono- and diglycerides influences its melting behaviour, where mono- and diglycerides composed of saturated fatty acids usually have higher melting points than the unsaturated version of the same carbon number. Mono- and diglycerides are oil-soluble molecules and have very low solubility in water, making dispersibility in water a challenging task in the industry.

In order to improve dispersion of mono- and diglycerides in water, co-emulsifiers are commonly added to mono- and diglycerides and have been marketed as a *self-emulsified* form for many years (Brokaw & Lyman, 1958). Effective co-emulsifiers for mono- and diglycerides are polar compounds and soluble in water, such as sodium stearate, which improves dispersibility of mono- and diglycerides in water (Krog & Vang Sparsø, 2003). Sodium stearate has both hydrophilic and hydrophobic components, which allows it to function as an emulsifier. Brokaw and Lyman (1958) discussed the effect of co-emulsifiers on gel preparation with mono- and diglycerides and the hydrophilic-lipophilic balance (HLB) system was developed to facilitate the selection of an emulsifier for a particular formulation, however, there is no literature to explain the effect of sodium stearate on emulsion properties and stability.

Most commercial products such as milks, ice-creams and spreads contain at least an emulsifier to stabilise the emulsion system. Complete separation of oil and water is rare, but the differences in density between oil droplets and the water phase often lead to formation of a ring at the top of the emulsion. This phenomenon is known as creaming (Taherian, Fustier, & Ramaswamy, 2006). Creaming is defined as the upward movement of oil droplets in the emulsion due to their lower density compared to the water phase (McClements, 2015). Creaming stability is the resistance of the oil droplets in the emulsion to rise to the top of the emulsion. Cream layers are easily dispersed back into the emulsion by shaking. Creaming is not a problem for products in a carton or can, however, creaming in clear packaging may give consumers the perception of inferior quality.

While both milk proteins and mono- and diglycerides adsorb to the oil-water interface, the interaction between them at the interface can influence droplet properties, flow behaviour and emulsion stability. The use of a low-molecular weight emulsifier has been found to affect emulsion stability due to the capability to displace milk protein from the

oil-water interface (Dickinson & Tanai, 1992; Matsumiya et al., 2010; Munk et al., 2013). However, oil-soluble emulsifiers such as mono- and diglycerides do not displace protein at low concentrations (Dickinson & Hong, 1994). Little information is available on the effect of mono- and diglyceride composition including fatty acid type, monoglyceride content and sodium stearate content, and their effect on physical properties and emulsion stability. The effect of monoglyceride content and sodium stearate content on emulsifiers' behaviour in emulsions remains a gap in literature. The objective of this study is to investigate the effect of different mono- and diglyceride compositions (unsaturation of fatty acids; monoglyceride content; and sodium stearate content) on physical properties and creaming stability in protein-stabilised emulsions.

3.2 Materials and Methods

3.2.1 Materials

Saturated mono- and diglycerides, Grindsted Mono-Di HP 40 and Dimodan HP, were donated by Danisco (Auckland, New Zealand) while unsaturated mono- and diglycerides, Radiasurf 7148 and Radiamuls MG 2905K, were a gift from Oleon (Klang, Malaysia) (**Table 3.1**). Sodium caseinate and whey protein concentrate 80% were provided by Tatua Co-operative Dairy Company Ltd. (Morrinsville, New Zealand). Commercially available hydrogenated coconut oil was obtained from Davis Food Ingredients (Auckland, New Zealand). Sodium stearate was provided by Peerage Product Ltd. (Christchurch, New Zealand). Caster sugar was purchased from the local supermarket. Sodium azide was purchased from Sigma-Aldrich (St. Louis, MO, USA).

The concentration of milk protein, fat level and caster sugar in the model emulsions were designed to mimic a lightly sweetened canned coffee beverage. Hydrogenated coconut oil was used to replace milk fat to avoid the effects of lipid oxidation on emulsion stability while it has a similar melting profile as milk fat. Sodium azide was used to protect the emulsions against microbial growth during storage. The use of commercially available ingredients to prepare the different mono- and diglyceride compositions means that the findings from this study can be applied directly by the food industry.

Table 3.1 Mono- and diglycerides composition

Sample	Description
Sat-40	Saturated mono- and diglycerides with low (40% w/w) monoglyceride content
Sat-90	Saturated distilled monoglyceride with high (90% w/w) monoglyceride content
Unsat-50	Unsaturated mono- and diglycerides with low (50% w/w) monoglyceride content
Unsat-90	Unsaturated distilled monoglyceride with high (90% w/w) monoglyceride content

3.2.2 Preparation of model emulsions

Twelve model emulsions with different mono- and diglyceride composition (2 levels of fatty acid saturation x 2 levels of monoglyceride content x 3 levels of sodium stearate content) and a control emulsion were prepared by microfluidisation. Each sample formulation was prepared in duplicate except for the control with four replicates. Samples were prepared across four days with a control on each day to evaluate emulsion repeatability across days. Water-phase ingredients consisted of sodium caseinate (0.8% w/w), whey protein concentrate (0.2% w/w), caster sugar (6% w/w) and sodium azide (0.02% w/w), reconstituted in Milli-Q water at 50 °C. The water-phase was continuously agitated using an Ultra-Turrax (IKA-Werke GmbH and Co. KG, Stufen, Germany) at 10,000 rpm and was simultaneously heated to 75 °C. The four compositions of mono- and diglycerides (0.2% w/w) (**Table 3.1**) were added to hydrogenated coconut oil (1.1% w/w) at 65 °C to form the oil-phase. Sodium stearate was added to mono- and diglycerides at 3% or 6% w/w level as required. The oil-phase was mixed with the water-phase solution, which was then homogenised for 3 min at 75 °C to form a coarse emulsion. The coarse emulsion was given a single pass through a microfluidiser (Microfluidics Corporation, Massachusetts, USA) at 55 MPa and 65 °C. The microfluidiser was pre-heated by circulating water at 70 °C. The control emulsion was prepared using the same method but with no mono- and diglycerides.

3.2.3 Droplet size and polydispersity index of model emulsions

Droplet size and size-range distributions of the model emulsions were measured by dynamic light scattering using a Malvern Zetasizer Nano S (Malvern Instruments Ltd.,

Malvern, Worchestershire, UK). The measurements were carried out using diluted samples of 1:1000 with a 6% sucrose solution. The diluted sample was analysed for 60 sec at 25 °C with duplicate measurements carried out for each sample. The average droplet size was expressed as the intensity-weighted mean diameter (Z-average), and the degree of non-uniformity of a droplet size distribution was presented as the polydispersity index.

3.2.4 Zeta potential

Zeta potential of the model emulsions was determined using the Malvern Zetasizer Nano S. Samples were diluted at 1:20 with a 6% sucrose solution. The diluted sample was analysed at 25 °C with duplicate measurements carried out for each sample.

3.2.5 Viscosity

Viscosity of the model emulsions was performed using a double gap geometry (measuring cup DG42 PO and rotor DG43 DIN53544 Ti) and 11.5 mL sample volume at 20 °C with a Rheostress 1 (Haake, Karlsruhe, Germany) rheometer coupled with a Haake DC-30 circulating bath. Measurements were carried out in duplicate for each sample at the shear rate range of 0 to 200 s^{-1}. The average viscosity was expressed as apparent viscosity at a shear rate of 100 s^{-1}. This shear rate resembles common processes such as pumping, pouring, mixing and stirring to prepare food emulsions (McClements, 2015; Shama & Sherman, 1973).

3.2.6 pH

The pH of model emulsions was tested at room temperature using a pH209 (HANNA Instruments, Woonsocket, RI, USA) pH meter.

3.2.7 Creaming index

The creaming index of the emulsion was obtained by a visual observation method (McClements, 2007). All model emulsions were transferred into clear test tubes (16 mm o.d. x 150 mm height) with screw caps and stored at 25 °C. Creaming index, which is the ratio of cream height over the total height of emulsion upon standing, was calculated according to McClements (2007).

3.2.8 Emulsion stability

Each sample was stored in 6 x 100 mL bottles with screw caps at 25 ℃ and one bottle was removed at the specific time point for analysis on droplet size, polydispersity index, zeta potential, viscosity and pH. For creaming index, each emulsion was stored in three test tubes and monitored visually with extra precaution not to disrupt the cream layer. All measurements were carried out over a period of 1-28 day intervals at 25 ℃. Sodium azide was used to protect the emulsions against microbial growth during storage.

3.2.9 Statistical analysis

IBM SPSS Statistics Version 23 (IBM Corporation, Armonk, New York, USA) was used for statistical comparison of the results. One-way analysis of variance (ANOVA) was carried out to determine significant differences between the results, followed by Tukey's post hoc test for pairwise comparison. All tests were performed at 95% confidence level.

3.3 Results and Discussion

3.3.1 Droplet size and polydispersity index of model emulsions

Table 3.2 shows the droplet size and polydispersity index of model emulsions prepared with different mono- and diglyceride compositions. The three different sodium stearate levels are combined as a single point because ANOVA showed that mono- and diglycerides with 0%, 3% and 6% sodium stearate were not significantly different for droplet size and polydispersity index (**Appendix A.1**). Sodium stearate had no influence on the droplet size and polydispersity index, which may due to the low concentration in the formulation.

Table 3.2 Droplet size and polydispersity index of emulsions prepared with different mono- and diglyceride compositions.

Sample	Droplet size (nm)		Polydispersity index	
	Day 0	*Day 28*	*Day 0*	*Day 28*
Control	178.4 ± 4.9^{d}	175.7 ± 4.1^{c}	0.221 ± 0.008^{c}	0.223 ± 0.006^{d}
Sat-40	151.3 ± 5.3^{bc}	150.4 ± 4.7^{b}	0.188 ± 0.008^{b}	0.198 ± 0.008^{c}
Sat-90	136.6 ± 5.3^{a}	136.5 ± 5.1^{a}	0.184 ± 0.007^{ab}	0.185 ± 0.008^{b}
Unsat-50	154.5 ± 4.3^{c}	151.5 ± 4.5^{b}	0.176 ± 0.010^{a}	0.174 ± 0.012^{ab}
Unsat-90	147.6 ± 6.8^{b}	145.9 ± 6.6^{b}	0.173 ± 0.013^{a}	0.169 ± 0.014^{a}

Values are mean ± standard deviation of 12 measurements (3 sodium stearate levels x 2 batches x 2 replicates), except for the control (n=8). Different letters in the same column indicate statistical significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test.

The droplet size of the control emulsions differed significantly from the emulsions prepared with mono- and diglycerides. Emulsions prepared with mono- and diglycerides were 13-23% smaller in droplet size compared to the control. Sat-90 was the most effective at reducing the droplet size of the emulsions. Monoglyceride content had a significant impact on the droplet size of model emulsions. Emulsions prepared with saturated mono- and diglycerides showed that high monoglyceride content produced significantly smaller droplet size than low monoglyceride content; the same trend was observed in the emulsions prepared with unsaturated mono- and diglycerides. This result showed that monoglyceride is more effective at reducing emulsion droplet size than diglyceride.

The droplet size distribution of the model emulsions is shown in **Figure 3.1**. The intensity-weighted size distribution in **Figure 3.1a** shows average sizes of monomodal peak between 150 nm and 250 nm with a small peak at 4-5 micron of large droplets. Some differences between samples were more distinct when converted to volume-weighted size distribution **Figure 3.1b**.

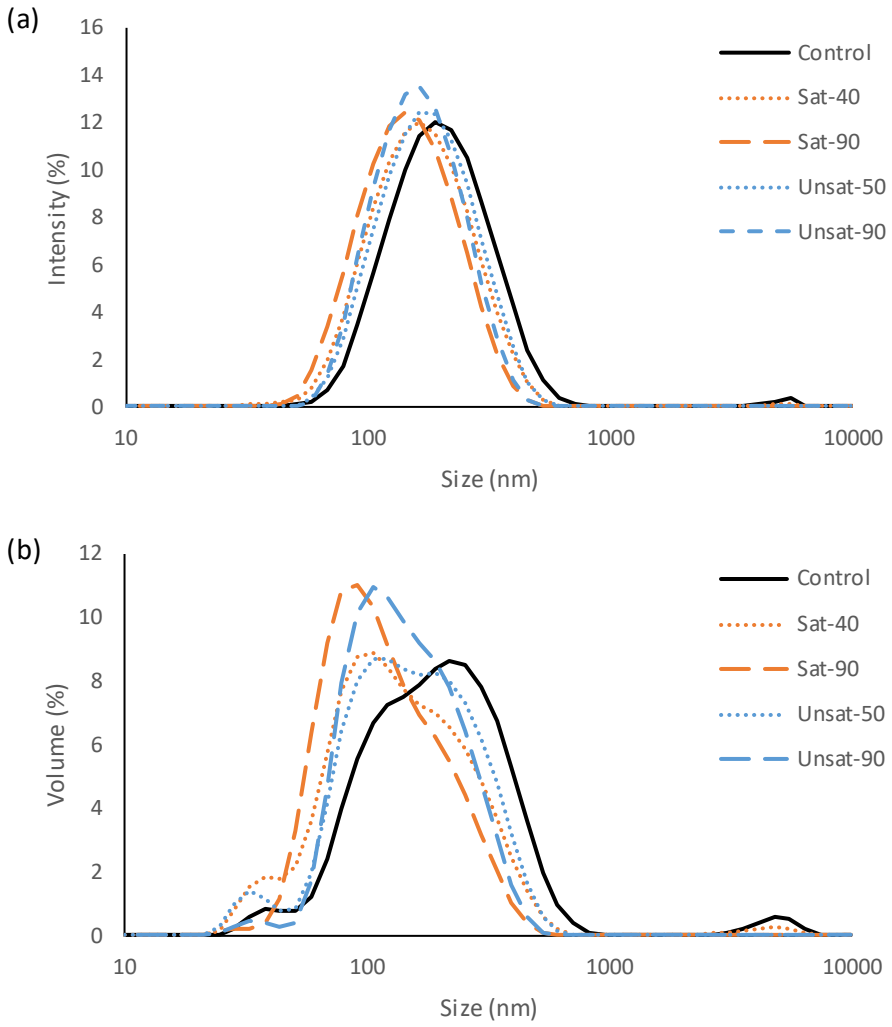


Figure 3.1 Frequency curve of the model emulsions according to (a) intensity-weighted size distribution and (b) volume-weighted size distribution.

The intensity-weighted size distribution was converted to the volume-weighted size distribution by Malvern software (**Figure 3.1b**). The volume-weighted size distribution of the control emulsion was multimodal and left-skewed. The size distribution became narrower and right-skewed in the emulsions with low monoglyceride content (Sat-40 and Unsat-50). Emulsions with a high monoglyceride content (Sat-90 and Unsat-90) showed a pseudo-monomodal size distribution. Emulsions with high monoglyceride have droplets with a narrow size distribution because there are fewer large molecules, i.e. diglycerides, which tend to form a larger droplet size. Diglycerides have higher affinity to oil than water, and thus require a larger oil surface to form stable oil-in-water droplets.

3.3.2 Zeta potential

The zeta potential of the model emulsions is shown in **Table 3.3**. For the ease of discussing the results, zeta potential refers to the magnitude and its negative sign is ignored because all model emulsions are in the negative zeta potential zone. In this context, an increase in zeta potential refers to the increase in negativity of the zeta potential.

Table 3.3 Effect of sodium stearate content in mono- and diglycerides on zeta potential (mV) of model emulsion.

Sample	Sodium stearate content %(w/w) in mono- and diglycerides		
	0	*3*	*6*
No-MG	-46.5 ± 1.2^{a}	n.a.	n.a.
Sat-40	-36.6 ± 1.1^{cA}	-42.5 ± 0.4^{aB}	-49.2 ± 1.9^{aC}
Sat-90	-28.3 ± 1.1^{dA}	-41.0 ± 0.4^{bB}	-45.6 ± 0.7^{bC}
Unsat-50	-41.8 ± 0.4^{bA}	-43.1 ± 0.4^{aB}	-43.6 ± 0.6^{bcB}
Unsat-90	-37.3 ± 1.2^{cA}	-39.0 ± 1.1^{cA}	-42.3 ± 1.9^{cB}

n.a., not available

Values are mean ± standard deviations of four (n=4) measurements. Different letters indicate statistical significant differences ($p<0.05$) according to a Tukey post-hoc multiple comparison test. Lower case letters indicate differences amongst mono- and diglycerides compositions for the same sodium stearate content (column). Upper case letters indicate differences amongst sodium stearate levels for the same mono- and diglyceride compositions (row).

The zeta potential of the emulsions prepared with mono- and diglycerides and no sodium stearate was 10-39% lower than control emulsions. Emulsions prepared with high monoglyceride content had lower zeta potential compared to low monoglyceride content. A reduction in zeta potential indicates the increase of attractive forces between droplets and a weakening of repulsive forces. An emulsion with a zeta potential more negative than -30 mV is usually considered to be a stable emulsion (Ross & Morrison, 1988). All emulsions, except the one prepared with Sat-90 without sodium stearate, had zeta potential values more negative than -30 mV.

The zeta potential of the emulsions increased linearly with sodium stearate content of the mono- and diglycerides (**Figure 3.2**). The zeta potential depends on the mono- and diglyceride composition and the effect of sodium stearate is more important in the

emulsions prepared with saturated mono- and diglycerides. Although sodium stearate is surface active, its participation at the oil-water interface has little effect on emulsion droplet size, whereas the ionic charges at the interface influence the droplet repulsive forces against one another.

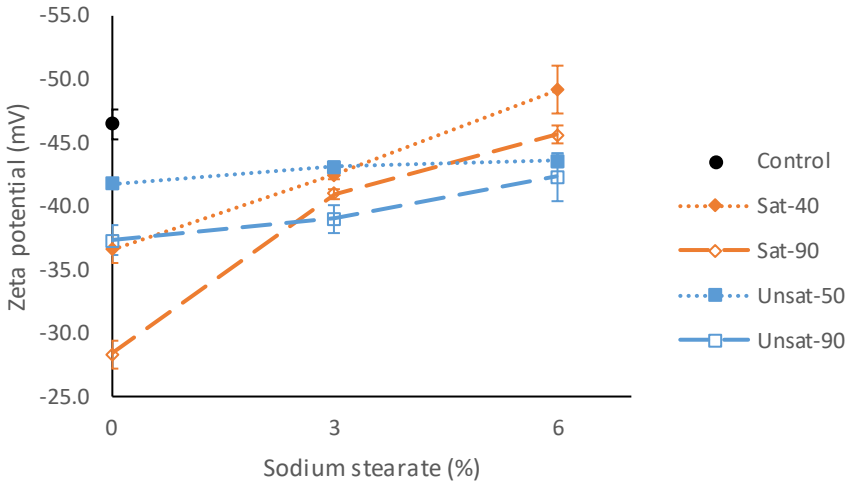


Figure 3.2 Relationship between zeta potential and sodium stearate content for different mono- and diglyceride compositions. Points represent mean zeta potential and the error bars are the standard deviation. All points have 4 replicates except for the control (n=8).

3.3.3 Viscosity

Figure 3.3a shows a linear relationship between shear stress and shear rate, which suggests that all emulsions observed the flow behaviour of a Newtonian fluid. Observation of Newtonian flow behaviour in these emulsions was expected given the low concentration of protein, absence of hydrocolloids such as starch and gums, and high water content in the formulation. The ratio of shear stress to shear rate derived a constant viscosity of 1.6 to 1.8 mPa s for these emulsions. Therefore, mono- and diglycerides have minimal effect on the viscosity of fresh emulsions. The apparent viscosity plotted against shear rate in **Figure 3.3b** shows that the apparent viscosity at low shear rate for all emulsions quickly reached a plateau. As a result, all emulsions were able to flow easily and did not exhibit shear-thinning or shear-thickening properties.

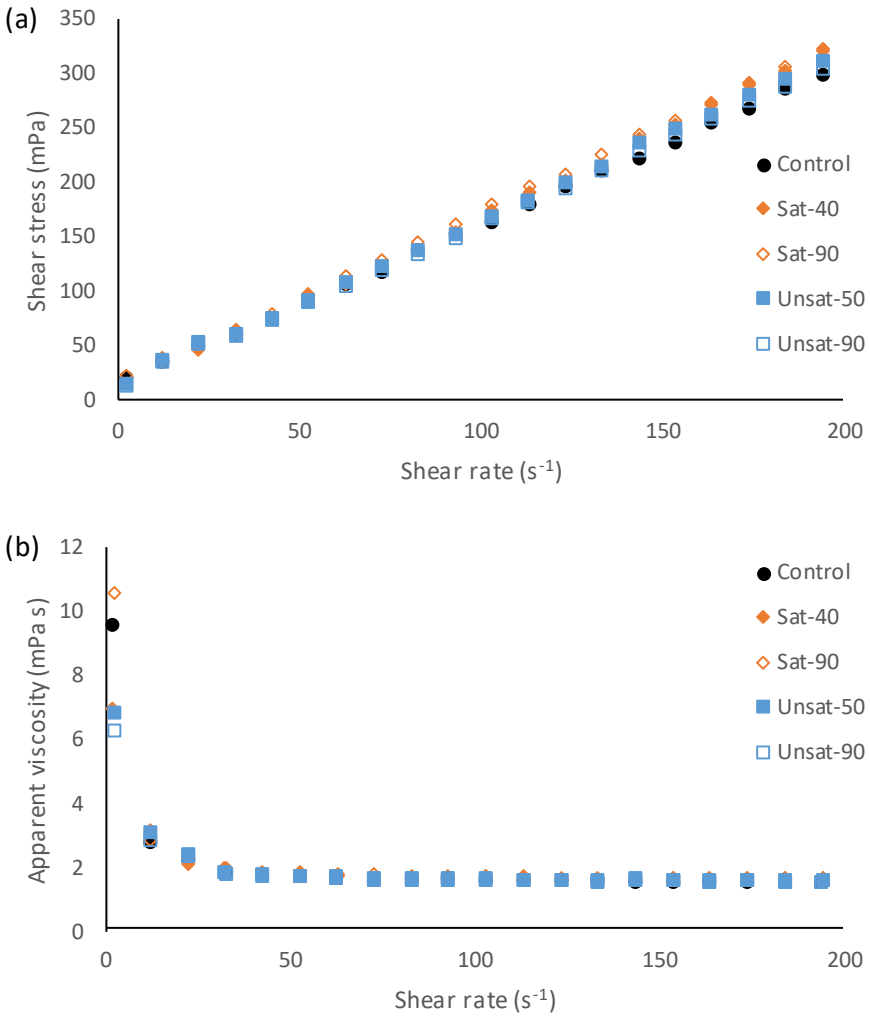


Figure 3.3 Rheological properties of emulsions prepared with different mono- and diglyceride composition. (a) Shear stress vs shear rate plot and (b) Viscosity vs shear rate plot.

3.3.4 pH

The pH of all fresh emulsions after microfluidisation was between 6.6 and 6.8. The inclusion of mono- and diglycerides had no effect on the pH of all model emulsions. This finding was expected because mono- and diglycerides are non-ionic emulsifiers and do not have a significant effect on the pH of emulsions. Although sodium stearate is an anionic emulsifier, the highest ratio used was 6% w/w in the mono- and diglycerides (equivalent to 0.012% w/w in the emulsion) and thus has negligible effect on the pH.

3.3.5 Emulsion stability

Emulsion stability was assessed by changes in physical properties over a period of 28 days at 25 °C. **Table 3.2** shows that the droplet size distribution of all model emulsions remained stable during storage. **Figure 3.4** shows the average droplet size during 28-day storage. There was no significant difference ($p>0.05$) according to independent t tests for the droplet size between the fresh emulsions and emulsion samples stored for 28 days. These results agree with Munk and Andersen (2015), where oil droplets stabilised by sodium caseinate or whey protein did not increase in droplet size over time. The polydispersity index for the emulsions in **Table 3.2** was also found to be stable over the storage period, with no significant differences. The stable droplet size suggests that there is little or no flocculation and coalescence happening during the storage, due to a stable oil-water interface.

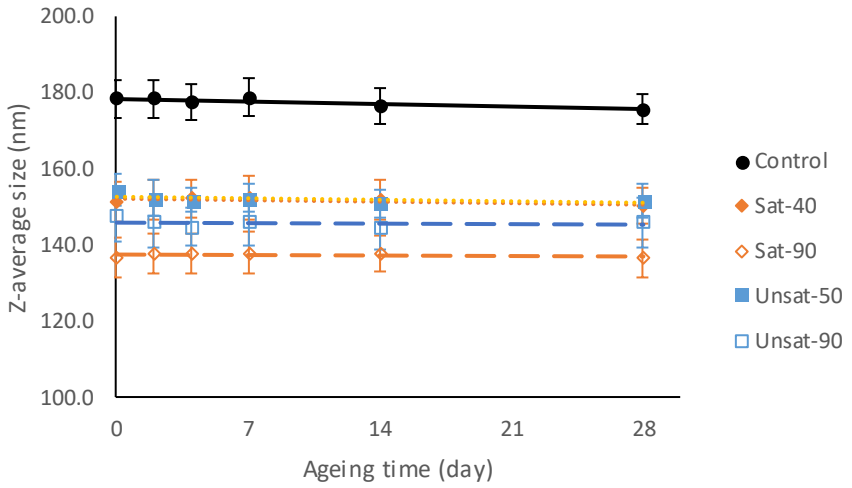


Figure 3.4 Effect of ageing time on average droplet size of model emulsions. Points are mean droplet size and the error bars are the standard deviation. All points have 12 replicates except for the control (n=8).

Figure 3.5 shows the changes in zeta potential for all emulsions during storage. A decreasing trend in zeta potential was observed, but this did not pose a serious threat to the emulsion stability. All emulsions, except Sat-90 with 0% sodium stearate, had a zeta potential above the critical limit of -30 mV throughout storage (Ross & Morrison, 1988) indicating that they were stable emulsions. This means that the emulsions have relatively strong repulsive forces that prevented the oil droplets merging or being attracted to each other. A reduction (becoming less negative) in the zeta potential by between 3-16% was observed in the samples over time.

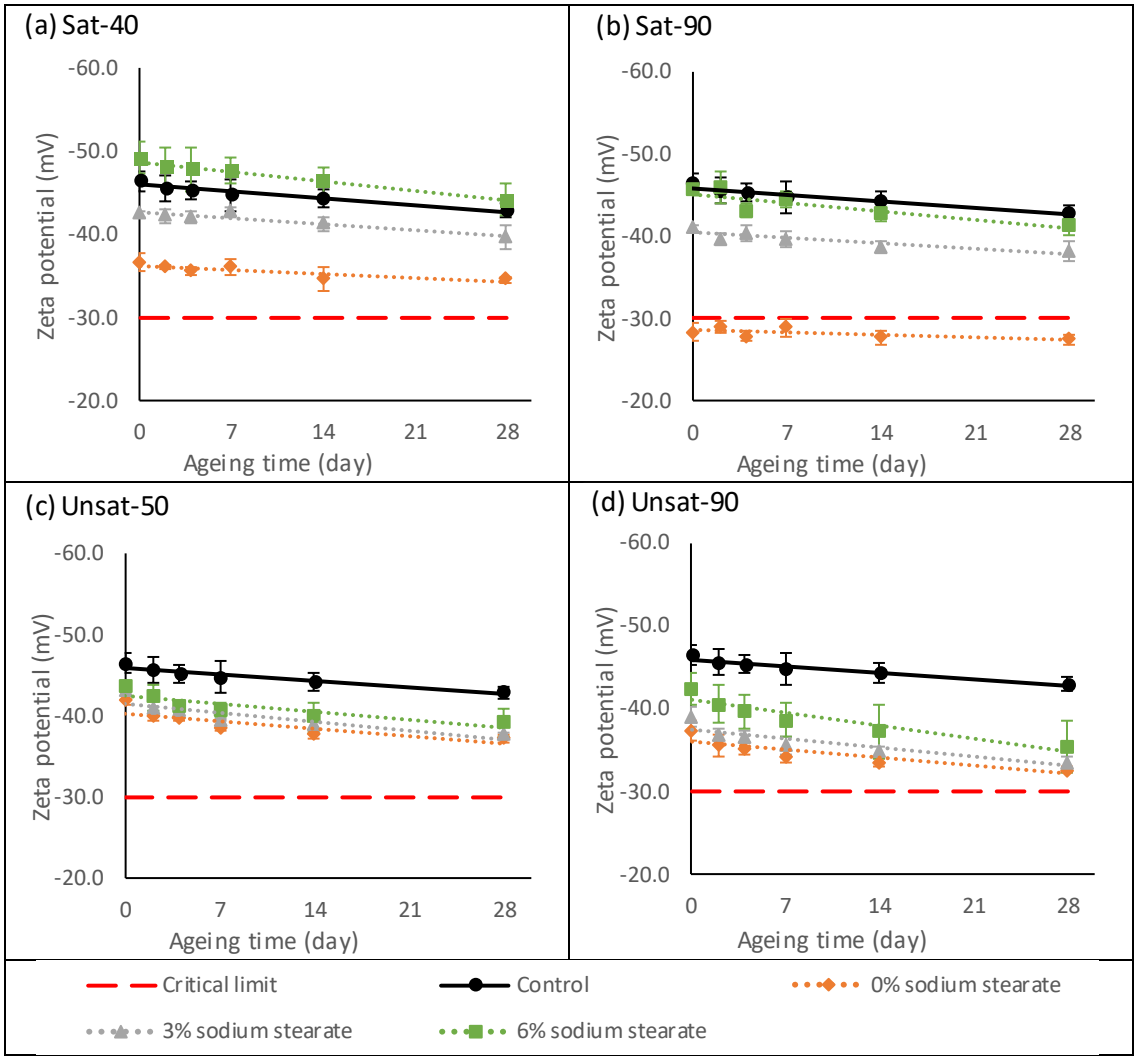


Figure 3.5 Zeta potential of emulsions prepared with different mono- and diglyceride compositions and sodium stearate content over 28 days of ageing. Points are mean zeta potential and the error bars are the standard deviation. All points have 4 replicates except for the control (n=8).

Figure 3.6 shows the apparent viscosity at 100 s^{-1} for all emulsions over the 28 days storage time. The viscosity of all samples, except control emulsion, changed significantly during storage ($p<0.05$). The change in viscosity could be due to the rearrangement of the milk proteins and mono- and diglycerides at the interface. Emulsions prepared with saturated mono- and diglycerides increased in viscosity during the storage, while emulsions with unsaturated mono- and diglycerides decreased in viscosity to a small degree. There was no change in the viscosity of the control emulsion. While the change in apparent viscosity in emulsions with mono- and diglycerides was statistically significant ($p<0.05$); however, the change in magnitude was small (1.5-2.0 mPa s).

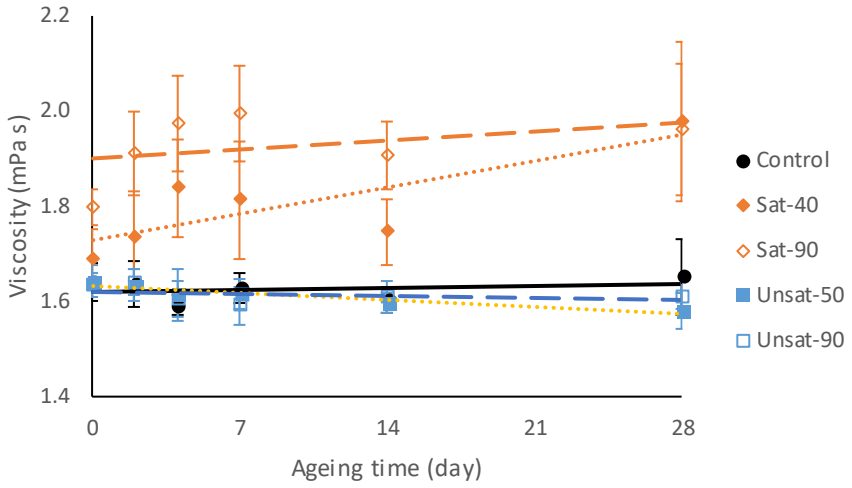


Figure 3.6 Apparent viscosity at 100 s^{-1} as a function of ageing time. Points are mean apparent viscosity and the error bars are the standard deviation. All points have 12 replicates except for the control (n=8).

All emulsions had a stable pH (6.6-6.8) over the storage period, suggesting that there was no occurrence of chemical reactions in the emulsions and that the amount of sodium azide (0.02%) was effective in preventing microbial activity.

Figure 3.7 shows the creaming index of the emulsions prepared with mono- and diglycerides and different sodium stearate content over 28 days storage time. The creaming index explains the thickness of the cream layer formed in the emulsions at a particular storage time. A high creaming index means an emulsion forms a thick cream layer and vice versa for the low creaming index. The ideal emulsion should have 0% creaming index, but all emulsions demonstrated an increase in creaming index over time.

The control formulation had the greatest creaming index, reaching 2.1% after 28 days storage. Any mono- and diglyceride composition that shows lower creaming index than the control is of interest to understand its properties.

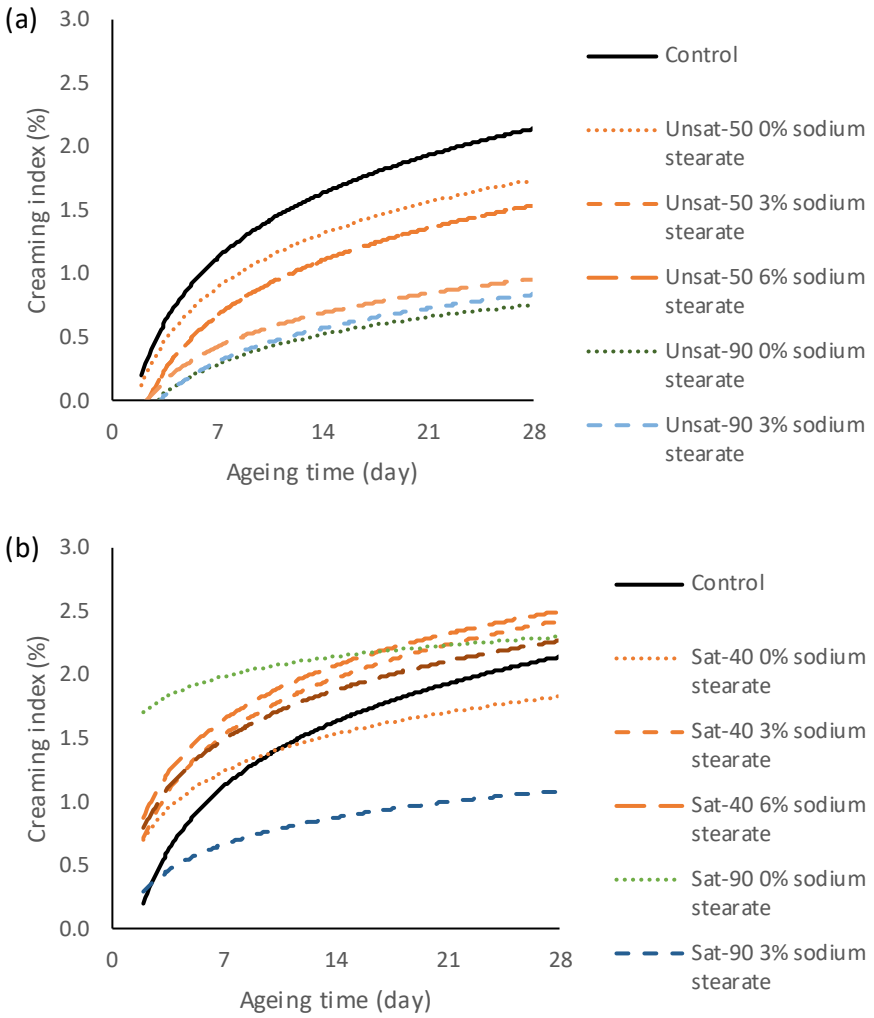


Figure 3.7 Creaming index as a function of ageing time for the emulsions prepared using (a) unsaturated and (b) saturated mono- and diglyceride composition with 0, 3 and 6% sodium stearate content. Points are mean creaming index with 4 replicates except for the control (n=8).

Figure 3.7a shows the change in creaming index over time for different unsaturated mono- and diglyceride compositions, while **Figure 3.7b** shows the change for saturated mono- and diglyceride compositions. All emulsions with unsaturated mono- and diglycerides (**Figure 3.7a**) had a lower creaming index compared to the control at 28 days storage. Unsaturated mono- and diglycerides with 90% monoglyceride content was

more effective in improving creaming stability compared to 50% monoglyceride content. There was no influence of sodium stearate on the unsaturated mono- and diglycerides. Creaming stability was significantly different between samples ($p<0.05$) and increased stability in this order: Control < 50% monoglyceride < 90% monoglyceride. The ability of unsaturated mono- and diglycerides to form droplets with a narrow distribution (lowest polydispersity index = 0.17) is speculated to contribute to the lower creaming index, and thus a more stable emulsion. A narrow size distribution means homogeneity in droplet size with few extremely small and large droplets. Large droplets have a faster rate of creaming compared to smaller ones and are more likely to separate due to gravity according to Stokes' Law (McClements, 2007). Thus, reducing the number of large droplets should delay the occurrence of creaming. This finding is in agreement with Liang et al. (2016), Matsumiya et al. (2010) and Krog (2011) in that droplet size distribution is a critical factor affecting creaming stability of oil-in-water emulsions.

Figure 3.7b shows that only the Sat-90 emulsion with 3% sodium stearate had an improved creaming index ($p<0.05$), i.e. the amount of creaming observed was very low, compared to the control at 28 days storage. The rest of the emulsions with saturated mono- and diglycerides did not improve stability of emulsion against creaming. Droplet size and the size-range distributions did not show any specific trend with creaming index; although an optimised level of monoglyceride content and sodium stearate may form a stable emulsion against creaming. This study shows that emulsions with saturated mono- and diglycerides work in a different manner compare to unsaturated mono- and diglycerides, which could be attributed to the high melting point of the saturated mono- and diglycerides leading to crystallisation of the emulsifier, protein desorption from the interface and the eventual cream separation. The stable droplet size and the size-range distributions and minimal viscosity change during storage suggested that the emulsion droplets did not undergo coalescence and flocculation, but this is unable to fully explain the creaming index, as there is emulsifier crystallisation potentially occurring at the interface.

McClements (2015) reported that the critical size to achieve stability for most beverages is around 300 nm and all emulsions produced in this study have mean droplet size between 130 and 180 nm, indicating that the emulsions are stable.

3.4 Conclusions

This study evaluated the effect of different mono- and diglyceride composition in a protein-stabilised model emulsion. Unsaturated mono- and diglycerides showed the best homogeneity in droplet size distribution and creaming stability when compared to saturated mono- and diglycerides and the control. High monoglyceride content was effective in achieving small droplet size and a narrow size distribution. This knowledge on the effect of monoglyceride content can be used to manipulate the droplet size distribution of the emulsion. Unsaturated mono- and diglycerides are more effective than saturated mono- and diglycerides in providing good emulsion stability to prevent creaming. Optimisation of sodium stearate is required to improve creaming stability when using saturated mono- and diglycerides, however, it had no effect with unsaturated mono- and diglycerides. This information is useful to develop a protein-stabilised emulsion with uniform particle suspension that allows it to remain stable on the shelf for a longer time.

Chapter 4

Effect of Milk Protein Composition on Physicochemical Properties, Creaming Stability and Volatile Profile of a Protein-Stabilised Oil-in-Water Emulsion

Part of the contents presented in this chapter has been published previously as a peer-reviewed journal article: Loi, C. C., Eyres, G. T., & Birch, E. J. (2019). Effect of milk protein composition on physicochemical properties, creaming stability and volatile profile of a protein-stabilised oil-in-water emulsion. *Food Research International, 120*, 83-91. doi:10.1016/j.foodres.2019.02.026

4.1 Introduction

Protein-stabilised oil-in-water emulsions are the basis for many food products, such as milk, yoghurt, whipping cream, ice-cream, gravies, mayonnaise and salad dressings (McClements, 2015). The oil droplets of these emulsions are formed and stabilised by milk protein molecules, which consist of hydrophilic and hydrophobic groups. Milk proteins are surface active molecules, which adsorb to the oil-water interface, lower the interfacial tension and form a protective layer surrounding the oil droplets (Walstra, 2002). These proteins at the interface also provide repulsive forces such as steric and electrostatic forces between droplets, which improve oil droplet stability in the emulsions (Tcholakova et al., 2006). The repulsive forces prevent oil droplets coming together, hence slowing the rate of creaming, flocculation and coalescence, and the eventual phase separation of emulsions.

Milk proteins such as casein and whey proteins are widely used as food ingredients with excellent emulsifying properties in food formulations (Dickinson, 1997; Sliwinski et al., 2003; Wilde et al., 2004). It is also common in food products to include an additional low-molecular-weight emulsifier, such as monoglycerides (Fredrick et al., 2013), to enhance physicochemical properties and emulsion stability of a protein-stabilised emulsion (McClements, 2004).

The most common destabilisation phenomenon in protein-stabilised emulsions is creaming, which is an upward movement of oil droplets due to their lower density compared to the aqueous phase (S. R. Euston & Hirst, 2000; McClements, 2015). In **Chapter 3**, the interaction between various monoglyceride compositions and milk proteins showed that emulsions with unsaturated distilled monoglyceride (Unsat-90) had the greatest stability against creaming after 28 days of ageing as a result of uniformly distributed small oil droplets at the interface (Dickinson & Hong, 1994; Matsumiya et al., 2010). Therefore, Unsat-90, which is also known as glycerol monooleate (GMO), was used as secondary emulsifier in protein-stabilised emulsions to investigate its interaction with different ratio of casein and whey protein affecting emulsion properties and stability.

Oxidative stability of lipid is an important quality parameter for food products. Protein-stabilised emulsions are generally known to protect oil droplets from oxidation (Donnelly

et al., 1998; Elisia & Kitts, 2011; Gumus, Decker, & McClements, 2017; Mei et al., 1999). Milk proteins such as casein and whey proteins have been reported to inhibit lipid oxidation via chelation of transition metal ions or scavenging free radicals (Donnelly et al., 1998; Faraji et al., 2004). The metal chelation of unadsorbed casein in the aqueous phase will increase oxidative stability (Berton et al., 2012; Gumus et al., 2017). However, it has been reported that metal chelation occurring at the interface may reduce oxidative stability (Villiere et al., 2005). Milk proteins can also form a barrier at the interface to isolate the interaction between oil and pro-oxidants (Donnelly et al., 1998) to increase oxidative stability. Different milk protein compositions may also exert different oxidative stability during ageing (Berton-Carabin et al., 2014). One of the methods to determine oxidative stability in emulsions is to measure volatile secondary products by headspace solid-phase microextraction (HS-SPME) with gas chromatography-mass spectrometry (GC-MS). This method is widely used to study secondary products of lipid oxidation (Damerau et al., 2014).

The high stability of all the model emulsions at room temperature in **Chapter 3** had resulted in a thin cream layer that lacked discrimination between samples. A pilot study was carried out to reformulate the model emulsions and storage conditions using different oil levels, oil types and storage temperatures to improve the discrimination between samples on creaming stability. In the pilot study, emulsions with 1.1% hydrogenated coconut oil (Chapter 3 formulation), 4% hydrogenated coconut oil and 4% canola oil at three different storage temperatures (4, 25 and 45 °C) for 28 days showed that emulsions with GMO consistently had smaller droplet size and greater creaming stability than control emulsions (no GMO). A model emulsion with canola oil is appropriate for the investigation on oxidative stability due to the present of unsaturated fatty acids that react with oxygen. The protein-stabilised emulsions without GMO is expected to show a thicker cream layer than the emulsions with GMO, however, was not carried out as part of this experiment. This study aims to understand the effect on milk protein composition on physicochemical properties, creaming stability and oxidative stability in protein-stabilised emulsions containing GMO. This study is useful to food technologists to optimise the use of ingredients in formulations to achieve good stability against creaming and increased oxidative stability in products.

4.2 Materials and Methods

4.2.1 Materials

Glycerol monooleate (Radiamuls MG 2905K) (containing at least 90% monoglyceride content) was a gift from Oleon (Klang, Malaysia). Sodium caseinate and whey protein concentrate 80% (WPC) were provided by Tatua Co-operative Dairy Company Ltd. (Morrinsville, New Zealand). Refined canola oil and caster sugar were purchased from the local supermarket. Sodium azide was purchased from Sigma-Aldrich (St. Louis, MO, USA).

4.2.2 Preparation of model emulsions

Five model emulsions with different milk protein compositions (5 different ratios of sodium caseinate and WPC) were prepared by microfluidisation. Each sample formulation was prepared in duplicate. Samples were prepared on the same day in a randomised block design to evaluate emulsion repeatability. Water-phase ingredients consisted of five different milk protein compositions (1.0% w/w) (**Table 4.1**), caster sugar (6% w/w) and sodium azide (0.02% w/w), reconstituted in Milli-Q water at 50 °C. The concentration of milk protein and caster sugar in the model emulsions were designed to mimic a ready-to-drink dairy beverage. Sodium azide was used to protect against effects of microbial contamination during creaming, emulsion stability and volatile profile analyses. The water-phase was continuously agitated using an Ultra-Turrax (IKA-Werke GmbH and Co. KG, Stufen, Germany) at 10,000 rpm and was simultaneously heated to 75 °C. GMO (0.2% w/w) was added to canola oil (4% w/w) at 60 °C to form the oil-phase. The oil-phase was mixed with the water-phase solution, which was then homogenised for three min at 75 °C to form a coarse emulsion. Oil content of the final emulsions was 4.2% w/w. The coarse emulsion was passed through a microfluidiser (Microfluidics Corporation, Massachusetts, USA) using a single pass at 55 MPa and 65 °C (same as **Section 3.2.2**). The microfluidiser was pre-heated by circulating water at 70 °C. All model emulsions were stored securely either in 100 mL bottles, clear test tubes (16 mm o.d. x 150 mm height) and 20 mL headspace vials with Teflon-lined septa for emulsion stability, creaming index and oxidative stability, respectively. The physical and oxidative stability of the emulsion droplets was evaluated under accelerated conditions at 45 °C over a period of 1-28 days.

Table 4.1 Milk protein composition for model emulsion samples.

Sample	Description
Cas-0	100% whey protein concentrate (WPC)
Cas-20	20% sodium caseinate and 80% WPC
Cas-50	50% sodium caseinate and 50% WPC
Cas-80	80% sodium caseinate and 20% WPC
Cas-100	100% sodium caseinate

4.2.3 Droplet size and polydispersity index of model emulsions

Droplet size and size-range distributions of the model emulsions were measured by dynamic light scattering using a Malvern Zetasizer Nano S (Malvern Instruments Ltd., Malvern, Worcestershire, UK). The measurements were carried out using procedures described previously in **Section 3.2.3**. Samples were diluted at 1:1000 with a 6% sucrose solution to keep the sample matrix and osmolarity constant. The diluted sample was analysed for 60 s at 25 °C with duplicate measurements carried out for each sample. The average droplet size was expressed as the intensity-weighted mean diameter (Z-average), and the degree of non-uniformity of a droplet size distribution was presented as the polydispersity index.

4.2.4 Zeta potential

Zeta potential of the model emulsions was determined using the Malvern Zetasizer Nano S. The procedure was described previously in **Section 3.2.4**. Samples were diluted at 1:20 with a 6% sucrose solution. The diluted sample was analysed at 25 °C with duplicate measurements carried out for each sample.

4.2.5 Viscosity

Viscosity of the model emulsions was performed using a double gap geometry (measuring cup DG42 RO and rotor DG43 DIN53544 Ti) and 11.5 mL sample volume at 20 °C with a Rheostress 1 (Haake, Karlsruhe, Germany) rheometer coupled with a Haake DC-30 circulating bath. Measurements were carried out in duplicate for each sample at the shear rate range of 1 to 500 s^{-1}.

4.2.6 pH

The pH of model emulsions was tested at room temperature using a pH209 (HANNA Instruments, Woonsocket, RI, USA) pH meter.

4.2.7 Creaming index

The creaming index of the emulsions was obtained by a visual observation method. The procedure was described previously in **Section 3.2.7**. Creaming measurements were made on the emulsions in clear test tubes sealed with screw cap lids, with extra precautions taken not to disrupt the cream layer. Creaming index, which is the ratio of cream height over total height of emulsion upon standing, was calculated according to McClements (2007).

4.2.8 Emulsion stability

All model emulsions stored in 100 mL bottles with screw caps were subjected to analysis on droplet size, polydispersity index, zeta potential, viscosity and pH. The bottles were shaken until the cream layer was well dispersed in the emulsion before performing any analysis at each time interval during the 28 day ageing period.

4.2.9 Headspace solid-phase microextraction (HS-SPME)

All emulsions were pre-weighed (5 g) into 20 mL headspace vials and the vial was capped with a Teflon-lined silicone rubber septum. The samples were aged at 45 °C over a period of 1-28 days and sampled at specified time intervals. The samples were then stored in a -20 °C freezer until all samples with different ageing times were collected, prior to analysis. All vials were covered with aluminium foil at all times to prevent chemical reaction with light. The extraction of volatiles was carried out with a PAL RSI 85 multipurpose sampler (CTC Analytics, Bern, Switzerland) at 45 °C. The samples were equilibrated at 45 °C for 5 min, before the SPME fibre (50/30 μm divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS)) (Supelco, Bellefonte, PA, USA), was exposed for 40 min. After the extraction, the SPME fibre was injected into the gas chromatograph inlet for desorption and analysis (see **Section 4.2.10**). Six headspace analyses were performed on each emulsion formulation (2 batches x 3 analytical replicates per batch).

4.2.10 GC-MS analysis

The analyses of headspace volatiles in the samples were carried out with an Agilent GC 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 5975B VL MSD quadrupole mass spectrometer (Agilent Technologies, Wilmington, DE, USA). SPME desorption was carried out at 240 °C for 2 min in splitless mode, followed by 3 min in split mode. Following sample desorption, the SPME fibre was conditioned for 2 min at 270 °C with a purge flow of 50.0 mL min^{-1}. Pure helium gas was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The chromatographic separation was performed on a 60 m x 0.32 mm i.d. x 0.5 μm Zebron ZB-WAX column (Phenomenex, Torrance, CA, USA). The oven temperature was held for 5 min at 40 °C, increased at a rate of 5 °C min^{-1} until 190 °C, then subsequently increased at a rate of 10 °C min^{-1} to 240 °C and held for 5 min. The transfer line to the MS was set to 230 °C and the quadrupole was set to 150 °C. The mass spectrometer was operated at a scan speed of 5.1 scans s^{-1} and mass spectra were recorded in the range of 29-300 m/z. Carryover between GC runs was assessed using 2 blank samples (an empty vial and a vial containing deionised water only) after every 7 or 8 samples. A randomised block of 15 samples was run on each day and the analyses were performed over a period of 6 days.

The Agilent Enhanced MSD Chemstation software (version F.01.01.2317, Agilent Technologies Inc., USA) was used to process the chromatogram and data from the mass spectrometer. Semi-quantification of volatile compounds was carried out on the total ion count (TIC) peak areas. Alignment of the chromatograms was carried out manually. Volatile compounds were identified based on their mass spectra and supported by their retention index (RI). Mass spectra were compared to the National Institute of Standards and Technology database (NIST14). Linear retention indices were calculated using a C9-C17 n-alkane series as reference compounds using the following equation:

$$\mathrm{RI}(x) = (100 \times z) + \frac{\mathrm{RT}(x) - \mathrm{RT}(z)}{\mathrm{RT}(z+1) - \mathrm{RT}(z)} \qquad \textbf{Equation 4.1}$$

Where RI(x) is the retention index of the unknown compound x, z is the number of carbon atoms of the n-alkane eluted before the unknown compound x, z+1 is the number of carbon atoms of the n-alkane eluted after the unknown compound x, RT(x) is the retention time of the unknown compound x, RT(z) is the retention time of the n-alkane eluted

before the unknown compound *x*, RT(*z*+1) is the retention time of the n-alkane eluted after the unknown compound *x*.

4.2.11 Statistical analysis

IBM SPSS Statistics Version 23 (IBM Corporation, Armonk, New York, USA) was used for statistical comparison of the results. One-way analysis of variance (ANOVA) was carried out to determine significant differences between the results, followed by Tukey's post hoc test for pairwise comparison. All tests were performed at the 95% confidence level.

4.3 Results and Discussion

4.3.1 Droplet size and polydispersity index of model emulsions

Table 4.2a shows the droplet size of model emulsions prepared with different milk protein compositions over 28 days of ageing at 45 °C. As GMO concentration is constant for all formulations, the change in droplet size is due to the protein type rather than GMO. Droplet sizes of fresh emulsions prepared with different milk protein compositions were significantly different ($p<0.05$). Fresh emulsions with only sodium caseinate (Cas-100) had a significantly smaller droplet size (176.1 nm) than the fresh emulsions with only WPC (Cas-0; 191.3 nm). This effect is due to the flexible structure of sodium caseinate, which can rapidly adsorb at the oil-water interface and form smaller oil droplets compared to the globular WPC (Cao & Damodaran, 1995; Damodaran, 2004; Dickinson, 1997). Fresh emulsions with different ratios of sodium caseinate and WPC formed oil droplets with intermediate sizes compared to Cas-0 and Cas-100.

All emulsions prepared with different milk protein compositions had a small reduction in droplet size (<4 nm) during ageing for 28 days at 45 °C. However, the changes in droplet size are so small that can be considered negligible. This finding agrees with the results on protein-stabilised emulsions in **Chapter 3** that indicated no change in droplet size over ageing time and no occurrence of coalescence (Berton, Genot, & Ropers, 2011; J. Chen & Dickinson, 1993).

Table 4.2b shows the polydispersity index of model emulsions over 28 days of ageing at 45 °C. In general, all emulsions achieved a polydispersity index below 0.2, which is an indication of a narrow size range distribution. The intensity-weighted size distribution

showed a monomodal size distribution for all fresh and aged emulsions (**Appendix B.1**). The monomodal size distribution is due to the sufficiently high pressure at the microfluidiser and is consistent with literature findings (Chung, Sher, Rousset, & McClements, 2017; Kuhn & Cunha, 2012). The polydispersity index of fresh emulsions prepared with different milk protein compositions was significantly different ($p<0.05$). Cas-0 had the highest polydispersity index and differed significantly from all the emulsions that contain sodium caseinate between 20% and 100%, except at 28 days of ageing. There was no significant difference ($p>0.05$) in the polydispersity index among the emulsions with different milk protein compositions at 28 days of ageing. This could be due to the rearrangement of the WPC structure at the oil-water interface (Nylander et al., 2008). The results on droplet size and polydispersity index of the model emulsions with different milk protein compositions showed that sodium caseinate had a greater ability to produce smaller, more uniform oil droplets compared to WPC.

Table 4.2 Droplet size and polydispersity index of model emulsions at different ageing times.

A) Droplet size (nm)

Sample	Day 0	Day 7	Day 14	Day 28
Cas-0	191.3 ± 4.3^{d}	192.5 ± 5.6^{c}	192.0 ± 5.9^{c}	189.6 ± 3.8^{c}
Cas-20	184.7 ± 1.2^{bc}	183.8 ± 1.1^{b}	183.8 ± 0.9^{b}	183.1 ± 0.8^{b}
Cas-50	187.5 ± 1.4^{cd}	185.5 ± 1.8^{b}	185.0 ± 0.8^{b}	183.3 ± 0.9^{b}
Cas-80	179.6 ± 2.4^{ab}	176.8 ± 1.1^{a}	177.0 ± 1.5^{a}	176.0 ± 2.0^{a}
Cas-100	176.1 ± 0.9^{a}	174.4 ± 0.7^{a}	174.7 ± 0.4^{a}	173.4 ± 1.0^{a}
F-value	25.8	27.8	24.2	40.9
p-value	<0.001	<0.001	<0.001	<0.001

B) Polydispersity index

Sample	Day 0	Day 7	Day 14	Day 28
Cas-0	0.201 ± 0.019^{b}	0.192 ± 0.014^{c}	0.193 ± 0.014^{b}	0.184 ± 0.018^{a}
Cas-20	0.159 ± 0.007^{a}	0.172 ± 0.002^{ab}	0.175 ± 0.003^{ab}	0.170 ± 0.010^{a}
Cas-50	0.174 ± 0.006^{a}	0.179 ± 0.005^{bc}	0.172 ± 0.010^{ab}	0.175 ± 0.008^{a}
Cas-80	0.162 ± 0.011^{a}	0.168 ± 0.004^{ab}	0.173 ± 0.011^{ab}	0.162 ± 0.009^{a}
Cas-100	0.158 ± 0.003^{a}	0.161 ± 0.004^{a}	0.158 ± 0.010^{a}	0.166 ± 0.012^{a}
F-value	11.5	10.9	5.9	2.1
p-value	<0.001	<0.001	<0.01	0.14

Values are means ± standard deviations of 4 measurements (2 batches x 2 replicates). Different letters in the same column indicate statistically significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test.

4.3.2 Zeta potential of model emulsions

Table 4.3 shows the zeta potential of model emulsions prepared with different milk protein compositions over 28 days of ageing at 45 °C. For the ease of discussing the results, zeta potential refers to the magnitude and its negative sign is ignored because all model emulsions display a negative zeta potential. In this context, an increase in zeta potential refers to the increase in the negativity of the zeta potential.

Table 4.3 Zeta potential (mV) of model emulsions at different ageing time.

Sample	Day 0	Day 7	Day 14	Day 28
Cas-0	-38.1 ± 0.7^{e}	-37.9 ± 0.4^{e}	-40.5 ± 1.1^{c}	-38.7 ± 0.5^{d}
Cas-20	-40.7 ± 0.3^{d}	-40.1 ± 0.4^{d}	-41.5 ± 1.8^{c}	-41.5 ± 0.6^{c}
Cas-50	-44.1 ± 0.4^{c}	-42.5 ± 1.2^{c}	-42.7 ± 0.5^{c}	-42.5 ± 0.4^{c}
Cas-80	-48.4 ± 1.0^{b}	-46.1 ± 0.6^{b}	-47.4 ± 0.9^{b}	-47.1 ± 0.7^{b}
Cas-100	-50.3 ± 0.7^{a}	-50.2 ± 0.8^{a}	-51.6 ± 0.5^{a}	-51.2 ± 0.5^{a}
F-value	223.9	176.9	75.9	325.1
p-value	<0.001	<0.001	<0.001	<0.001

Values are means ± standard deviations of 4 measurements (2 batches x 2 replicates). Different letters in the same column indicate statistically significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test.

The zeta potential provides information about changes of the environment around the oil droplet caused by changes in milk protein composition. The zeta potentials of fresh emulsions prepared with different milk protein compositions were significantly different ($p<0.05$). Cas-0 emulsions showed the lowest zeta potential while the highest zeta potential was in Cas-100 emulsions. An increase in zeta potential was observed with the increased ratio of sodium caseinate in the emulsions. The same phenomenon was observed in the emulsions after ageing at 45 °C.

The zeta potential of emulsions prepared with different milk protein compositions was significantly different ($p<0.05$) over 28 days of ageing. The changes in the zeta potential value (<2 mV) during ageing were small and may not have any practical implication. Regression analysis was applied to find out if there is any correlation between the ratio of sodium caseinate and zeta potential in the fresh and aged emulsions. **Figure 4.1** shows a linear relationship between the ratio of sodium caseinate and zeta potential in the (a) fresh emulsions ($R^2 = 0.98$) and (b) after 28 days of ageing ($R^2 = 0.94$). In the absence of sodium caseinate (Cas-0), both fresh and aged emulsions had a mean zeta potential of -38 mV and this value can be assigned to WPC as the control emulsion. Both figures demonstrate that zeta potential increased when more sodium caseinate was present in the emulsions with little or no influence from GMO. In a high fat (25%) emulsion system, Munk et al. (2014) showed that 0.2% GMO has little effect on zeta potential, which can be explained by the low displacement of protein from the oil-water interface. Dalgleish

et al. (1995) reported that low-molecular-weight emulsifier at 0.2% level had minimal effect on protein adsorption rate at the interface of a 20% fat emulsion, and increasing the concentration of proteins had no effect to the structures formed at the interfacial layer. The structure at the interface affects zeta potential, droplet size and viscosity of the emulsions (Dalgleish et al., 1995).

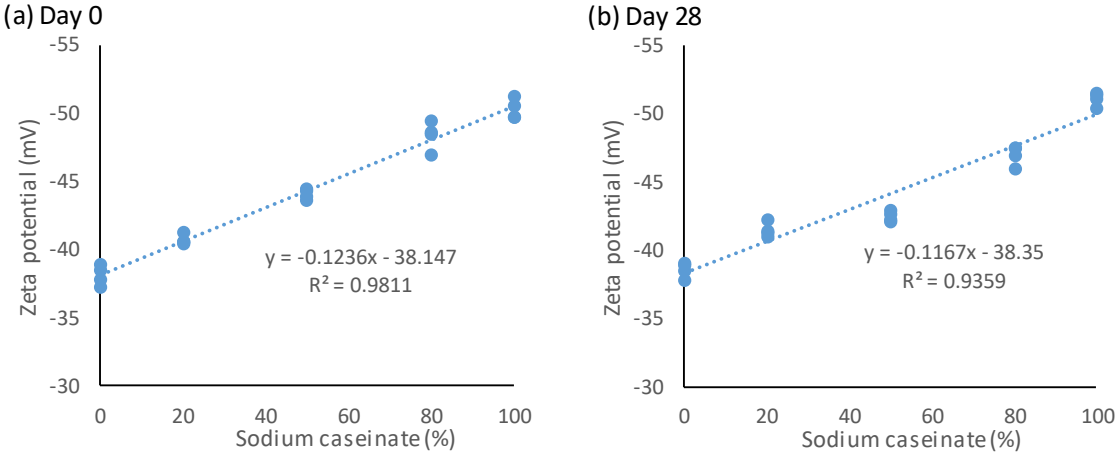


Figure 4.1 Relationship between zeta potential and sodium caseinate content in the (a) fresh model emulsions and (b) after 28 days of ageing (n=4).

4.3.3 Viscosity of model emulsions

Figure 4.2 shows a linear relationship between shear stress and shear rate, which implies that all fresh emulsions observed the flow behaviour of a Newtonian fluid. This observation was anticipated due to the low protein concentration, absence of thickening agents such as starch and gum, and contain high water content in the formulation. The same flow behaviour was observed in the emulsions at different ageing time (**Appendix B.2**). Thus, all emulsion viscosities were reported as the adjusted value according to the Newtonian model (R^2>0.99).

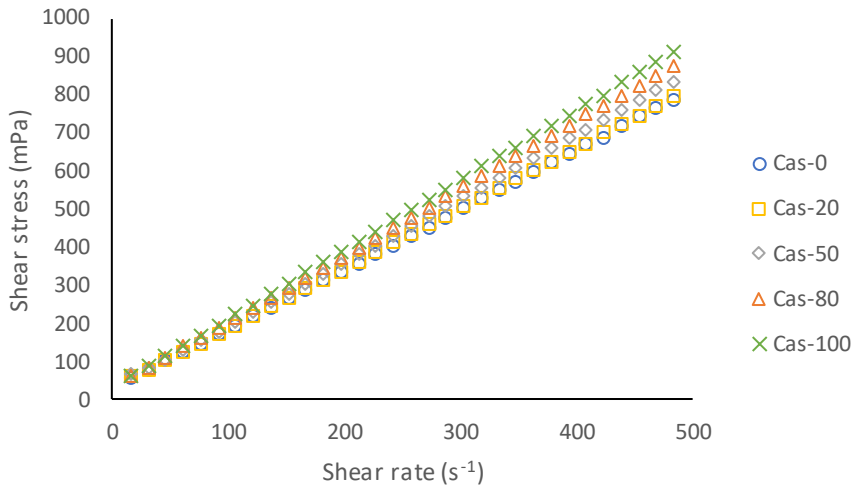


Figure 4.2 Rheological properties of fresh emulsions prepared with different milk protein compositions.

Figure 4.3 shows the viscosity of model emulsions with different milk protein compositions over 28 days of ageing at 45 °C. The viscosity of the fresh emulsions ranged from 1.6-1.9 mPa s. Statistical analysis shows that the viscosity of fresh model emulsions with different milk protein compositions was significantly different ($p<0.05$). The viscosity increased by up to 14% as the proportion of sodium caseinate in the emulsion was increased from 0% to 100% sodium caseinate. The viscosities of the emulsions were affected by sodium caseinate level in the formulation. Dickinson and Ritzoulis (2000) also reported that higher sodium caseinate concentrations increased viscosity of emulsions. The increase in viscosity can also be explained as a function of droplet size. In a constant volume, the number of droplets will increase when sodium caseinate reduces the droplet size in the emulsion, and also increase the interactions between droplets. This interaction will eventually cause an overall increase in viscosity.

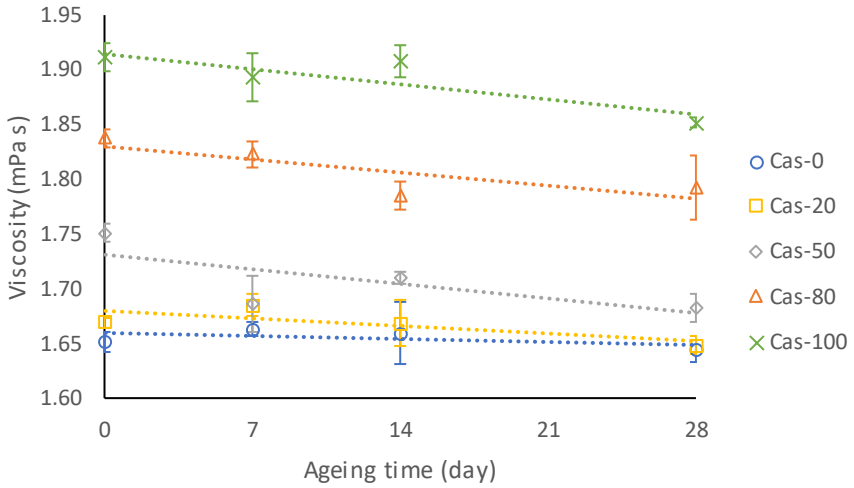


Figure 4.3 Viscosity of model emulsions with different milk protein compositions as a function of ageing time. Points are mean viscosity and the error bar are the standard deviation (n=4).

4.3.4 pH of model emulsions

The pH of all fresh emulsions was similar (pH 6.6-6.8), decreasing slightly (<0.2) during ageing with no obvious oil separation or protein aggregation in the emulsions. The different ratio of sodium caseinate and WPC had no effect on the pH of all model emulsions. This finding was expected because sodium caseinate and WPC have similar pH in the solution according to information provided by the supplier.

4.3.5 Creaming index of model emulsions

Unlike the findings in **Chapter 3** on emulsion systems with different mono- and diglyceride compositions, no differences in creaming index were found for the emulsions with different protein compositions over 28 days of ageing at 45 °C. GMO was selected as a low-molecular-weight emulsifier in this study as a continuation from **Chapter 3**, where GMO demonstrated good creaming stability in protein-stabilised emulsions, similar or better than other monoglycerides and control (without monoglycerides). Although the emulsions with different protein compositions showed significant differences in term of droplet size, zeta potential and viscosity, its effects on the cream layer were too small for a visual observation in this study. This finding was in agreement with S. R. Euston and Hirst (2000), where emulsions containing low levels of milk protein and GMO normally show no significant relationship between protein

composition and creaming index (S. R. Euston & Hirst, 2000). A thin layer of cream (creaming index at 0.9) started to develop after 2 days at 45 °C for all emulsions. The cream layer did not develop further after 7 days (maximum creaming index at 1.7) and indicated that the emulsions were stable for the rest of ageing until 28 days. This observation was supported by the stable readings in term of droplet size, zeta potential and viscosity. The small change in pH after ageing also did not affect emulsion droplet properties and creaming stability. Many studies have shown that creaming does not significantly change the mean droplet size (Dickinson, 1997; Ye & Singh, 2006), which manifested in this study. Emulsions with small droplets and a narrow size distribution are also stable during long-term storage (Huang, Kakuda, & Cui, 2001).

4.3.6 Oxidative stabilty in model emulsions measured using volatile analysis

The formation of volatile compounds was monitored for all emulsions containing GMO with different protein compositions for various ageing times. Volatile compounds that increased significantly ($p<0.05$) during storage and had been reported as markers of lipid oxidation in the literature were regarded as compounds of interest in this study. Compounds with reduced or no change in concentrations during storage were not reported. Analysis of the volatile compounds using headspace SPME with GC-MS identified 18 compounds of interest in the model emulsions. **Figure 4.4** shows the summed peak areas for the 18 identified compounds of interest from emulsions with different milk protein compositions over 28 days of ageing at 45 °C. Peak areas from the GC total ion chromatogram (TIC) were reported as area units (au). All fresh emulsions with different milk protein compositions had the same amount of total selected volatiles (mean peak area of 994 million au) and a similar volatile profile. Four groups of chemical compounds were identified in all fresh emulsions, namely aldehydes, alcohols, ketones and furans. Aldehydes (88.6% of total peak area) were the largest group of chemical compounds in the volatiles of fresh emulsions, followed by alcohols (7.0%), ketones (4.2%) and furans (0.2%). Hexanal was the aldehyde with the largest peak area in fresh emulsions (81.3% of total peak area for all the identified compounds).

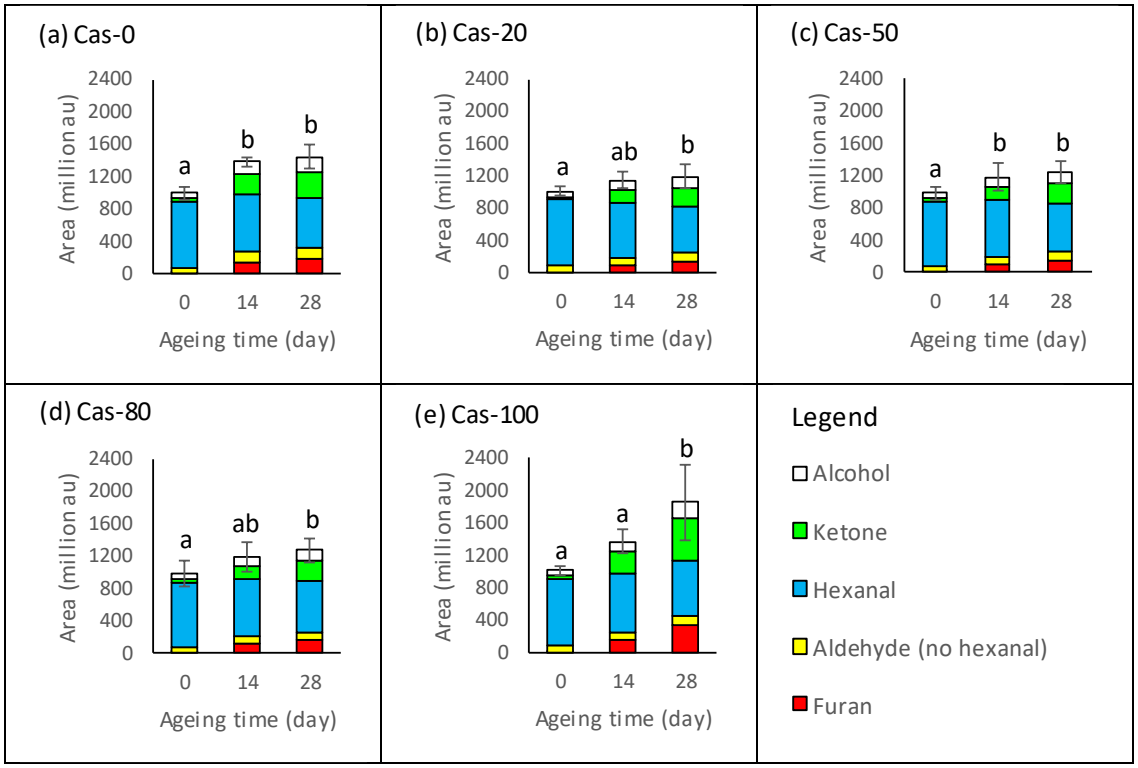


Figure 4.4 Summed peak area of 18 identified compounds of interest from emulsions with different milk protein compositions during ageing. Different letters in the same bar chart indicate statistically significant differences ($p<0.05$) between days for the same sample by Tukey post-hoc multiple comparison test. Error bars are standard deviations of 6 measurements (2 batches x 3 replicates).

The total peak area for all samples changed significantly during ageing ($p<0.05$). Cas-100 showed the largest change in total peak area after 28 days of ageing, where the total peak area increased by 83.7% compared to the fresh emulsion, followed by Cas-0 (45.9% increase), Cas-80 (30.2%), Cas-50 (26.4%) and Cas-20 (18.2%). The changes in total volatiles were greater in a single type of milk protein, i.e. sodium caseinate or WPC, rather than with a protein blend. Others have shown similar differences in oxidative stability between single and multiple protein emulsifiers at the interfaces (Berton-Carabin, Genot, Gaillard, Guibert, & Ropers, 2013; Berton et al., 2012; Faraji et al., 2004). The total volatile development in the emulsions with mixtures of sodium caseinate and WPC were at least 35% lower than the single type milk protein (Cas-0; Cas-100).

The milk protein composition present at the interface may influence the development of volatiles in the model emulsions. The highest volatile development rate after 28 days of

ageing occurred in the Cas-100 emulsion, followed by Cas-0 emulsion. Cas-100 emulsions exhibited smaller droplet size than Cas-0 emulsions at the various ageing time (**Table 4.2**). The smaller droplet size of the Cas-100 emulsions can be translated into a larger oil surface area available for interaction with oxygen, and thus, undergo a higher rate of oxidation. Others have shown that oxidation rate is affected by droplet size (Berton et al., 2012; Frankel, Satué-Gracia, Meyer, & German, 2002; Genot et al., 2003; Lethuaut, Métro, & Genot, 2002; van Ruth, Roozen, Posthumus, & Jansen, 1999). The protein composition contributing to the charges at the interface is another factor affecting lipid oxidation. The zeta potential results in **Table 4.3** showed that Cas-100 emulsion had the highest charge at interface and Cas-0 had the lowest charge, which in turn resulted in higher oxidation rate in Cas-100 emulsion. Faraji et al. (2004) demonstrated that emulsions with sodium caseinate had better oxidative stability than whey protein isolate, and this was attributed to the higher zeta potential in sodium caseinate than whey protein isolate. The emulsion with only WPC in this study had a lower zeta potential than sodium caseinate and as a result, it showed a lower oxidation rate than sodium caseinate. This can be explained by the inherent negative charge of sodium caseinate making it a good iron-binder (Faraji et al., 2004; Villiere et al., 2005). When the adsorbed sodium caseinate binds with iron, the free iron will get very close to the oil droplet to promote oxidation and increase oxidation rate.

Droplet size and zeta potential may explain the higher oxidation rate in the emulsions with only sodium caseinate or WPC, but they cannot explain the lower oxidation rate in the emulsion with mixtures of sodium caseinate and WPC. It seems that the interface with multiple protein emulsifiers behaved differently from the single protein emulsifier. This could be explained by the synergistic effect of sodium caseinate, WPC and GMO at the interface to form a multilayer interface due to the different molecular sizes. GMO and sodium caseinate are small molecules that are able to fill between the gaps of WPC during adsorption to the interface. This would form a compact interface that acts as a barrier to reduce oil droplet interaction with oxygen. Many studies showed that a thicker interface formed by protein-stabilised emulsion will enhance oxidative stability of the emulsions (Donnelly et al., 1998; Haahr & Jacobsen, 2008; McClements & Decker, 2000). Dalgleish et al. (1995) showed that the structures formed at the interface by casein and emulsifiers cannot simply be characterised by a ratio of adsorbed casein to emulsifier

at the interface, but rather exists as complex interactions between protein, emulsifier and oil due to the flexible molecules of the adsorbed casein.

The presence of unsaturated fatty acids such as oleic acid, linoleic acid and linolenic acid in canola oil and GMO indicate susceptibility to oxidation during preparation and ageing time. The mechanisms of lipid oxidation, in particular, free radical chain reactions, have been studied extensively (Kamal-Eldin et al., 2003). Unsaturated fatty acids react with radical oxygen to form unstable hydroperoxides, which are quickly decomposed to more stable secondary products such as carbonyl compounds, hydrocarbons, furans and other materials (Genot et al., 2003; Min & Lee, 1999).

Amongst the chemical groups detected (**Figure 4.4**), furan compounds increased by the greatest degree, from the mean peak area of 1.6 million au in the fresh emulsions to a mean peak area of 195.1 million au after 28 days of ageing. Furan compounds were detected at very low levels in the fresh emulsions and were mostly formed during ageing at 45 °C, which explains the dramatic increase in the emulsions over time. Furan compounds can be formed through thermal degradation, Maillard reaction and lipid oxidation via reactions with sugars, amino acids and unsaturated fatty acids (H. Cho & Lee, 2014; Van Lancker, Adams, Owczarek-Fendor, De Meulenaer, & De Kimpe, 2011; Yaylayan, 2006).

Ketone compounds increased from a mean peak area of 41.6 million au in the fresh emulsions to 311.3 million au after 28 days, alcohol compounds increased from 69.8 million au to 166.4 million au, and aldehyde compounds (without hexanal) increased from 73.1 million au to 105 million au due to lipid oxidation. However, hexanal decreased from 807.8 million au to 623.7 million au. ANOVA also showed that there was no significant difference ($p>0.05$) in hexanal among the emulsions with different milk protein compositions at 28 days (**Appendix B.3**). The reduction of hexanal after 28 days of ageing might due to the competitive adsorption between volatile compounds to the SPME fibre (Coleman, 1996). Hexanal was not useful as a marker in this study due to its high peak area in the fresh emulsions, despite many previous studies reporting it as a good marker for lipid oxidation (Aprea et al., 2006; Berton et al., 2012; Böttcher, Steinhäuser, & Drusch, 2015; Elisia & Kitts, 2011; Graciano-Verdugo et al., 2010; Sarkar, Arfsten, Golay, Acquistapace, & Heinrich, 2016; Zou & Akoh, 2015). The

separation of hexanal from the rest of the aldehyde compounds was also carried out to prevent the overshadowing effect of hexanal due to its large peak area.

Eleven compounds were identified as markers for lipid oxidation in this study that showed a significant difference between protein compositions during ageing (**Table 4.4**). The key criteria for the selection of lipid oxidation markers is that the volatile compounds must have been identified and used in the literature as secondary products of lipid oxidation. **Figure 4.5** shows the total peak area of the 11 lipid oxidation markers in emulsions with different milk protein compositions at 14 and 28 days of ageing. Cas-100 and Cas-0 emulsions showed the highest oxidation marker content at 14 days of ageing. The analysis on the oxidation markers showed similar findings with the volatile development rate where Cas-100 and Cas-0 emulsions with single protein type showed higher oxidation rates. Although Cas-0 emulsion had a higher oxidation rate until 14 days of ageing, the oxidation rate slowed down considerably at 28 days of ageing and it had similar oxidation markers with emulsions with mixtures of protein types. Cas-100 emulsion continued to have significantly higher oxidation markers ($p<0.05$) from the rest of the emulsions and this suggested oxidation is still actively taking place.

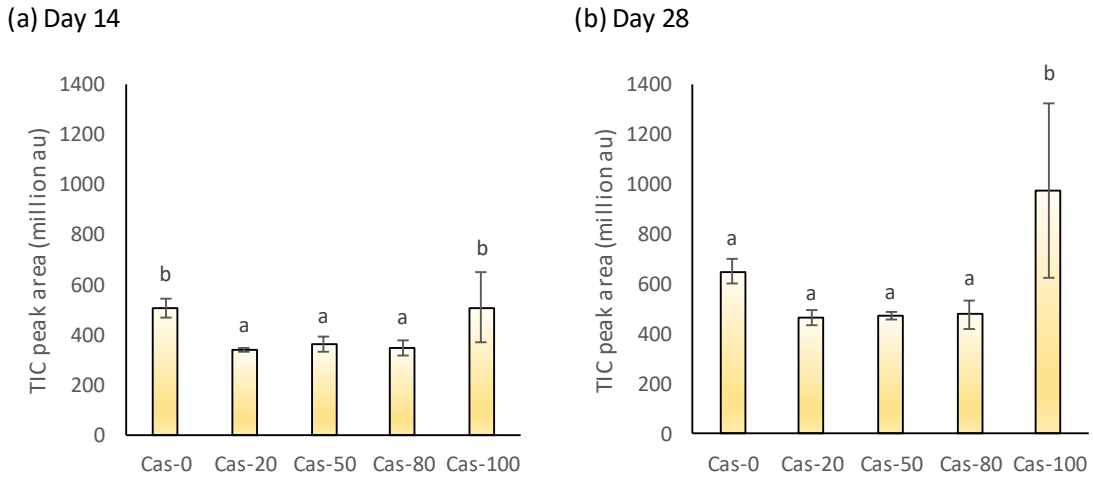


Figure 4.5 Total lipid oxidation markers for the emulsions with different milk protein compositions at (a) 14 days and (b) 28 days of ageing. Different letters in the bar chart indicate statistically significant differences ($p<0.05$) between emulsions with different milk protein compositions by Tukey post-hoc multiple comparison test. Error bars are standard deviations of 6 measurements (2 batches x 3 replicates).

Table 4.4 shows the peak area values and retention index of the oxidation markers at 28 days of ageing together with the unsaturated fatty acid as the likely source of oxidation. Five markers (2,4-heptadienal, 3,5-octadien-2-one isomer 1 and 2 (secondary oxidation products of linolenic acid) and 3-octen-2-one and 2-pentyl furan (linoleic acid)) had the highest relative peak area in Cas-100 emulsion. Two markers (octanal and nonanal derived from oleic acid) had the highest peak area in Cas-0 emulsion and the remaining four markers showed mixed results. Besides protein composition at the interface (discussed earlier), the unadsorbed protein in the emulsion may also affect the development of volatile markers during ageing. Faraji et al. (2004) showed that oxidation of omega-3 fatty acids can be protected by the unadsorbed protein in the aqueous phase. The location of the protein (adsorbed or unadsorbed) in the emulsions will also affect the lipid oxidation (Mei et al., 1999; Villiere et al., 2005).

While sodium caseinate was good at dispersing smaller and more uniform oil droplets, it had the lowest oxidative stability among the model emulsions at 28 days of ageing. The emulsions with only WPC had larger and less uniform oil droplets, and it did not show the best oxidation stability among the emulsions. The results showed that emulsions with mixtures of protein (Cas-20, Cas-50 and Cas-80) had intermediate oil droplet properties between Cas-0 and Cas-100 and much-improved lipid oxidation properties. This is potentially good news for the food industry, where product technologists can formulate beverages between 20% and 80% sodium caseinate without compromising the physical and chemical stability. This also offers an opportunity for the food industry to manage their material costs by lowering the sodium caseinate content, which is usually at a higher price than WPC.

Table 4.4 Lipid oxidation markers for emulsions after 28 days of ageing (45 °C) as a function of milk protein composition.

Retention index	Volatile compound	TIC peak area (million au)					Unsaturated fatty acid contributing to lipid oxidation*
		Cas-0	*Cas-20*	*Cas-50*	*Cas-80*	*Cas-100*	
959	2-Ethyl furan	39.06 ± 4.84^{ab}	20.07 ± 4.86^{a}	18.17 ± 3.89^{a}	17.53 ± 4.09^{a}	73.05 ± 49.74^{b}	n-3 linolenic acid
1239	2-Pentyl furan	113.01 ± 23.45^{a}	91.63 ± 10.21^{a}	99.47 ± 9.51^{a}	99.17 ± 16.82^{a}	191.79 ± 52.58^{b}	n-6 linoleic acid
1304	Octanal	67.24 ± 12.47^{b}	50.14 ± 4.60^{a}	50.20 ± 5.02^{a}	46.12 ± 8.07^{a}	50.10 ± 8.20^{a}	n-9 oleic acid
1410	Nonanal	61.46 ± 9.30^{b}	44.05 ± 6.17^{a}	43.37 ± 4.00^{a}	39.90 ± 6.07^{a}	45.33 ± 10.37^{a}	n-9 oleic acid
1429	3-Octen-2-one	12.92 ± 1.39^{a}	8.66 ± 0.44^{a}	9.27 ± 0. 47^{a}	9.92 ± 1.34^{a}	43.63 ± 28.90^{b}	n-6 linoleic acid
1451	1-Octen-3-ol	26.96 ± 1.19^{ab}	23.05 ± 0.96^{a}	21.35 ± 0.99^{a}	21.43 ± 1.26^{a}	33.53 ± 11.22^{b}	n-6 linoleic acid
1459	1-Heptanol	84.98 ± 9.51^{b}	61.23 ± 2.35^{a}	56.92 ± 5.44^{a}	53.03 ± 4.95^{a}	73.59 ± 25.75^{ab}	n-9 oleic acid
1536	2,4-Heptadienal	2.87 ± 0.63^{a}	1.42 ± 0.42^{a}	2.27 ± 0.93^{a}	3.26 ± 1.42^{a}	17.23 ± 8.66^{b}	n-3 linolenic acid
1544	3,5-Octadien-2-one isomer 1	37.70 ± 4.45^{a}	26.75 ± 2.01^{a}	30.46 ± 5.89^{a}	35.97 ± 8.29^{a}	109.56 ± 34.33^{b}	n-3 linolenic acid
1560	1-Octanol	44.79 ± 6.12^{b}	30.64 ± 4.41^{ab}	28.97 ± 4.26^{a}	26.48 ± 5.74^{a}	39.59 ± 17.07^{ab}	n-9 oleic acid
1588	3,5-Octadien-2-one isomer 2	161.15 ± 19.18^{a}	110.53 ± 9.63^{a}	112.47 ± 14.16^{a}	128.16 ± 22.81^{a}	299.09 ± 108.81^{b}	n-3 linolenic acid

Values are means peak area (million au) ± standard deviations of 6 measurements (2 batches x 3 replicates). Different letters in the same row indicate statistically significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test.
*(Genot et al., 2003)

4.4 Conclusions

This study evaluated the effect of different milk protein compositions on physicochemical properties, emulsion physical stability and oxidative stability of protein-stabilised emulsions. Emulsions with sodium caseinate showed a smaller droplet size, higher zeta potential and higher viscosity, which corresponded to an increase in sodium caseinate content. All the emulsions had good emulsion stability against creaming and different milk protein compositions did not affect creaming stability during ageing. All fresh emulsions had the same volatile profile after preparation, however, the emulsions demonstrated significant changes during ageing as a function of milk protein composition. Emulsions prepared with mixtures of sodium caseinate and WPC showed greater oxidative stability as compared to the emulsions prepared with only sodium caseinate or WPC. It was hypothesised that the greater oxidative stability may be attributed to the formation of a compact multilayer interface to separate oil droplets from oxygen and/or the antioxidant effect of the unadsorbed protein in the aqueous solution. This knowledge on the interaction of milk protein composition with GMO is useful to develop a protein-stabilised emulsion with uniform droplet suspension that has high creaming stability and oxidative stability. The role of GMO on emulsion properties will be investigated in **Chapter 6** using the same model emulsion applied in this chapter and **Chapter 7** in an application study using a model milk coffee emulsion.

Chapter 5

Preparation and Characterisation of a Novel Emulsifier System Based on Glycerol Monooleate by Spray-Drying

5.1 Introduction

Monoglycerides are common food emulsifiers typically used in food products such as coffee creamers, creams, margarine, ice cream and dairy beverages (Krog & Vang Sparsø, 2003). Monoglycerides are amphiphilic molecules with one hydrophobic fatty acid esterified to the hydrophilic glycerol molecule, making them surface-active molecules (Krog & Vang Sparsø, 2003). Monoglycerides are readily adsorbed to the oil-water interface and lower the interfacial tension to facilitate emulsion formation. However, monoglycerides are oil-soluble molecules with poor dispersion in water, which limits their application in the industry.

While there is a wide range of commercial monoglycerides available, glycerol monostearate (GMS) and glycerol monooleate (GMO) are both typically used as emulsifiers. GMS is a saturated monoglyceride (mostly comprised of stearic acid and palmitic acid) and is used in many food applications due to its high oxidative stability. In contrast, GMO is an unsaturated (oleic acid) monoglyceride, which has been under-utilised for food formulation. In **Chapter 3**, results showed that there is a potential use of GMO to achieve both good physicochemical properties and creaming stability in protein-stabilised emulsions. GMS can incorporate co-emulsifiers such as sodium stearate to improve dispersion in water (Krog & Vang Sparsø, 2003). As GMO is a waxy solid at room temperature, the incorporation of sodium stearate is not practical, and this has therefore restricted its use in many food formulations. In addition, the unsaturated fatty acid in GMO is prone to oxidation, which can lead to the formation of undesirable aroma compounds. Thus, there is a need to transform GMO into a powder with good dispersibility in water and with protection against oxidation for use in food formulations.

Spray-drying is a process widely used in the food industry for encapsulation of oils rich in unsaturated fatty acids (Carneiro et al., 2013; Jafari, Assadpoor, He, et al., 2008; Turchiuli, Fuchs, et al., 2005). The powders resulting from spray-drying possess low water activity, uniform dispersion in food formulations, improved stability for long-term storage and are easy to handle and store (Bae & Lee, 2008; Vega & Roos, 2006). Maltodextrin is commonly used as a wall material in spray-drying due to its relatively low cost, neutral odour and taste, minimal effect on viscosity, and good protection against undesired reactions (Carneiro et al., 2013; Vega & Roos, 2006). Due to the low emulsifying properties of maltodextrin, it is usually used together with sodium caseinate

to improve the emulsifying and interface properties of lipophilic core materials (Hogan, McNamee, O'Riordan, et al., 2001). The rheological and functional properties of maltodextrin are characterised by dextrose equivalent (DE), which is an indication on the degree of starch hydrolysis (Pycia, Juszczak, Gałkowska, Witczak, & Jaworska, 2016). Hogan, McNamee, O'Riordan, et al. (2001) reported that at higher DE values maltodextrin showed a higher encapsulation efficiency of soybean oil emulsions during spray-drying. Dried powder using maltodextrin of DE 25 was also more stable than DE 10, as the former showed a lower rate of droplet size growth in reconstituted coconut oil emulsions (Matsuura et al., 2015). Dried powders with high monoglyceride levels are able to deliver emulsification and stabilisation of oil droplets in emulsions.

There are many studies in the literature on the effect of emulsifier type and concentration on the spray-drying process and resulting powder properties (Vega & Roos, 2006), but none have specifically examined the encapsulation of emulsifiers. The encapsulation of emulsifiers such as GMO has the potential to produce valuable ingredients to the food and ingredient industries. In this study, we report on a spray-drying process for the preparation of a novel emulsifier system based on GMO. This study also investigates the influence of monoglyceride level and DE value of maltodextrin on emulsion properties, particle properties and oxidative stability of an instantised GMO.

5.2 Materials and Methods

5.2.1 Materials

Glycerol monooleate (Radiamuls MG 2905K) (containing at least 90% monoglyceride content) was a gift from Oleon (Klang, Malaysia). Sodium caseinate was provided by Tatua Co-operative Dairy Company Ltd. (Morrinsville, New Zealand), while sodium stearate was supplied by Peerage Product Limited (Christchurch, New Zealand). Maltodextrins with dextrose equivalent (DE) values of 10 and 18 were purchased from Hawkins Watts Ltd. (Auckland, New Zealand) and Davis Food Ingredients (Auckland, New Zealand), respectively. Refined canola oil was purchased from the local supermarket. The organic solvents (hexane, chloroform and methanol) used were of analytical grade. In pilot experiments, it was found that an emulsion with high GMO content suitable for spray-drying (oil-in-water emulsion with low viscosity and no phase separation within 24 hours) can be formulated by incorporating canola oil, sodium caseinate, sodium stearate and maltodextrin. Canola oil serves as the oil-phase for the

emulsion and used as a carrier oil for GMO. The addition of melted GMO (without canola oil) to water phase will form a gel instead of an emulsion. The addition of sodium stearate reduced the viscosity of the emulsion, while maltodextrin increased emulsion stability at 45 °C. As the main aim of this chapter was to prepare instantised GMO powder by spray-drying for further investigation of the material in a beverage application, the consideration to optimise the processing parameters and formulation will not be covered by this thesis. Therefore, all emulsions were spray-dried using the same drying conditions to produce sufficient material for powder characterisation and subsequent tests in a beverage application (**Chapter 6**).

5.2.2 Preparation of emulsions for spray-drying

Four emulsions with two GMO levels (33.6% (low) and 47.0% (high) on the dry weight basis) and two types of maltodextrin (DE 10 and DE 18) were prepared by microfluidisation (**Table 5.1**). Each emulsion-type was spray-dried the same day the emulsion was prepared. All emulsions were prepared at a constant total solids of 40% w/w. Sodium stearate was added as a component of GMO at 4% w/w. The ratios of GMO to canola oil and GMO to sodium caseinate were calculated at 33.6:15 and 33.6:3, respectively. Maltodextrin made up the balance of the dry weight. Water-phase ingredients, consisting of maltodextrin, sodium caseinate and sodium stearate, were reconstituted in Milli-Q water at 50 °C. The water-phase was continuously agitated using an Ultra-Turrax (IKA-Werke GmbH and Co. KG, Stufen, Germany) at 13,000 rpm and was simultaneously heated to 75 °C. GMO was added to canola oil at 45 °C to form the oil-phase. The oil-phase was mixed with the water-phase solution, which was then homogenised for 15 min at 75 °C to form a coarse emulsion. The coarse emulsion was passed through a microfluidiser (Microfluidics Corporation, Massachusetts, USA) for a single pass at 55 MPa and 65 °C. The microfluidiser was pre-heated by circulating water at 70 °C. The batch size for each emulsion was 1300 mL. All emulsions were stored in either 100 mL bottles or clear test tubes (16 mm o.d. x 150 mm height) for droplet size and viscosity measurements, and emulsion stability, respectively.

Table 5.1 Formulation of instantised GMO powder based on % dry weight.

Sample*	LowMG-DE10	LowMG-DE18	HighMG-DE10	HighMG-DE18
Core materials	**48.6%**	**48.6%**	**68.0%**	**68.0%**
i) GMO	33.6%	33.6%	47.0%	47.0%
ii) Canola oil	15.0%	15.0%	21.0%	21.0%
Wall materials	**51.4%**	**51.4%**	**32.0%**	**32.0%**
iii) NaCas	3.0%	3.0%	4.2%	4.2%
iv) Na stearate	1.4%	1.4%	2.0%	2.0%
v) MD DE 10	47.0%		25.8%	
vi) MD DE 18		47.0%		25.8%
Core:Wall	**0.95**	**0.95**	**2.13**	**2.13**
NaCas:MD	**0.06**	**0.06**	**0.16**	**0.16**

*GMO: Glycerol monooleate, NaCas: Sodium caseinate, Na stearate: Sodium stearate, MD: Maltodextrin, MG: Monoglyceride, DE: Dextrose equivalent. GMO:Canola oil:NaCas ratio was fixed at 33.6:15:3 for all samples. Total solids were adjusted to 40% w/w solids during emulsion preparation.

5.2.3 Emulsion characterisation

5.2.3.1 Droplet size and polydispersity index of emulsions

Droplet size and size-range distributions of the emulsions were measured by dynamic light scattering using a Malvern Zetasizer Nano S (Malvern Instruments Ltd., Malvern, Worcestershire, UK). The measurements were carried out using samples diluted 1:1000 with Milli-Q water. The diluted sample was analysed for 30 s at 25 °C with triplicate measurements carried out for each sample. The average droplet size was expressed as the intensity-weighted mean diameter (Z-average), and the degree of non-uniformity of a droplet size distribution was presented as the polydispersity index.

Droplet size and size-range distributions of the reconstituted emulsions were measured using the same procedure as the emulsions. In order to reach the same concentration of the diluted emulsions, 0.04 g of powder sample was mixed with Milli-Q water in a 100 mL volumetric flask.

5.2.3.2 Viscosity

Viscosity of the model emulsions was performed using a cone and plate geometry (DC60/1° Ti) at 45 °C with a Rheostress 1 (Haake, Karlsruhe, Germany) rheometer. Measurements were carried out in triplicate for each sample at the shear rate range of 0.1 to 100 s^{-1}. Haake RheoWin Data Manager Version 3.5 (Haake, Karlsruhe, Germany) was used for the rheological data analysis. The measurement data were fitted to the Bingham model (**Equation 5.1**).

$$\tau = \tau_o + \eta_p \dot{\gamma} \qquad \textbf{Equation 5.1}$$

where τ is the shear stress (Pa), τ_o is the yield stress (Pa), η_p is the plastic viscosity (Pa s), and $\dot{\gamma}$ is the shear rate (s^{-1}).

5.2.3.3 Emulsion stability

The stability of the emulsions was monitored visually with care taken not to disrupt the separated layer (Frascareli, Silva, Tonon, & Hubinger, 2012). All measurements were carried out at 24 hours at 45 °C.

5.2.4 Spray-drying of emulsions

Instantised GMO powders were prepared using a laboratory scale spray-dryer, LabPlant SD-05 model (Keison Products, Essex, England), fitted with a 1.0 mm diameter atomiser nozzle. The emulsion was fed to the atomiser with a peristaltic pump at a feed rate of 0.76 L h^{-1} and the feed transfer line maintained at 55 °C. The drying air flow rate through the chamber was at maximum setting (50 m^3 h^{-1}) and the atomiser pressure used was 110 kPa. The inlet air temperature was set at 180 °C, which resulted in the measured outlet air temperature of 88 ± 5 °C. The instantised GMO powder was stored in a low-density polyethylene zip-lock bag, sealed inside an aluminium foil laminate bag and kept stored at 4 °C until required for use.

5.2.5 Powder characterisation

5.2.5.1 Encapsulation efficiency

Encapsulation efficiency of the instantised GMO powders is an important parameter in spray-drying. Encapsulation efficiency refers to the amount of oil-soluble ingredients encapsulated by wall materials and can be calculated using the following equation:

$$\text{Encapsulation efficiency} = \frac{\text{Total oil} - \text{Surface oil}}{\text{Total oil}} \times 100\% \qquad \textbf{Equation 5.2}$$

5.2.5.2 Surface oil

Surface oil content of the powders was determined according to González, Martínez, Paredes, León, and Ribotta (2016) with some modifications. A sample (1 g) was mixed with 30 mL hexane in a 50 mL conical flask, stirred for 1 min and filtered using a Whatman filter paper No. 1 into a pre-weighed flask. The solids retained on the filter paper were washed twice with 30 mL hexane and all the organic phases were combined. The solvent was removed using a rotary evaporator, Rotavapor-R (Büchi, Flawil, Switzerland) and kept in the oven at 60 °C until a constant weight was reached. The surface oil content was calculated based on the difference between the weight of the initial clean flask and that containing the extracted oil.

5.2.5.3 Total oil

Total oil content of the powders was determined according to Sarkar et al. (2016) with some modifications. Briefly, a sample (1 g) was mixed with 25 mL methanol:chloroform 2:1 in a 50 mL centrifuge tube and vortexed for 5 min. The powder-solvent mixture was subjected to centrifugation at 3000 x g for 10 min before recovering the liquid phase by filtering through a Whatman filter paper No. 1 into a pre-weighed flask. Methanol:chloroform 1:1 (15 mL) was added to the remaining powder in the centrifuge tube and vortexed for another 5 min. The powder-solvent mixture was centrifuged and filtered under the same conditions as above and the liquid phase was added to the flask containing the solvent from the first extraction. A third extraction was carried out in the same manner with 15 mL methanol:chloroform 1:1. The solvent in the flask was removed using a rotary evaporator, Rotavapor-R (Büchi, Flawil, Switzerland) and kept in the oven at 60 °C until a constant weight was reached. The total oil was calculated based on the difference between the weight of the initial clean flask and that containing the extracted oil.

5.2.5.4 Moisture content

Moisture content of the powders was determined by loss on drying using 1 g of the powder at 103 ± 2 °C until a constant weight was reached. The moisture content was calculated using the following equation:

$$\text{Moisture} = \frac{\text{Initial weight} - \text{Dried weight}}{\text{Initial weight}} \times 100\% \qquad \textbf{Equation 5.3}$$

5.2.5.5 Water activity

Water activity of the powders refers to the ratio of the partial vapour pressure of water in the powder to the partial vapour pressure of pure water at the same temperature. The water activity was measured using an AquaLab Series 4TE water activity meter (Decagon Devices, Inc., WA, USA) at 25 °C.

5.2.5.6 Bulk density and flowability

Bulk density of the powders was determined according to the procedure by Kha, Nguyen, Roach, and Stathopoulos (2014) with some modifications. The powder (2 g) was gently added into an empty 10 mL graduated cylinder, and then the volume occupied by the mass was measured. The same sample in the cylinder was then tapped for 100 times to determine the tapped density. Both the bulk and tapped densities were calculated as the ratio of powders mass and volume occupied by the powder.

The flow behaviour of the powders is affected by its own physical properties and external conditions such as temperature, humidity and equipment. Empirical calculations such as Carr's index and Hausner ratio based on the bulk and tapped densities using the equations below were used to estimate the powder compressibility and flowability for each sample (Jinapong, Suphantharika, & Jamnong, 2008; Turchiuli, Eloualia, El Mansouri, & Dumoulin, 2005).

$$Carr's \text{ index} = \frac{(\text{tapped density} - \text{bulk density})}{\text{tapped density}} \times 100\% \qquad \textbf{Equation 5.4}$$

$$\text{Hausner ratio} = \frac{\text{tapped density}}{\text{bulk density}} \qquad \textbf{Equation 5.5}$$

5.2.5.7 Dispersibility

Dispersibility of the powders was determined according to the procedure described in GEA Niro Method No. A 6 a (Jinapong et al., 2008) with some modifications. A sample (1 g) was added into a 50 mL beaker, followed by 10 g of Milli-Q water. The stopwatch was started, and the sample was stirred with a spatula for 20 s at 1 complete stirring movement per second. One complete stirring movement was the complete rotation of

clockwise and anticlockwise across the whole diameter of the beaker. The sample was allowed to stand for 30 s before pouring them over a 250-µm test sieve. The emulsion (2 g) that passed through the sieve was transferred to a pre-weighed petri dish and dried for 4 hours at 103 ± 2 °C. The dispersibility of the powder was calculated using the following equation:

$$\text{Dispersibility} = \frac{(a+b) \times d}{b \times \frac{100-c}{100}} \qquad \textbf{Equation 5.6}$$

where a is the weight of water added to the powder in g, b is the weight of powder used in g, c is the moisture content (%) in the powder, and d is the dry matter (%) in the reconstituted emulsion after it has passed through the sieve.

5.2.5.8 Solubility

Solubility of the powders was determined according to (Kha et al., 2014) with some modifications. A sample (1 g) and 25 mL of milli-Q water were added into a 50 mL centrifuge tube and vortex for 1 min. Then the sample was incubated in a 45 °C water bath for 30 min, followed by vortex for 1 min and centrifugation at 3000 rpm for 10 min. The supernatant was carefully poured into a pre-weighed petri dish and dried at 103 ± 2 °C until a constant weight was reached. The solubility was calculated using the following equation:

$$\text{Solubility} = \frac{\text{Weight of dried matter in supernatant}}{\text{Weight of powder used}} \times 100\% \qquad \textbf{Equation 5.7}$$

5.2.5.9 Thermal oxidative stability of bulk GMO and instantised GMO powders at 80 °C by thermogravimetric analysis (isothermal)

The thermal oxidative stability of the GMO powders was compared to the bulk oil using TA Instruments Q50 Thermogravimetric Analyser (TA Instruments, New Castle, DE, USA). A sample (15-25 mg) was placed into a platinum pan and heated to 80 °C at 5 °C min^{-1} under an air atmosphere. The changes in sample weight as a result of lipid peroxidation were monitored for 4320 min at 80 °C. TA Universal Analysis 2000 software (version 4.5A, TA Instruments-Waters LLC, USA) was used to process all the data from TGA.

5.2.5.10 Headspace solid-phase microextraction (HS-SPME)

All GMO samples were pre-weighed (1 g for bulk GMO or its equivalent to 1 g of bulk GMO in powder form) into 20 mL headspace vials and the vial was capped with a Teflon-lined silicone rubber septum. The samples were aged at 45 °C over a period of 1 to 56 days, three bottles of each sample were removed from 45 °C and transferred to -20 °C at specified time intervals. The samples were stored at -20 °C until all time points were collected and until analysis. All vials were covered with aluminium foil at all times to prevent light-induced reactions. The extraction of volatiles was carried out with a PAL RSI 85 multipurpose sampler (CTC Analytics, Bern, Switzerland) at 45 °C. The samples were equilibrated at 45 °C for 5 min, before the SPME fibre (50/30 μm divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS)) (Supelco, Bellefonte, PA, USA), was exposed for 40 min. After the extraction, the SPME fibre was injected into the gas chromatograph inlet for desorption and analysis (see **Section 5.2.5.11**). Analysis of each time point for each sample was carried out in triplicate.

5.2.5.11 GC-MS analysis

Analysis of headspace volatiles above the samples was carried out with an Agilent GC 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 5975B VL MSD quadrupole mass spectrometer (Agilent Technologies, Wilmington, DE, USA). SPME desorption was carried out at 240 °C for 2 min in splitless mode, followed by 3 min with a split flow of 50 mL min^{-1}. Following sample desorption, the SPME fibre was conditioned for 2 min at 270 °C with a purge flow of 50.0 mL min^{-1}. Pure helium gas was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The chromatographic separation was performed on a 60 m x 0.32 mm i.d. x 0.5 μm Zebron ZB-WAX column (Phenomenex, Torrance, CA, USA). The oven temperature was held for 5 min at 40 °C, increased at a rate of 5 °C min^{-1} until 190 °C, then subsequently increased at a rate of 10 °C min^{-1} to 240 °C and held for 5 min. The transfer line to the MS was set to 230 °C and the quadrupole was set to 150 °C. The mass spectrometer was operated at a scan speed of 5.1 scans s^{-1} and mass spectra were recorded in the range of m/z 29-300. Carryover between GC runs was assessed using an empty vial after every 3 or 4 samples. Sample analysis was randomised and blocked by replicate. Samples were analysed in triplicate.

The Agilent Enhanced MSD Chemstation software (version F.01.01.2317, Agilent Technologies Inc., USA) was used to process the chromatogram and data from the mass spectrometer. Alignment of the chromatograms was carried out manually. Volatile compounds were identified based on their mass spectra and supported by their retention index (RI). Mass spectra were compared to the National Institute of Standards and Technology (NIST14) database. Linear retention indices were calculated using a C7-C18 n-alkane series as reference compounds using the following equation:

$$\mathrm{RI}(x) = (100 \times z) + \frac{\mathrm{RT}(x) - \mathrm{RT}(z)}{\mathrm{RT}(z+1) - \mathrm{RT}(z)} \qquad \textbf{Equation 5.8}$$

Where RI(x) is the retention index of the unknown compound x, z is the number of carbon atoms of the n-alkane eluted before the unknown compound x, z+1 is the number of carbon atoms of the n-alkane eluted after the unknown compound x, RT(x) is the retention time of the unknown compound x, RT(z) is the retention time of the n-alkane eluted before the unknown compound x, RT(z+1) is the retention time of the n-alkane eluted after the unknown compound x. Semi-quantification of the volatile compounds was carried out using the total ion count (TIC) for each compound.

5.2.6 Statistical analysis

IBM SPSS Statistics Version 23 (IBM Corporation, Armonk, New York, USA) was used for statistical comparison of the results. One-way analysis of variance (ANOVA) was carried out to determine significant differences between the results, followed by Tukey's post hoc test for pairwise comparison. All tests were performed at the 95% confidence level. Principal component analysis (PCA) was carried out on all volatile compounds that increased significantly over time using The Unscrambler X Version 10.5 (CAMO, Oslo, Norway) and all variables in the PCA were standardised (1/standard deviation).

5.3 Results and Discussion

5.3.1 Characterisation of the pre-drying emulsion

5.3.1.1 Droplet size

Droplet size of the emulsions (initial and aged for 24 hours) was not affected by the DE value of maltodextrin but was dependent on the MG level (**Table 5.2c**). Droplet size increased significantly when MG level increased from low to high. The increase in the ratio of core to wall material from 0.95 to 2.13 could lead to coalescence or merging of oil droplets during atomisation in spray-drying to form bigger droplets.

The polydispersity indices for all emulsions (initial and aged for 24 hours) were below 0.2 (**Table 5.2c**), which indicated narrow droplet size distributions. The volume-weighted droplet size distribution graph showed that all initial and aged emulsions had monomodal distributions (results not shown), which explains the low polydispersity index. Emulsion LowMG-DE18 had a significantly higher polydispersity index than the rest of the emulsions ($p<0.05$), however, this difference was small and may not have any practical implication.

Table 5.2 Physical properties of the pre-drying emulsion at various stages.

Sample	LowMG-DE10	LowMG-DE18	HighMG-DE10	HighMG-DE18
a) Emulsion stability at 24 hours	Stable	Stable	Stable	Stable
b) Rheological properties (fitted to Bingham plastic model)				
Yield stress (mPa)	946 ± 135^{a}	842 ± 61^{a}	3428 ± 424^{b}	4672 ± 838^{c}
Viscosity (mPa s)	41.0 ± 3.7^{b}	21.6 ± 1.7^{a}	64.8 ± 1.0^{c}	61.6 ± 7.3^{c}
Correlation coefficient, R*	0.99	0.97	0.99	0.99
c) Droplet size				
Initial (nm)	153.1 ± 2.7^{aA}	153.9 ± 2.8^{aA}	180.7 ± 2.5^{cA}	167.6 ± 4.3^{bA}
24 hours (nm)	156.7 ± 2.8^{aA}	158.5 ± 2.1^{aA}	177.9 ± 2.1^{bA}	172.2 ± 3.3^{bA}
Reconstituted (nm)	267.1 ± 8.4^{aB}	295.0 ± 11.8^{aB}	453.2 ± 16.6^{bB}	426.9 ± 10.4^{bB}
d) Polydispersity index				
Initial	0.119 ± 0.008^{aA}	0.160 ± 0.010^{bA}	0.123 ± 0.005^{aA}	0.122 ± 0.011^{aA}
24 hours	0.116 ± 0.008^{aA}	0.149 ± 0.010^{bA}	0.099 ± 0.011^{aA}	0.109 ± 0.010^{aA}
Reconstituted	0.148 ± 0.007^{aB}	0.144 ± 0.010^{aA}	0.241 ± 0.016^{bB}	0.176 ± 0.018^{aB}

Values are means ± standard deviations of three measurements. Different letters (small) in the same row and different letters (capital) in the same column for the same measurement indicate statistically significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test.
*The correlation coefficient (R) for the fit of the Bingham plastic model for experimental data.

5.3.1.2 Emulsion stability

Table 5.2 shows the resistance to phase separation, rheological properties, droplet size and polydispersity index of all pre-drying emulsions. All emulsions (initial and aged at 45 °C for 24 hours) after microfluidisation in three independent test tubes showed no sign of phase separation. This observation indicated that all microfluidised emulsions were kinetically stable at 45 °C for 24 hours.

The shear stress and shear rate data for all emulsions were best fitted to a Bingham plastic model ($R>0.97$). All emulsions were flowable at 45 °C with a yield stress of 800-4700 mPa and viscosity of 20-65 mPa s. Yield stress is the critical stress for the emulsion to start flowing, which indicates the resistance of an oil droplet to move and collide with other droplets. The high yield stress and viscosity of the emulsions can reduce the collision rate between droplets, which may minimise droplet coalescence or flocculation, and subsequently delay phase separation (McClements, 2007).

The evaluation of droplet size and polydispersity index of all emulsions showed that there was no difference between the initial and aged emulsions ($p>0.05$). The stable droplet size and polydispersity index provided evidence that the oil droplets of the emulsions did not undergo flocculation, coalescence or Ostwald ripening within 24 hours ageing. The stable droplet properties prevented phase separation in all emulsions.

Adding a thickening agent to increase the viscosity of the initial emulsion or using a homogeniser to reduce droplet size and its size distribution can improve emulsion stability (McClements, 2015). Hogan, McNamee, O'Riordan, et al. (2001) reported that emulsion stability improved in a soybean oil/protein emulsion through the formation of smaller droplets by homogenisation. A stable emulsion with small oil droplet size is desirable during spray-drying as it typically leads to better encapsulation of oil in the dried powders (X.-D. Liu et al., 2001). Emulsion instability could also adversely affect the physical properties and stability of the dried oil powders (Tan, Chan, & Heng, 2005). All the emulsions showed stable oil droplets for 24 hours, and thus were deemed suitable for spray-drying within the evaluated time.

5.3.1.3 Viscosity

The yield stress and viscosity (Bingham plastic model) of emulsions increased significantly when MG level was increased from 33.6% (low) to 47.0% (high). The

increased ratio of sodium caseinate to maltodextrin from 0.06 to 0.16 could explain the increased viscosity and yield stress. Depending upon DE, maltodextrin has a limited effect on emulsion viscosity even at high concentrations (Carneiro et al., 2013). Sodium caseinate is known to increase the viscosity of emulsions (Dickinson & Ritzoulis, 2000).

Increasing the DE value from 10 to 18 resulted in a mixed effect on yield stress and viscosity. In the low MG emulsions, the DE 18 showed a 50% reduction in emulsion viscosity with no change in yield stress compared to DE 10. However, in the high MG emulsions, changing the maltodextrin from DE 10 to DE 18 did not change the emulsion viscosity but increased the yield stress by 35%. The viscosity reduction in the low MG emulsions as a function of increasing DE value of maltodextrin agrees with the findings by Hogan, McNamee, O'Riordan, et al. (2001). The increasing DE value of maltodextrin did not change the viscosity of the high MG emulsions, and this may be due to the high yield stress caused by an increase in the dispersed phase volume that has masked the effect of the maltodextrin DE value. Furthermore, the amount of maltodextrin in the high MG emulsions was halved compared to low MG emulsions, hence any effect on the rheological properties would be smaller.

The low viscosity of the low MG emulsion is advantageous in encapsulation by spray-drying, as it allows high total solids concentration in the feed emulsion (Hogan, McNamee, O'Riordan, et al., 2001). The emulsion must have a suitable viscosity to be pumped and sprayed for rapid and efficient drying. The work by Jafari, Assadpoor, He, et al. (2008) revealed that a higher viscosity in-feed formed large and irregular particles after drying due to the difficulties in droplet formation at high viscosity. Maintaining a low emulsion viscosity is an important parameter as it can influence the spray-drying process (drying and formation rate of a semipermeable membrane) and properties of the dried powders (movement of core material to the surface of powder particles, powder size and thickness of the wall) (Hogan, McNamee, O'Riordan, & O'Sullivan, 2001; Jafari, Assadpoor, He, et al., 2008).

5.3.2 Physical properties of instantised GMO powders

5.3.2.1 Encapsulation efficiency

Table 5.3a shows the physical properties of instantised GMO powders. Encapsulation efficiency is the percentage of encapsulated oil to total oil, which indicates the amount

of oil encapsulated during the spray-drying process. A high encapsulation efficiency also means that there is a lower amount of surface oil on the powder. Samples LowMG-DE10 and LowMG-DE18 had the highest encapsulation efficiency, followed by HighMG-DE18, and then HighMG-DE10 had the lowest ($p<0.05$). The low GMO (33.6%) powders had a low surface oil content around 3% whereas the high GMO (47%) powders had 20-30% surface oil. More than 90% of the oil-phase was successfully encapsulated when MG content was low. A literature review by Jafari, Assadpoor, He, et al. (2008) showed that higher oil content generally results in lower encapsulation efficiency and higher surface oil content of the powder.

Table 5.3 Physical, handling and flow, and reconstitution properties of instantised GMO powders.

Sample	LowMG-DE10	LowMG-DE18	HighMG-DE10	HighMG-DE18
a) Physical properties				
Encapsulation efficiency (%)	93.3 ± 1.0^{c}	93.9 ± 1.2^{c}	56.2 ± 2.7^{a}	70.4 ± 1.4^{b}
Surface oil (%)	3.0 ± 0.4^{a}	3.1 ± 0.6^{a}	29.5 ± 1.8^{c}	21.5 ± 1.0^{b}
Moisture (%)	2.1 ± 0.6^{a}	2.1 ± 0.5^{a}	2.1 ± 1.7^{a}	1.8 ± 1.6^{a}
Water activity	0.126 ± 0.005^{a}	0.128 ± 0.003^{a}	0.157 ± 0.007^{b}	0.137 ± 0.003^{a}
b) Handling and flow properties				
Bulk density (g cm^{-3})	0.22 ± 0.01^{a}	0.30 ± 0.01^{b}	0.24 ± 0.01^{a}	0.25 ± 0.01^{a}
Tapped density (g cm^{-3})	0.44 ± 0.01^{c}	0.45 ± 0.01^{c}	0.33 ± 0.01^{a}	0.37 ± 0.01^{b}
Carr's index (%)	50 ± 2^{b}	32 ± 2^{a}	26 ± 3^{a}	32 ± 4^{a}
Hausner ratio	1.99 ± 0.08^{b}	1.47 ± 0.03^{a}	1.36 ± 0.05^{a}	1.48 ± 0.10^{a}
c) Reconstitution properties				
Solubility (%)	96.4 ± 1.1^{a}	96.5 ± 0.3^{a}	95.3 ± 1.2^{a}	97.5 ± 0.4^{a}
Dispersibility (%)	74.0 ± 0.8^{b}	87.3 ± 2.0^{c}	66.2 ± 1.1^{a}	69.0 ± 1.3^{a}

Values are means ± standard deviations of three measurements. Different letters in the same row for the same measurement indicate statistically significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test.

There was no significant effect of DE value on encapsulation efficiency for the low MG powders. At high MG content, DE 18 had a 25% higher encapsulation efficiency compared to DE 10. The greater encapsulation efficiency due to increasing DE value of maltodextrin has been previously reported for wall materials with only maltodextrin (Nayak & Rastogi, 2010), mixtures of sodium caseinate and maltodextrin (Hogan, McNamee, O'Riordan, et al., 2001), and mixtures of whey protein and maltodextrin (Sheu & Rosenberg, 1995). Maltodextrins with different DE values denote that they have different compositions of oligosaccharides and may exhibit different physicochemical properties in emulsion systems (Pycia et al., 2016). This may alter the drying kinetics and form powders with different properties. In addition, the smaller droplet size of the emulsions with low MG level could result in higher oil retention and lower surface oil in the dried powder (Hogan, McNamee, O'Riordan, et al., 2001). The increase in core:wall material ratio (determined by %MG level) had a greater influence on the encapsulation efficiency (Akhavan Mahdavi, Jafari, Assadpoor, & Dehnad, 2016; Hogan, McNamee, O'Riordan, et al., 2001). Higher surface oil in powders can adversely affect flowability, compressibility, dispersibility and oxidative stability (Bae & Lee, 2008; Jafari, Assadpoor, Bhandari, & He, 2008; Turchiuli, Fuchs, et al., 2005).

5.3.2.2 Moisture content and water activity

Moisture contents of all GMO powders did not differ significantly and were in the range of 1.8% to 2.1%, which indicated that that the neither the DE value of maltodextrin nor the MG level affected the completeness of the spray-drying process. This is consistent with the literature that moisture content was not affected by the type of wall material and the ratio of core to wall (Hogan, McNamee, O'Riordan, et al., 2001).

Water activities for all GMO powders were below 0.2, which indicated that all dried powders have low microbiological risk (Can Karaca, Low, & Nickerson, 2013; Fuchs et al., 2006). Although the water activity of sample HighMG-DE10 was statistically higher than the others, all the powders are prone to oxidation and have a very low microbiological risk.

5.3.3 Handling and flow properties of instantised GMO powders

Table 5.3b shows the handling and flow properties of instantised GMO powders. The powder bulk and tapped densities are useful parameters to determine its commercial and economic viability (Sharma, Jana, & Chavan, 2012). For instance, a powder with high tapped density saves product volume and packaging material. Furthermore, a lower bulk density value correlates to greater amounts of air voids/pockets in the powders and can therefore increase oxygen exposure to lipids and promote lipid oxidation (Dantas, Pasquali, Cavalcanti-Mata, Duarte, & Lisboa, 2018). All GMO powders had a bulk density ranging from 0.22 to 0.30 g cm^{-3} and a tapped density from 0.33 to 0.45 g cm^{-3}. The tapped density is the degree of packing as a result of tapping, which is related to particle flow and movement in the bed. The tapped density of the powders was lowered by 20% when MG level increased from low to high, but there was no significant influence due to the DE value of maltodextrin.

Compressibility and flowability are flow properties that affect practical applications such as mixing, handling and storage. Carr's index and Hausner ratio are two common empirical values to describe the compressibility and flowability of the powders. The LowMG-DE10 sample had the highest value of Carr's index (50%) and Hausner ratio (1.99), which means that the powder had poor compressibility and was not free-flowing (Turchiuli, Eloualia, et al., 2005). The rest of the instantised GMO powders had moderate compressibility (26-32%) and were not free-flowing (1.36-1.48) according to the same classification. The poor flow properties are common characteristics of powders with lipid-like core materials and have been reported in dried powders based on flaxseed oil (Quispe-Condori, Saldaña, & Temelli, 2011), vegetable oil (Fuchs et al., 2006), α-tocopherol (Turchiuli, Jimenez Munguia, Hernandez Sanchez, Cortes Ferre, & Dumoulin, 2014), soymilk (Jinapong et al., 2008) and avocado (Dantas et al., 2018).

The increase of DE value from 10 to 18 in low MG powders showed a reduction in Hausner ratio and Carr's index, resulting in improved compressibility and flowability. This finding agrees with results reported by Akhavan Mahdavi et al. (2016) regarding the effect of maltodextrin DE value on the flowability of encapsulated anthocyanins. The smaller carbohydrate molecules in maltodextrin DE 18 have a better molecular packing than bigger molecules (Roussenova, Townrow, Murith, Ubbink, & Alam, 2013) and therefore improved flowability. Due to the higher MG:maltodextrin ratio in high MG,

the effect of the DE value of maltodextrin on flowability was masked by the effect of the increased surface oil and reduced maltodextrin level.

5.3.4 Reconstitution properties of instantised GMO powders

5.3.4.1 Solubility

Table 5.3c shows the reconstitution properties of instantised GMO powders, specifically the solubility, dispersibility and reconstituted droplet size. Solubility is defined as the ability of the solid particles to dissolve in water. The solubility of the powders in water was not affected by the DE value of maltodextrin and MG level. All GMO powders had more than 95% solubility in water, which means good reconstitution in water. This demonstrates that the wall materials, maltodextrin and sodium caseinate are suitable for the encapsulation of MG. The encapsulation was also successful in transforming the oil-soluble MG into a water-dispersible ingredient.

5.3.4.2 Dispersibility

Dispersibility is defined as the ease of a powder to form a suspension that can pass through a 250-µm sieve when dispersed in water under gentle mixing conditions. The LowMG-DE18 sample showed the highest dispersibility at room temperature, followed by LowMG-DE10. Both HighMG-DE10 and HighMG-DE18 had the lowest dispersibility in water. The dispersibility of the powder was influenced by the MG:maltodextrin ratio. A greater dispersibility was observed when MG level was reduced from high to low. The high dispersibility in the low MG powders may have been due to high encapsulation efficiency and low surface oil. Surface oil composes of GMO that has poor dispersion in water could adversely affect the dispersibility of the powders. Increasing the DE value of maltodextrin from 10 to 18 enhanced the dispersibility in low MG powders, however, had no significant effect in high MG powders. The reduced amount of maltodextrin and high surface oil in high MG powders could mean that any effect on the dispersibility is negligible.

5.3.4.3 Droplet size of reconstituted emulsions

The powder was completely reconstituted in water in a volumetric flask prior to droplet size analysis. High shear dispersion techniques such as homogenisation were not applied to avoid changing the oil droplet size distribution of the reconstituted emulsions. All reconstituted emulsions showed significantly larger droplet size than the emulsion before

spray-drying ($p<0.05$) (**Table 5.2c**). Similar findings of droplet growth after reconstitution were observed in powders containing milk fat (Danviriyakul, McClements, Decker, Nawar, & Chinachoti, 2002), soya oil (Hogan, McNamee, O'Riordan, et al., 2001) and flaxseed oil (Can Karaca et al., 2013).

A larger increase in reconstituted droplet size was observed in the high MG formulations (increased by 150%) than low MG (increased by 50-70%). Hogan, McNamee, O'Riordan, et al. (2001) also reported a similar finding on the droplet size growth of reconstituted emulsions when the ratio of core to wall materials was increased. When insufficient wall material is present to prevent coalescence of oil droplets during spray-drying this could lead to higher droplet growth in the high MG formulation. Increasing the MG level from low to high resulted in a bigger mean droplet sizes in the reconstituted emulsions. There was little influence on the DE value of maltodextrin on the droplet size of the reconstituted emulsions. Hence, the droplet growth is most likely due to oil droplets coalescing during spray-drying rather than during reconstitution.

The polydispersity indices for all reconstituted emulsions increased significantly compared to the initial emulsions ($p<0.05$), except for LowMG-DE18 (**Table 5.2d**). While the polydispersity indices of the fresh emulsions were similar for all samples except LowMG-DE18, the polydispersity index of reconstituted HighMG-DE10 (0.241) was the highest of all samples. **Figure 5.1** shows the influence of the DE value of maltodextrin and MG level on droplet size distribution. Increasing the MG level from low to high in the dried powders resulted in the droplet size distribution changing from monomodal to bimodal due to the presence of larger droplets around 5 μm.

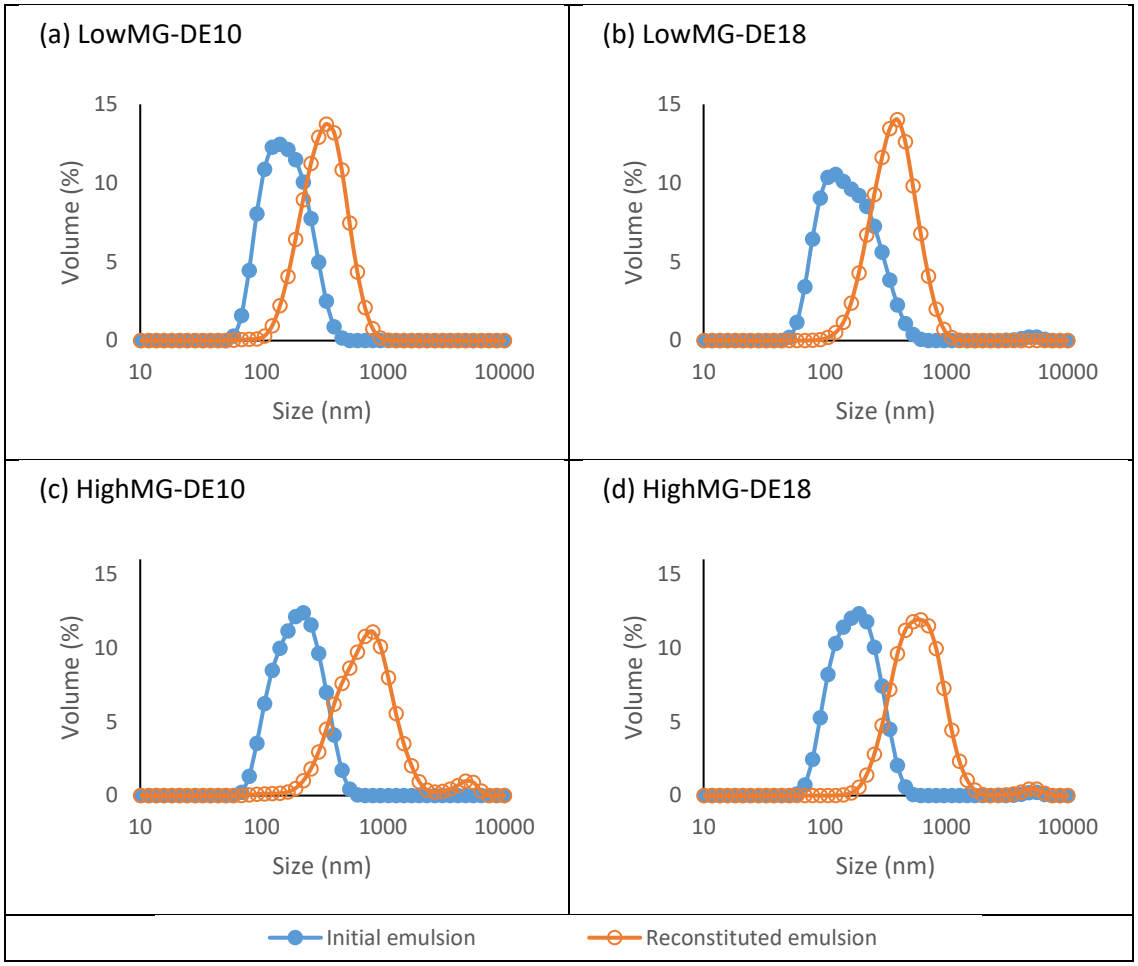


Figure 5.1 Influence of DE value of maltodextrin and MG level on droplet size distribution before spray-drying, and after spray-drying and reconstitution.

5.3.5 Comparison of thermal oxidative stability between bulk GMO and instantised GMO powders

Figure 5.2 shows the effect of encapsulation on the oxidative stability of GMO via TGA. TGA was performed isothermally (80 °C) for 72 hours to compare the weight change of the bulk GMO and instantised GMO powders. All the GMO powders showed an initial weight loss of about 2-3%, which corresponded to the powder moisture content. The initial weight loss (<0.5%) in bulk GMO was due to its hydrophobic nature. The bulk GMO showed a small increase in weight (99.56% to 99.69%) that may correspond to primary lipid oxidation starting from 58 min, due to the formation of hydroperoxides from unsaturated fatty acids. The subsequent weight loss in the bulk GMO was due to further oxidation of the hydroperoxides to form secondary lipid oxidation volatiles, such

as alkanes, aldehydes, ketones, acids and furans. There was no weight gain observed in any of the GMO powders and most likely, any weight loss after 58 min was primarily due to the formation of secondary lipid oxidation volatile compounds. All GMO powders reached a plateau in term of weight around 96-98%, while the bulk GMO still showed a steady weight loss until the end of the analysis. This indicates that encapsulation can protect GMO powders against thermal oxidation. A closer examination on the slope showed that increasing MG level from 35% to 49% resulted in a higher rate of oxidation after omitting the sudden weight loss between 2000-2500 min in sample HighMG-DE10. This higher rate of weight loss might due to the lower encapsulation efficiency and higher surface oil, leading to a more rapid rate of oxidation.

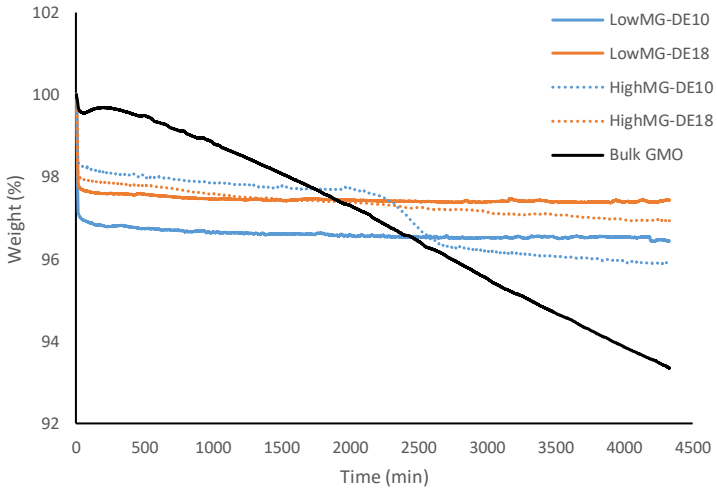


Figure 5.2 Oxidative stability of bulk GMO and instantised GMO powders at 80 °C by TGA.

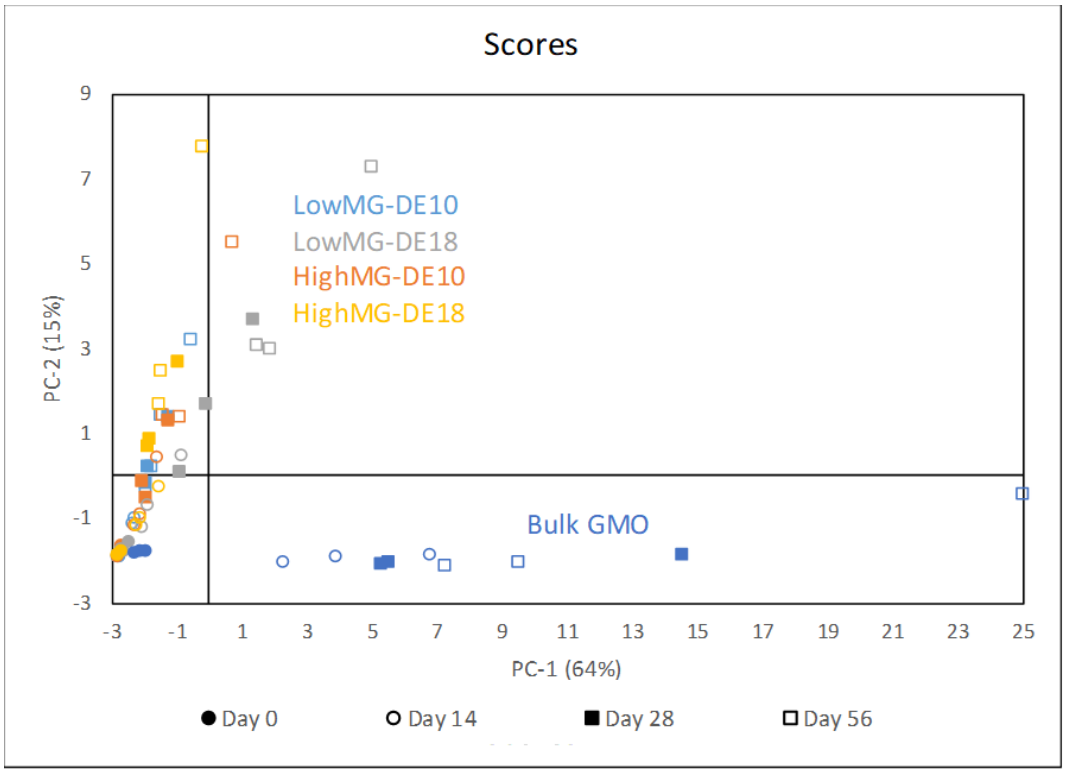
5.3.6 Oxidative stability of bulk and instantised GMO measured using volatile analysis

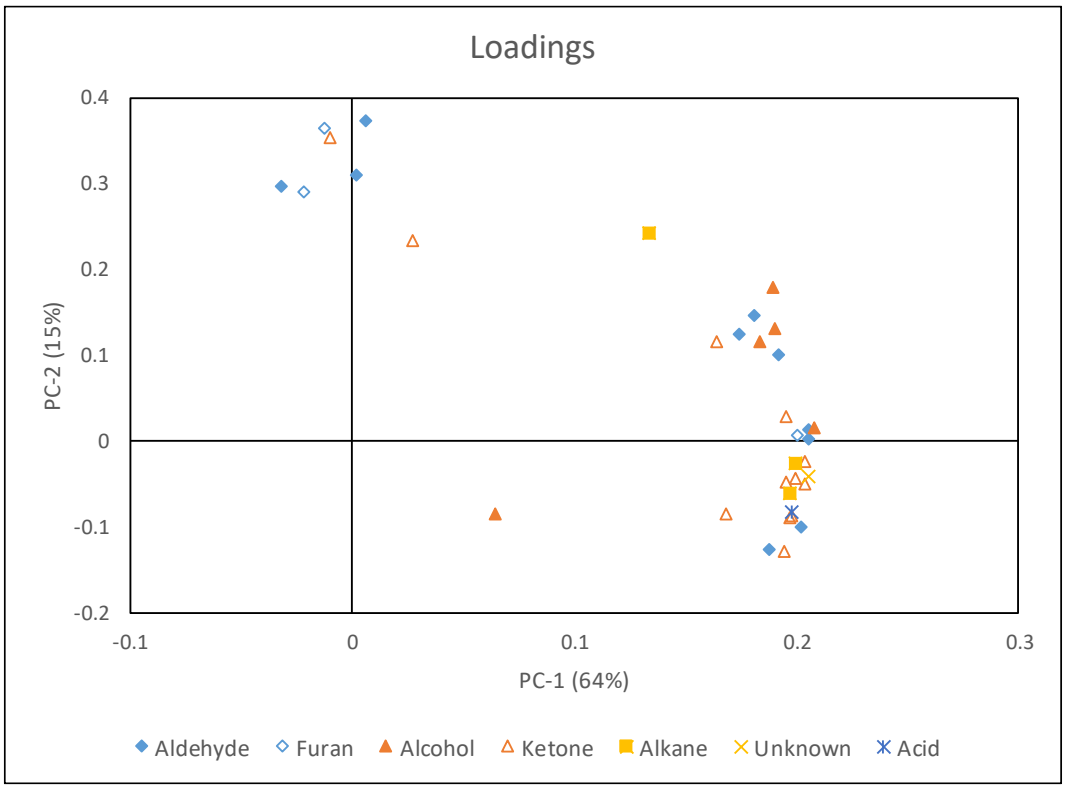
The oxidative stability of the bulk and instantised GMO was also investigated by measuring the formation of secondary lipid oxidation compounds in the headspace volatiles of the samples. The focus of the study was to evaluate the oxidative stability of GMO with different formulations, therefore only volatile compounds that increased

significantly over time ($p<0.05$) were selected and visualised using principal components analysis (PCA).

A total of 59 volatile compounds were detected in all samples, but only 35 compounds showed a significant ($p<0.05$) increase in peak area with time, which were subsequently analysed by PCA. The PCA model was used to transform the data set with multiple variables (formulation, storage time) that may be correlated with one another into a new dimension. This method could be used to identify the principal component of the data set that can explain the variability among the samples. **Figure 5.3a** shows the PCA scores and loadings plots of the volatile compounds that increased significantly in instantised GMO powders and bulk GMO over 56 days of storage at 45 °C. PC-1 and PC-2 explain 64% and 15% of the variation in the different types of GMO, respectively. The scores plot indicates that the different types of GMO can be grouped into three clusters, namely bulk GMO only, LowMG-DE18 powder only and other GMO powders. The difference between bulk GMO and all GMO powders as well as increases in bulk GMO with storage time was discriminated on PC-1, while the differences between the GMO powders were separated on PC-2.

(a) Instantised GMO powders and bulk GMO





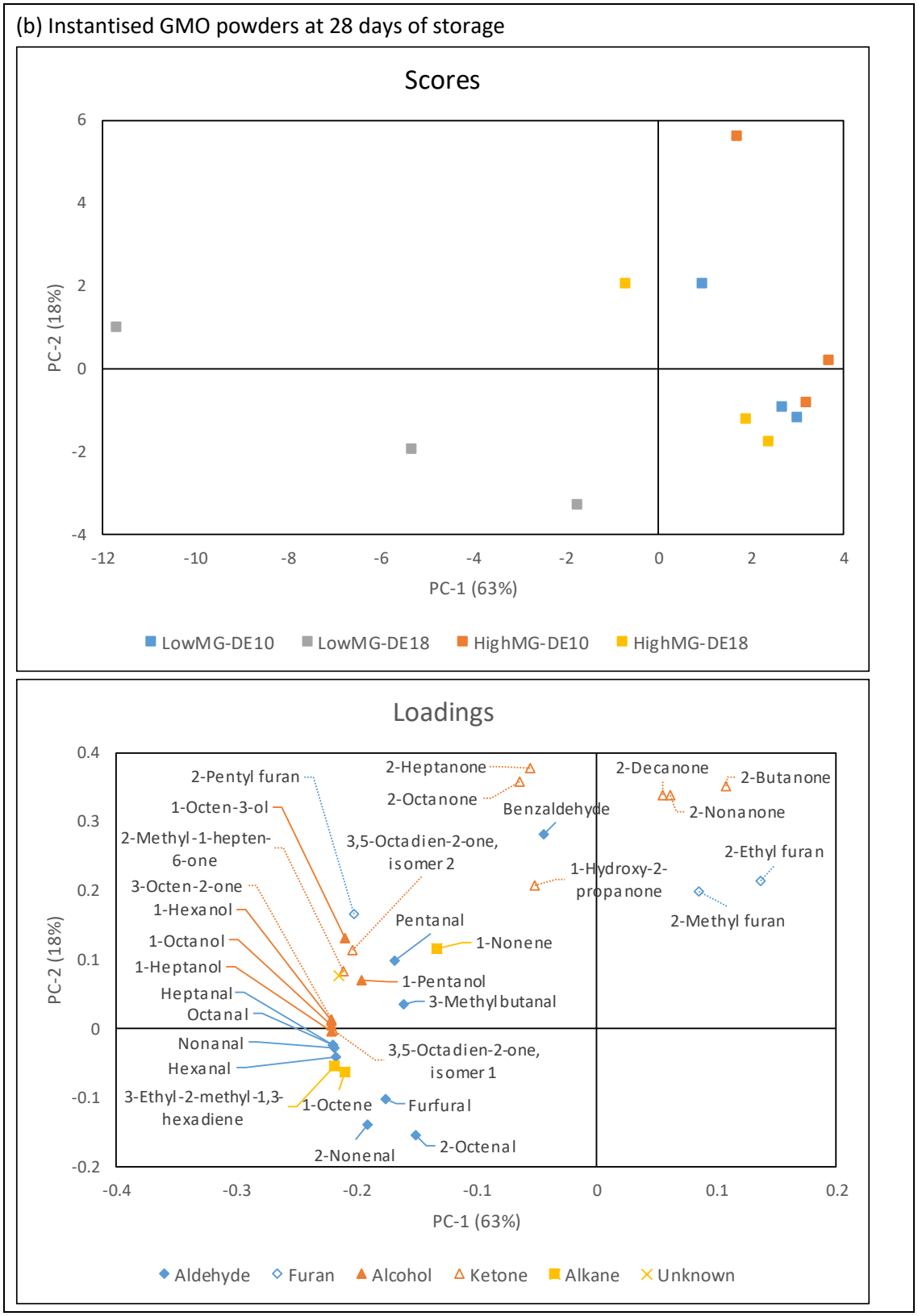


Figure 5.3 PCA chart of the volatile compounds that increased significantly in (a) instantised GMO powders and bulk GMO at the various storage time at 45 °C, and (b) instantised GMO powders at 28 days of storage.

On the loadings plot the compounds were grouped according to chemical class, including aldehydes, furans, alcohols, ketones, alkanes and acids. The majority of the compounds had high loadings on PC-1, which correlated to the increased lipid oxidation in bulk GMO over time. However, acetic acid, 3-octanone and 3-nonen-2-one were only detected in the bulk GMO and not in the instantised powders. The volatile profile of bulk GMO changed after spray-drying with different formulations. The high temperature during homogenisation or spray-drying could increase or reduce the concentration of the volatile compounds. It is also possible for chemical interaction between the ingredients in the formulation to form new compounds. The caseins and maltodextrin could also form a physical structure that support and protect the encapsulated GMO against oxidation when the emulsion is spray-dried (Augustin & Oliver, 2014; Danviriyakul et al., 2002). Canola oil used in the preparation of instantised powders was refined and without added antioxidant and hence there should be minimal influences of the oil on oxidative stability of the powder. There were also compounds with high loadings on PC-2 and associated with the increased oxidation in the instantised GMO powders. These compounds were uniquely present in the instantised GMO powders such as 2-methylfuran, 2-ethylfuran, furfural, 3-methylbutanal, benzaldehyde and 3,5-octadien-2-one isomer 1. The furan compounds in the instantised powder have been reported to be formed by Maillard reaction between sugars, proteins and lipid (Y. Xu et al., 2011), and this can occur during the spray-drying process at high temperature (Van Lancker et al., 2011). The bulk GMO, being a single component material only, experienced lipid oxidation and degradation (Karangwa et al., 2015). The encapsulated oils had a barrier that affects the diffusion of small molecules such as oxygen into the wall materials and resulted in different oxidative stability and volatile profile compared to the bulk oils (Carneiro et al., 2013).

In order to understand the effect of DE value of maltodextrin and MG level on the volatile compounds that increased significantly during storage, instantised GMO powders at 28 days of storage were further analysed by PCA (**Figure 5.3b**). Powders stored for 28 days were selected because they had sufficient lipid oxidation compounds that can discriminate the powder formulation, but had a lower degree of variation among the replicates compared to Day 56 samples. The scores plot at 28 days of storage showed that the LowMG-DE18 sample was different from the other GMO powders, consistent with **Appendix C.1**. The volatile compounds in the loadings plot were in the same direction as LowMG-DE18, except the methyl ketones (2-alkanones), benzaldehyde and

furan compounds. The aldehydes and aliphatic alcohols negatively loaded on PC-1 were lipid oxidation products derived from the unsaturated fatty acids in GMO and canola oil.

Eight compounds positively correlated on PC-1 and one negatively correlated were selected to discriminate further the powder formulation. The compounds were hexanal, octanal, nonanal, 2-ethylfuran, 2-pentylfuran, 1-heptanol, 1-octanol, 3-octen-2-one and 3,5-octadien-2-one isomer 1, and they made up 25% of the total oxidation compounds in the samples. Hence, these compounds were representative to the behaviour of lipid oxidation in the powder formulation. Furthermore, many studies have used these compounds as oxidation markers (Damerau et al., 2014; Genot et al., 2003) including **Chapter 4**. **Figure 5.4** shows the changes in total ion chromatogram (TIC) peak area for the nine oxidation markers over 56 days of storage at 45°C. The LowMG-DE18 sample had the highest rate of increase over time for all oxidation markers except 2-ethylfuran, which exhibited low loadings on PC-1. Other GMO powders had a similar rate of increase over time. The peak area at 28 days of storage (**Table 5.4**) also shows that the LowMG-DE18 sample had the largest peak area for hexanal, octanal, nonanal, 1-heptanol, 1-octanol and 3,5-octadien-2-one isomer 1.

The LowMG-DE18 sample showed higher secondary lipid oxidation than other GMO powders. The determination of encapsulated oil position in whole milk powder using X-ray photoelectron spectroscopy revealed that about 68% of encapsulated oils are deposited near to the surface after removal of surface oil (E. H. J. Kim, Chen, & Pearce, 2002). GMO, being an emulsifier, could adsorb to the semi-permeable membrane of oil droplets and become deposited in the wall material. Therefore, the encapsulation of high level of emulsifier (>30% MG) may not show the same oxidative stability trend compared to oil encapsulation due to their position in the powder structure. The free-flowing and small droplet size of the LowMG-DE18 sample provided a large surface area for contact with oxygen, which could explain the higher lipid oxidation. Although the LowMG-DE10 sample had a small mean droplet size, the lower oxidation could be due to a lower surface area due to powder agglomeration. The powder agglomeration in LowMG-DE10 can be hypothesised from the high Carr's index and Hausner ratio, which indicated the powder had poor compressibility and not free-flowing (Cortés-Rojas, Souza, & Oliveira, 2014). The high MG powders had larger oil droplets, which correspond to lower surface area for oxidation.

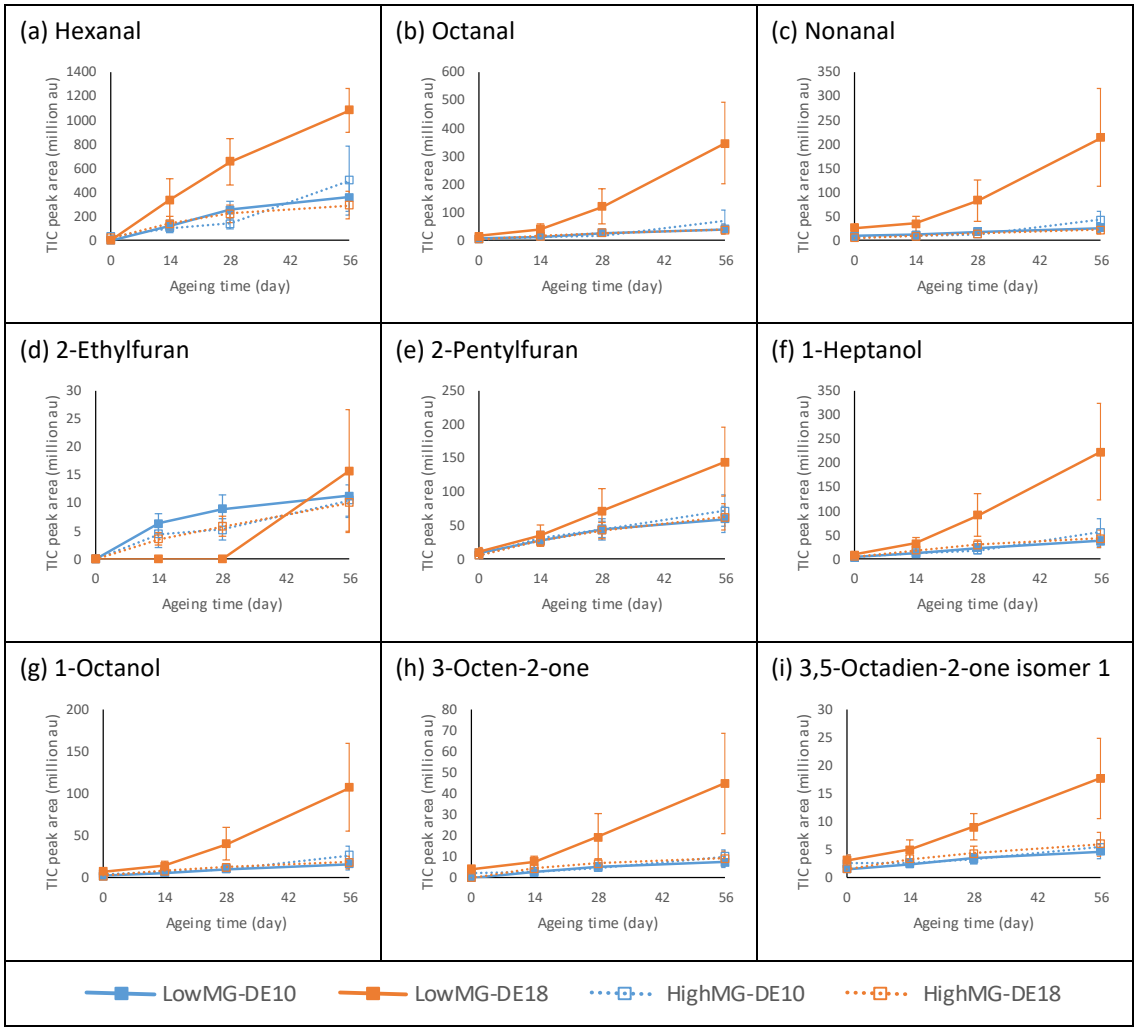


Figure 5.4 Lipid oxidation markers at the various storage time at 45 °C. Each point represents the mean value and the error bar represents the standard deviations (n=3).

Table 5.4 Lipid oxidation markers for GMO powders at 28 days of storage at 45 °C as a function of DE value of maltodextrin and MG level.

Compound	TIC peak area (million au)			
	LowMG-DE10	*LowMG-DE18*	*HighMG-DE10*	*HighMG-DE18*
Hexanal	253.95 ± 68.76[a]	**656.52 ± 195.51[b]**	140.32 ± 39.75[a]	221.65 ± 72.82[a]
Octanal	26.70 ± 8.63[a]	**121.40 ± 61.09[b]**	17.90 ± 4.40[a]	26.28 ± 9.22[a]
Nonanal	17.81 ± 5.67[a]	**83.57 ± 42.91[b]**	12.54 ± 2.95[a]	14.02 ± 3.88[a]
2-Ethylfuran	8.89 ± 2.46[a]	not detected	5.26 ± 1.84[a]	5.86 ± 1.71[a]
2-Pentylfuran	44.53 ± 12.36[a]	72.07 ± 32.13[a]	44.08 ± 15.02[a]	43.16 ± 12.19[a]
1-Heptanol	23.56 ± 7.43[a]	**91.75 ± 44.88[b]**	18.60 ± 6.62[a]	29.73 ± 9.97[a]
1-Octanol	10.63 ± 3.25[a]	**40.29 ± 19.49[b]**	10.09 ± 3.30[a]	12.90 ± 4.26[a]
3-Octen-2-one	5.36 ± 1.52[ab]	19.43 ± 10.82[b]	4.75 ± 1.36[a]	6.68 ± 2.15[ab]
3,5-Octadien-2-one isomer 1	3.44 ± 0.94[a]	**9.03 ± 2.38[b]**	3.34 ± 0.88[a]	4.32 ± 1.23[a]

Values are means ± standard deviations of three measurements. Different letters in the same row for the same measurement indicate statistically significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test.
Compounds in bold show that only one sample has the highest mean peak area.

5.4 Conclusions

This study evaluated the influence of DE value of maltodextrin and MG level on emulsion properties, particle properties and oxidative stability of an instantised GMO prepared by spray-drying. The DE value of maltodextrin had a smaller effect on the emulsion and powder properties compared to the MG level. Increasing the MG level from low to high resulted in emulsions with larger droplets and higher viscosity and dried powders with poorer reconstitution properties (higher emulsion droplet growth and lower dispersibility). All GMO powders showed greater oxidative stability than bulk GMO. Among the instantised GMO powders, the LowMG-DE10, HighMG-DE10 and HighMG-DE18 samples showed the greatest oxidative stability. A good instantised GMO powder should have good dispersibility in water, form small and stable emulsion droplets upon reconstitution and have high oxidative stability. This study demonstrated that spray-drying is a potential process to transform lipid soluble GMO into an instantised ingredient with good dispersibility properties in water and enhanced oxidative stability. This successful preparation of instantised GMO powder is an advancement in ingredient science, where a typical oil-soluble emulsifier can now be used conveniently in the food industry with enhanced functionality and oxidative stability.

Chapter 6

Effect of Novel Instantised Glycerol Monooleate by Spray-Drying on Physicochemical Properties, Creaming Stability and Oxidative Stability of a Protein-Stabilised Oil-in-Water Emulsion

6.1 Introduction

Protein-stabilised emulsions are an example of oil-in-water emulsions that have been used to deliver nutrients, bioactive compounds and flavours (Hu et al., 2017). The most common food emulsions are yoghurt, cream, coffee creamer, milk and plant-based beverages (McClements, 2015). Proteins are the main emulsifier that stabilises the oil droplets in the aqueous phase. Many proteins are amphiphilic molecules containing both hydrophilic and hydrophobic structures, which make them good emulsifiers. The amphiphilic nature of protein allows them to adsorb to the oil-water interface and form a protective film surrounding the oil droplets (Walstra, 2002). The adsorbed proteins at the interface provide repulsive forces such as steric and electrostatic forces to stabilise the oil droplets in the aqueous phase (Bos & van Vliet, 2001). However, oil droplets can simultaneously undergo destabilisation mechanisms such as creaming, flocculation and coalescence that eventually lead to complete separation of oil and aqueous layers.

Casein and whey proteins are the milk proteins primarily used in food formulations due to their outstanding emulsifying properties (Damodaran, 2005; Dickinson, 1997). Milk proteins can also adsorb to the air-water interface under agitation leading to foam formation. Foaming in milk beverages is not desired as it can lead to inconsistent product quality, higher product loss and lower productivity. Low-molecular-weight emulsifiers, such as monoglycerides, can be added to the emulsion to increase emulsion stability (Fredrick et al., 2013; McClements, 2004) and reduce foam formation during homogenisation (Krog, 2011).

Creaming is the most common destabilisation mechanism that occurs in oil-in-water emulsions such as milk beverages (S. R. Euston & Hirst, 2000; McClements, 2015). Creaming is an upward movement of dispersed oil droplets in the emulsion due to the density difference between oil and aqueous phases at quiescence state. The cream layer is easy to redisperse back into the emulsion upon agitation, as the oil droplets are still intact with no physical and chemical interactions. **Chapter 3** investigated various monoglyceride compositions in model emulsions, and the results showed that glycerol monooleate (GMO) produced smaller oil droplets with narrow size distribution and greater stability towards creaming during ageing. Dickinson and Hong (1994) and Matsumiya et al. (2010) also reported that GMO adsorption at the oil-water interface led to smaller droplet formation that improved creaming stability of the emulsion.

GMO is an unsaturated monoglyceride with only one of the hydroxyl groups in glycerol esterified to unsaturated fatty acids, i.e. oleic acid. The structure of GMO is analogous to a triglyceride molecule, contributing to its high solubility in oil and poor dispersibility in water and is thus not suitable for many food formulations. Therefore, GMO could be transformed into a suitable food format, i.e. an instantised powder with good dispersibility in water, using spray-drying. Spray-drying is the most commonly available technique in the food industry to transform hydrophobic materials, such as oil and bioactive compounds, into instantised powders with good water dispersibility and protection against lipid oxidation (Augustin & Oliver, 2014). The unsaturated fatty acids in GMO are prone to oxidation, which is the root cause of rancidity and off-flavour in the ingredients. Headspace solid-phase microextraction (HS-SPME) with gas chromatography-mass spectrometry (GC-MS) has been used extensively to study lipid oxidative stability by measuring the volatile secondary oxidation products (Damerau et al., 2014).

Chapter 5 characterised the physicochemical properties and oxidative stability of the instantised GMO powders produced using spray-drying with different formulations. This chapter investigates the application of the GMO powders in protein-stabilised emulsions. This study aims to understand the effect of instantised GMO powder on physicochemical properties, creaming stability and oxidative stability in protein-stabilised emulsions.

6.2 Materials and Methods

6.2.1 Materials

Glycerol monooleate (Radiamuls MG 2905K) (containing at least 90% monoglyceride content) was a gift from Oleon (Klang, Malaysia). Sodium caseinate and whey protein concentrate 80% (WPC) were provided by Tatua Co-operative Dairy Company Ltd. (Morrinsville, New Zealand). Refined canola oil and caster sugar were purchased from the local supermarket. Sodium azide was purchased from Sigma-Aldrich (St. Louis, MO, USA). Instantised GMO powders, GMO+DE10 and GMO+DE18, were prepared using spray-drying as described in **Section 5.2.2** and the powder characterisation results were presented in **Table 5.3**. Both the instantised GMO powders with low monoglyceride content (LowMG-DE10 and LowMG-DE18) in **Chapter 5** were selected for this study because the powders had the lowest surface oil (3%), good dispersibility in water (74-87%) and the smallest change in droplet size after reconstitution. The key comparisons

were between emulsions with bulk GMO and instantised GMO and between emulsions with GMO and control. The same model emulsion (4% canola oil with 0.8% caseinate and 0.2% whey protein) as the pilot study was used so that the results can be related back to previous chapters.

6.2.2 Preparation of model emulsions

Five model emulsions with different emulsifier systems were prepared by microfluidisation and their formulations are presented in **Table 6.1**. Each sample formulation was prepared in duplicate. Samples were prepared on the same day in a randomised block design to evaluate emulsion repeatability. Water-phase ingredients consisted of sodium caseinate, whey protein concentrate, caster sugar and sodium azide, reconstituted in Milli-Q water at 50 °C. Instantised GMO powder or maltodextrin only was added to the water-phase when required in the formulation. The concentration of sodium caseinate, whey protein concentrate and caster sugar in the model emulsions were designed to mimic a ready-to-drink dairy beverage. Sodium azide was used to prevent of microbial growth during storage affecting the results of creaming, emulsion stability and volatile profile analyses. The water-phase was continuously agitated using a high-shear homogeniser, Ultra-Turrax (IKA-Werke GmbH and Co. KG, Stufen, Germany), at 10,000 rpm and was simultaneously heated to 75 °C. GMO, when required, was added to canola oil at 60 °C to form the oil-phase. The oil-phase was mixed with the water-phase solution, which was then homogenised for three min at 75 °C to form a coarse emulsion. The visual appearance of the foaming behaviour after formation of the coarse emulsion was recorded by using the camera (8 MP) of an iPhone 5S (Apple Inc., Cupertino, CA, USA). The coarse emulsion was passed through a microfluidiser (Microfluidics Corporation, Massachusetts, USA) using a single pass at 55 MPa and 65 °C. The microfluidiser was pre-heated by circulating water at 70 °C for 2 min. All model emulsions were stored securely either in 100 mL bottles, clear test tubes (16 mm o.d. x 150 mm height) and 20 mL headspace vials with Teflon-lined septa for emulsion stability, creaming index and oxidative stability, respectively. The physical and oxidative stability of the emulsion droplets was evaluated under accelerated conditions at 45 °C over a period of 1-28 days.

Table 6.1 Formulation table for preparing model emulsion samples.

Sample	bGMO	GMO+ DE10	GMO+ DE18	DE10	DE18
(a) Oil phase					
Canola oil	4.0%	4.0%	4.0%	4.0%	4.0%
GMO	0.2%				
(b) Water phase					
Caster sugar	6.0%	6.0%	6.0%	6.0%	6.0%
Sodium caseinate	0.80%	0.80%	0.80%	0.80%	0.80%
WPC	0.20%	0.20%	0.20%	0.20%	0.20%
Sodium azide	0.02%	0.02%	0.02%	0.02%	0.02%
Maltodextrin DE 10				0.28%	
Maltodextrin DE 18					0.28%
LowMG-DE10 powder		0.60%			
LowMG-DE18 powder			0.60%		
Milli-Q water	88.8%	88.4%	88.4%	88.7%	88.7%

GMO: Glycerol monooleate, WPC: Whey protein concentrate, DE: Dextrose equivalent

6.2.3 Droplet size and polydispersity index of model emulsions

Droplet size and size-range distributions of the model emulsions were measured by dynamic light scattering using a Malvern Zetasizer Nano S (Malvern Instruments Ltd., Malvern, Worcestershire, UK). The measurements were carried out using the procedures described previously in **Section 3.2.3**. Samples were diluted at 1:1000 with a 6% sucrose solution to keep the sample matrix and osmolarity constant during analysis. The diluted sample was analysed for 60 s at 25 °C with duplicate measurements carried out for each sample. The average droplet size was expressed as the intensity-weighted mean diameter (Z-average), and the degree of non-uniformity of a droplet size distribution was presented as the polydispersity index.

6.2.4 Zeta potential

Zeta potential of the model emulsions was determined using the Malvern Zetasizer Nano S. The procedure was described previously in **Section 3.2.4**. Samples were diluted at

1:20 with a 6% sucrose solution. The diluted sample was analysed at 25 °C with duplicate measurements carried out for each sample.

6.2.5 Viscosity

Viscosity of the model emulsions was performed using a double gap geometry (measuring cup DG42 RO and rotor DG43 DIN53544 Ti) and 11.5 mL sample volume at 20 °C with a Rheostress 1 (Haake, Karlsruhe, Germany) rheometer coupled with a Haake DC-30 circulating bath. The procedure was described previously in **Section 4.2.5**. Measurements were carried out in duplicate for each sample at the shear rate range of 1 to 500 s^{-1}.

6.2.6 pH

The pH of model emulsions was tested at room temperature using a pH209 (HANNA Instruments, Woonsocket, RI, USA) pH meter.

6.2.7 Creaming index

The creaming index of the emulsions was obtained by a visual observation method as described previously in **Section 3.2.7**. Creaming measurements were taken on emulsions stored in clear test tubes sealed with screw cap lids, with extra care taken not to disrupt the cream layer. Creaming index, which is the ratio of cream height over total height of emulsion upon standing, was calculated according to McClements (2007).

6.2.8 Emulsion stability

All model emulsions were stored in 100 mL bottles with screw caps and subjected to analysis on droplet size, polydispersity index, zeta potential, viscosity and pH. The bottles were agitated until the cream layer was well redispersed in the emulsion before conducting any analysis at each time interval during the 28-day ageing period.

6.2.9 Headspace solid-phase microextraction (HS-SPME)

The extraction of volatiles in the samples were carried out using the procedures described previously in **Section 4.2.9**. All emulsions were pre-weighed (5 g) into 20 mL headspace vials and the vial was capped with a Teflon-lined silicone rubber septum. The samples were stored in the sealed headspace vials at 45 °C over a period of 1-28 days and removed at specified time intervals. The samples were then stored in a -20 °C freezer until all

samples with different ageing times were collected, prior to volatile analysis. All vials were covered with aluminium foil at all times to prevent impact of light on chemical reactions. The extraction of volatiles was carried out with a PAL RSI 85 multipurpose sampler (CTC Analytics, Bern, Switzerland) at 45 °C. The samples were equilibrated at 45 °C for 5 min, before the SPME fibre (50/30 μm divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS)) (Supelco, Bellefonte, PA, USA), was exposed for 40 min. After the extraction, the SPME fibre was injected into the gas chromatograph inlet for desorption and analysis (see **Section 6.2.10**). Six HS-SPME analyses were performed on each emulsion formulation (2 emulsion batches x 3 analytical replicates per batch).

6.2.10 GC-MS analysis

The analyses of headspace volatiles in the samples were carried out as described previously in **Section 4.2.10** with some modifications using an Agilent GC 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 5975B VL MSD quadrupole mass spectrometer (Agilent Technologies, Wilmington, DE, USA). SPME desorption was carried out at 240 °C for 2 min in splitless mode, followed by 3 min in split mode. Following sample desorption, the SPME fibre was conditioned for 2 min at 270 °C with a purge flow of 50.0 mL min^{-1}. Pure helium gas was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The chromatographic separation was performed on a 60 m x 0.32 mm i.d. x 0.5 μm Zebron ZB-WAX column (Phenomenex, Torrance, CA, USA). The oven temperature was held for 5 min at 40 °C, increased at a rate of 5 °C min^{-1} until 190 °C, then subsequently increased at a rate of 10 °C min^{-1} to 240 °C and held for 5 min. The transfer line to the MS was set to 230 °C and the quadrupole was set to 150 °C. The mass spectrometer was operated at a scan speed of 5.1 scans s^{-1} and mass spectra were recorded in the range of 29-300 m/z. Carryover between GC runs was assessed using 2 blank samples (an empty vial and a vial containing deionised water only) after every 7 or 8 samples. A randomised block of 15 samples was run on each day and the analyses were performed over a period of 6 days.

The Agilent Enhanced MSD Chemstation software (version F.01.01.2317, Agilent Technologies Inc., USA) was used to process the chromatogram and data from the mass spectrometer. Semi-quantification of volatile compounds was carried out on the total ion count (TIC) profile. Alignment of the chromatograms was carried out manually. Volatile

compounds were identified based on their mass spectra and supported by their retention index (RI). Mass spectra were compared to the National Institute of Standards and Technology (NIST14) database. Linear retention indices were calculated using a C9-C17 n-alkane series as reference compounds using the following equation:

$$\mathrm{RI}(x) = (100 \times z) + \frac{\mathrm{RT}(x) - \mathrm{RT}(z)}{\mathrm{RT}(z+1) - \mathrm{RT}(z)} \qquad \textbf{Equation 6.1}$$

Where RI(x) is the retention index of the unknown compound x, z is the number of carbon atoms of the n-alkane eluted before the unknown compound x, z+1 is the number of carbon atoms of the n-alkane eluted after the unknown compound x, RT(x) is the retention time of the unknown compound x, RT(z) is the retention time of the n-alkane eluted before the unknown compound x, RT(z+1) is the retention time of the n-alkane eluted after the unknown compound x. Calibration curves for hexanal, octanal and 1-octen-3-ol were prepared using a standard addition method and the linear region in the range of 0-900 mg/mL was used for semi-quantification. All samples were measured in six replicates (n=6).

6.2.11 Statistical analysis

IBM SPSS Statistics Version 23 (IBM Corporation, Armonk, New York, USA) was used for statistical comparison of the results. One-way analysis of variance (ANOVA) was carried out to determine significant differences between the results, followed by Tukey's post hoc test for pairwise comparison. All tests were performed at the 95% confidence level. Principal component analysis (PCA) was carried out on all volatile compounds that increased significantly over time using The Unscrambler X Version 10.5 (CAMO, Oslo, Norway) and all variables in the PCA were standardised (1/standard deviation).

6.3 Results and Discussion

6.3.1 Observation of foaming behaviour during homogenisation

Figure 6.1 shows the visual appearance of model emulsions prepared with different emulsifier systems during high-shear homogenisation. The foams in the emulsions disappeared after addition GMO (bGMO, GMO+DE10 and GMO+DE18) during homogenisation, but a substantial amount of foam remained in the control emulsions with maltodextrin only (DE10 and DE18). According to Hanselmann and Windhab (1998),

the agitation between protein and sugar in solution led to foam formation. Besides emulsification, GMO also exhibits anti-foaming properties in protein-stabilised emulsions that could increase productivity through foam reduction.

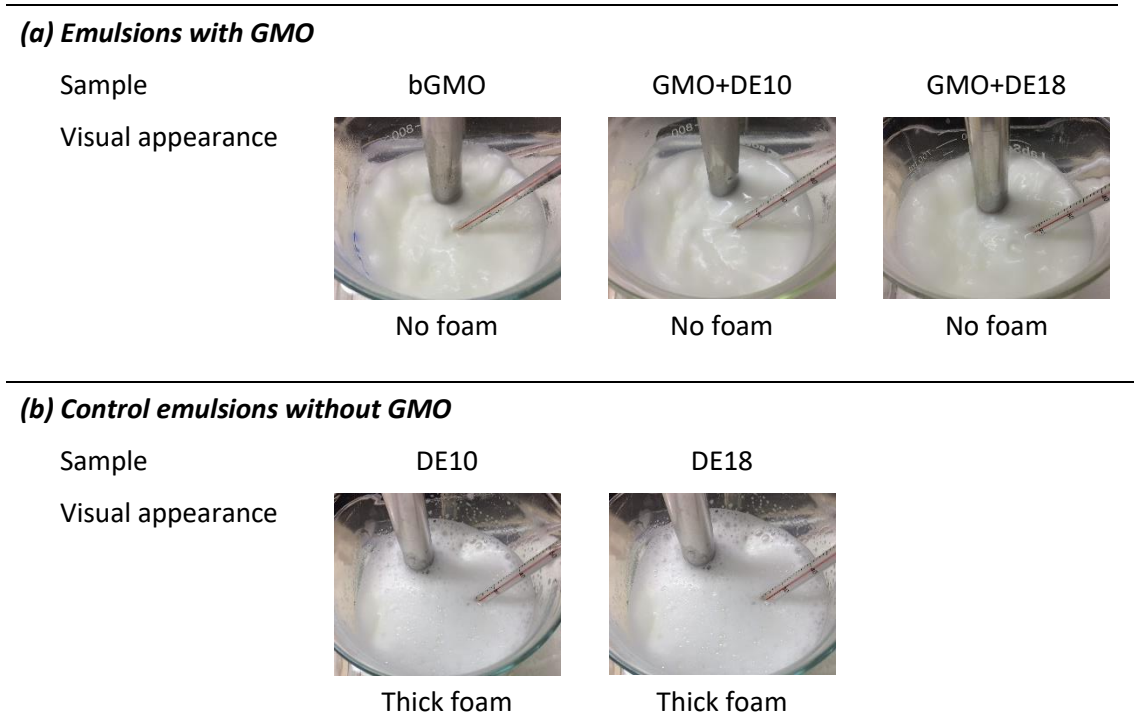


Figure 6.1 Visual appearance of model emulsions (a) with and (b) without GMO during high-shear homogenisation.

6.3.2 Droplet size and polydispersity index of model emulsions

Table 6.2 shows the droplet size and polydispersity index of model emulsions prepared with different emulsifier systems over 28 days of ageing at 45 °C. Droplet size of control emulsions (DE10 and DE18) differed significantly from emulsions with GMO (bGMO, GMO+DE10 and GMO+DE18) ($p<0.05$). Fresh and aged emulsions with GMO (172-186 nm) had a significantly smaller droplet size than controls (194-200 nm). This observation agrees with the findings in **Chapter 3** that emulsions with monoglycerides form smaller droplets than controls. However, there was no significant difference in term of droplet size for the emulsions with bulk GMO and instantised GMO powders of different DE values of maltodextrin. The sodium stearate from the GMO powders was very low (around 80 ppm in the emulsions) and did not affect the droplet size. Both controls with maltodextrin DE10 and DE18 also had the same droplet sizes.

Table 6.2 Droplet size and polydispersity index of model emulsions prepared with different emulsifier systems at various ageing times.

(a) Droplet size (nm)

Sample	Day 0	Day 7	Day 14	Day 28
bGMO	186.0 ± 5.6^{ab}	183.2 ± 5.2^{a}	182.3 ± 4.0^{a}	184.1 ± 3.1^{a}
GMO+DE10	178.1 ± 1.5^{a}	175.7 ± 2.3^{a}	175.1 ± 3.2^{a}	177.9 ± 2.3^{a}
GMO+DE18	175.8 ± 0.8^{a}	174.1 ± 0.6^{a}	172.3 ± 1.0^{a}	174.0 ± 0.9^{a}
DE10	198.4 ± 8.5^{c}	199.8 ± 10.5^{b}	196.4 ± 9.2^{b}	199.5 ± 9.6^{b}
DE18	196.9 ± 6.1^{bc}	196.1 ± 5.0^{b}	194.7 ± 4.4^{b}	198.4 ± 3.5^{b}
F-value	15.2	16.3	18.7	22.7
p-value	<0.001	<0.001	<0.001	<0.001

(b) Polydispersity index

Sample	Day 0	Day 7	Day 14	Day 28
bGMO	0.162 ± 0.022^{ab}	0.170 ± 0.012^{a}	0.177 ± 0.009^{a}	0.177 ± 0.019^{a}
GMO+DE10	0.163 ± 0.019^{ab}	0.162 ± 0.015^{a}	0.162 ± 0.008^{a}	0.165 ± 0.010^{a}
GMO+DE18	0.158 ± 0.014^{a}	0.158 ± 0.006^{a}	0.161 ± 0.009^{a}	0.160 ± 0.006^{a}
DE10	0.201 ± 0.022^{b}	0.204 ± 0.022^{b}	0.202 ± 0.021^{b}	0.202 ± 0.018^{b}
DE18	0.194 ± 0.015^{ab}	0.209 ± 0.009^{b}	0.198 ± 0.011^{b}	0.200 ± 0.018^{b}
F-value	4.6	11.5	10.0	6.6
p-value	<0.05	<0.001	<0.001	<0.01

Values are means ± standard deviations of four measurements (2 batches x 2 replicates). Different letters in the same column indicate statistically significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test.

All emulsion samples, except GMO+DE18, showed no significant change in droplet size over 28 days of ageing at 45 °C ($p>0.05$). Although the GMO+DE18 sample changed significantly over ageing time, the magnitude of the change in their droplet sizes was negligible (<3%). This finding concurs with the results on protein-stabilised emulsions in **Chapter 3** that indicated stable droplet size during storage without any significant droplet growth due to flocculation, coalescence or Ostwald ripening (Berton et al., 2011; J. Chen & Dickinson, 1993; McClements & Rao, 2011; Munk & Andersen, 2015).

Table 6.2b shows the polydispersity index of model emulsions over 28 days of ageing at 45 °C. All emulsions with GMO and controls had a polydispersity index of 0.2 or lower,

which indicated narrow size-range distributions. The volume-weighted droplet size distribution showed that all fresh and 28-day aged emulsions had a pseudo-monomodal size distribution (**Figure 6.2**) and supported the polydispersity index results. The fine droplets around 20 nm in the 28-day aged sample DE10 was less than 1.5%, hence did not affect its polydispersity index. The polydispersity index values were 18% lower for emulsions prepared with GMO compared to the controls. Regardless of ageing time, emulsions with bulk GMO and instantised GMO had smaller mean oil droplet sizes and a narrower droplet size distribution than controls. Both control emulsions with different DE value of maltodextrin did not show any significant difference in term of droplet size and polydispersity index from each other. This observation reaffirmed that maltodextrin at this level had no significant effect on the droplet size and its size-range distribution, which is in line with the findings by (Gharsallaoui et al., 2010). All emulsions had similar protein composition and level, therefore suggesting GMO is responsible for the reduction in droplet size of the emulsions.

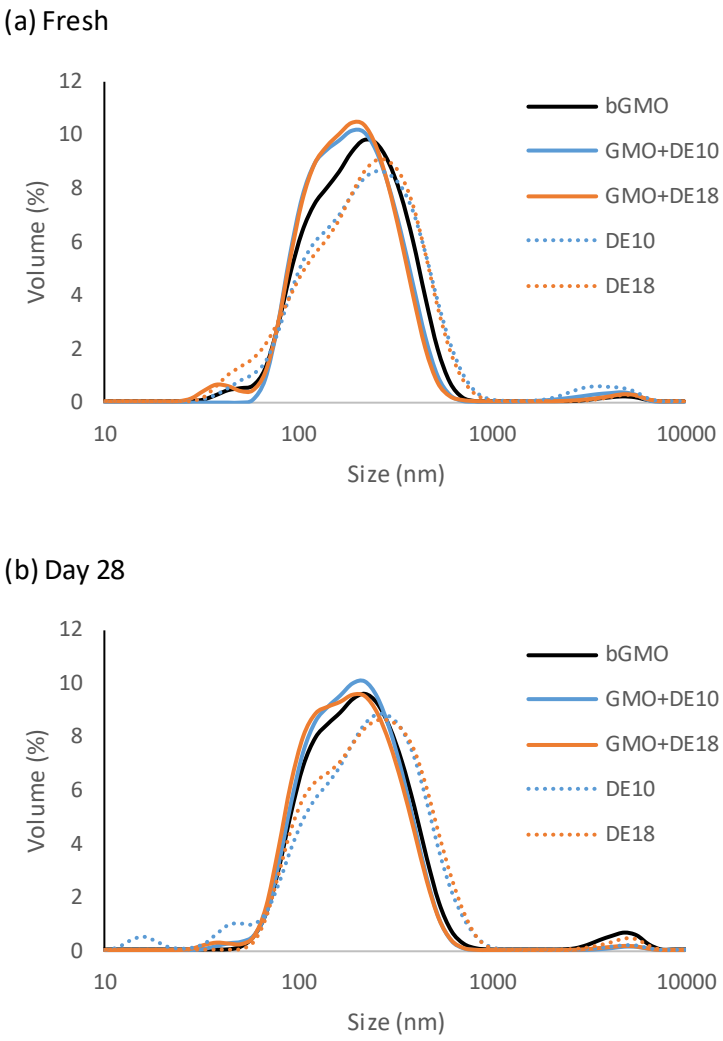


Figure 6.2 Volume-weighted size distribution of (a) fresh and (b) 28 days aged model emulsions.

6.3.3 Zeta potential of model emulsions

Table 6.3 shows the zeta potential of model emulsions prepared with different emulsifier systems over 28 days of ageing at 45 °C. All fresh emulsions (Day 0) prepared with different emulsifier systems had a zeta potential in the range of -51 to -53 mV. In order to simplify the discussion of the results, zeta potential refers to the magnitude and its negative sign is ignored due to all emulsions exhibiting negative values. Hence, an increase in zeta potential refers to the increase in the negativity of the zeta potential. The zeta potential provides information on the repulsive forces at the oil-water interface which can explain droplet stability in the emulsions (Tcholakova et al., 2006). All the

aged emulsions with different emulsifier systems showed a statistically significant difference ($p<0.05$), but the small differences in zeta potential (<3 mV) are not useful to distinguish the emulsifier systems. Sodium stearate in the instantised powders increased the zeta potential of the emulsions, similar to the findings observed in **Chapter 3**. The increase in zeta potential by sodium stearate is speculated to be due to the adsorption of anionic molecules at the oil-water interface increasing the ionic repulsive force among the oil droplets. The similarity in zeta potential indicates that the emulsion droplets are predominantly stabilised by protein (Post, Arnold, Weiss, & Hinrichs, 2012) and not much protein displacement by the emulsifier is taking place. It is hypothesised that sodium caseinate contributes to the negative zeta potential in the soluble form and the slightly lower zeta potential value after storage might due to precipitation of sodium caseinate (Mezdour & Korolczuk, 2010). All emulsions showed a decreasing trend in zeta potential with ageing time ($p<0.05$) and the small reduction of zeta potential (<5 mV) over 28 days of ageing. However, this may not have any practical implication because the oil droplets still retained high repulsive forces (-47 to -50 mV). Ross and Morrison (1988) stated that oil droplets with a zeta potential below -30 mV have excellent stability against flocculation or coalescence. This observation was supported by stable droplet size distribution during the ageing time.

Table 6.3 Zeta potential of model emulsions prepared with different emulsifier systems at various ageing times.

Sample	Day 0	Day 7	Day 14	Day 28
bGMO	-51.6 ± 1.4^{a}	-47.8 ± 0.5^{c}	-47.7 ± 0.4^{a}	-46.9 ± 1.1^{b}
GMO+DE10	-51.9 ± 0.2^{a}	-49.1 ± 0.3^{b}	-49.0 ± 0.5^{b}	-47.6 ± 0.2^{ab}
GMO+DE18	-52.1 ± 0.4^{a}	-49.0 ± 0.3^{b}	-49.2 ± 0.5^{b}	-47.7 ± 0.1^{ab}
DE10	-53.0 ± 0.3^{a}	-49.8 ± 0.5^{ab}	-49.9 ± 0.7^{b}	-48.1 ± 0.9^{ab}
DE18	-52.9 ± 0.8^{a}	-50.3 ± 0.0^{a}	-50.0 ± 0.8^{b}	-49.3 ± 1.1^{a}
F-value	2.7	16.9	10.0	4.8
p-value	0.07	<0.001	<0.001	<0.05

Values are means ± standard deviations of four measurements. Different letters in the same column indicate statistically significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test.

6.3.4 Viscosity of model emulsions

All five emulsion systems showed a linear relationship between shear stress and shear rate (**Appendix D.1**), which indicated that all emulsions observed the behaviour of a Newtonian fluid. This observation was expected due to the high water content, low protein concentration and absence of polysaccharides and gums in the emulsions. The experimental data best fitted to a Newtonian model (R^2>0.99), hence emulsion viscosity was reported as the adjusted value according to the Newtonian model.

Figure 6.3 shows the viscosity of model emulsions prepared with different emulsifier systems over 28 days of ageing at 45 °C. The fresh emulsion viscosity was not significantly affected by the emulsifier systems (p>0.05) and were in the range of 1.8-2.1 mPa s. Hence, GMO did not affect the viscosity of emulsions and did not change the flow behaviour, which agrees with the findings in **Chapter 3**.

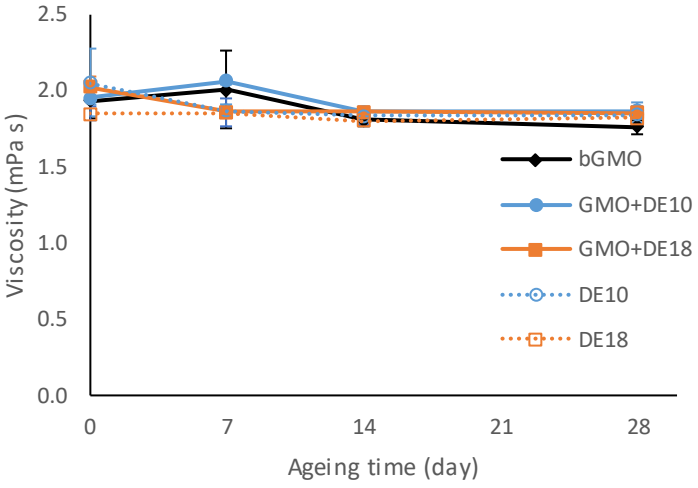


Figure 6.3 Viscosity of model emulsions prepared with different emulsifier systems at various ageing times. Points represent means viscosity and the error bars are the standard deviations (n=4).

The viscosity of all emulsions, except with instantised GMO, did not change significantly over ageing time (p>0.05). The emulsions with instantised GMO changed in the same magnitude over time as other emulsions. All the emulsions showed a small decline in viscosity (1.7-1.9 mPa s) after 28 days of ageing.

6.3.5 pH of model emulsions

The pH of all fresh emulsions was similar (pH 6.7-6.9), decreasing slightly (<0.2) over 28 days of ageing with no apparent oil separation or protein aggregation. The inclusion of GMO at 0.2% did not affect the pH of the model emulsions. This finding was expected because GMO is a non-ionic emulsifier that does not change the pH of the solution. The slight reduction in pH over the storage period could be due to precipitation of soluble caseinate, which also accounts for a small reduction in zeta potential.

6.3.6 Creaming index of model emulsions

Figure 6.4 shows the creaming index of the model emulsions prepared with different emulsifier systems over 28 days of ageing at 45 °C. A thin layer of cream started to develop in the emulsions with bulk GMO and instantised GMO powders (GMO+DE10 and GMO+DE18) after 2 days of ageing at 45 °C and did not increase further after 7 days (maximum creaming index at 1.3%). The results also revealed that the sodium stearate from the instantised GMO powders did not affect creaming. In contrast, the control emulsions with DE10 and DE18 showed that the cream layer continued to develop after 7 days at a slower rate and reached a creaming index of 2.4% at 28 days. This observation showed that emulsions with GMO had greater creaming stability than the control and agrees with the findings on creaming stability in protein-stabilised emulsions in **Chapter 3**. The results showed that the DE value of maltodextrin had no effect on creaming stability. All emulsions had a similar protein composition, which indicated the GMO component was the factor responsible for the improved creaming stability.

The formation of small droplets with narrow size distribution in the emulsions was hypothesised to be due to the interaction between GMO and milk proteins, and was the key to form stable emulsions against creaming. According to Stokes' Law, small droplets have a slower rate of creaming than larger ones and are less likely to separate due to gravitational forces (McClements, 2007). Thus, the small droplet size with narrow droplet size distribution improves creaming stability for long-term storage (Huang et al., 2001; Krog, 2011; Liang et al., 2016; Matsumiya et al., 2010).

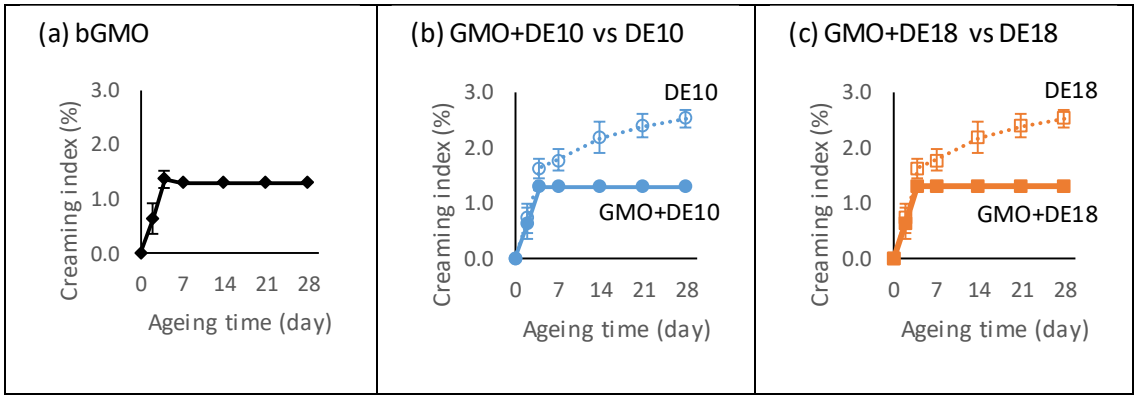


Figure 6.4 Creaming index of model emulsions prepared with different emulsifier systems at various ageing times. Points represent means creaming index and the error bars are the standard deviation (n=6).

6.3.7 Oxidative stability of model emulsions measured using volatile analysis

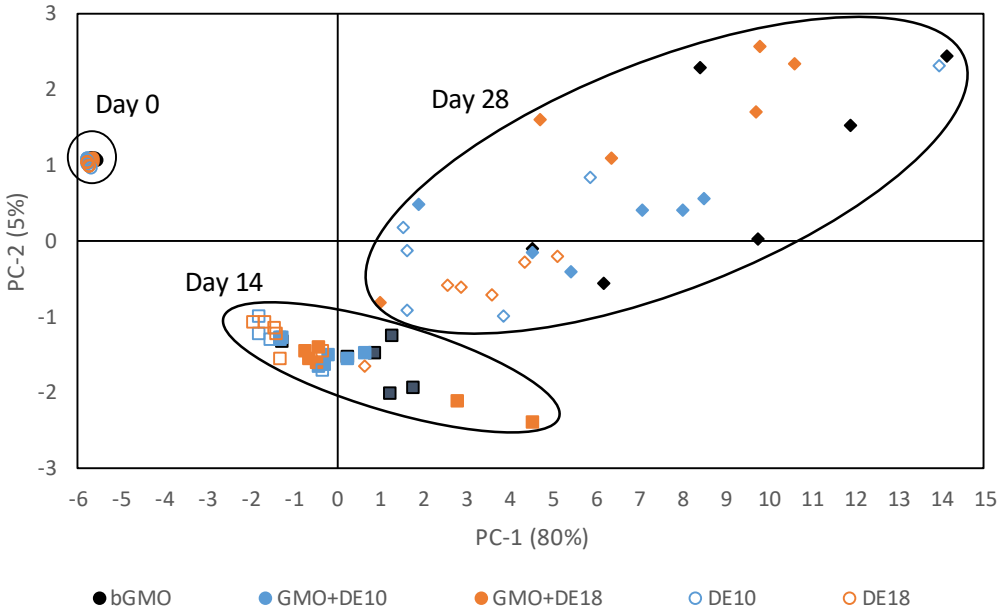
The formation of volatile compounds was monitored in all model emulsions with different emulsifier systems at various storage times using headspace SPME with GC-MS as a tool to evaluate changes in secondary oxidation products during storage. Volatile compounds that increased significantly ($p<0.05$) during storage and had been reported as markers of lipid oxidation in the literature were reported in this study. Compounds with reduced or no change in concentrations during storage were not reported. Analysis of the volatile compounds detected 36 compounds (including two unknowns) that increased significantly in the model emulsions during storage, from four chemical classes, namely aldehydes, furans, alcohols and ketones. Hexanal, was present with the highest abundance relative to other identified compounds.

Figure 6.5 shows the scores and loadings plot of principal component analysis (PCA) for model emulsions at various storage times. The first principal component (PC-1) explained most of the variation (80%) in the data and PC-2 explains 5% of the variation. Sample freshness (fresh vs stored) was the main cause of variation in the PCA model, and by using the first two principal components, the samples can be grouped into three clusters according to the sample freshness, namely fresh, 14-day storage and 28-day storage. Storage at 45 °C was the main cause of variation for PC-1 in the scores plot to differentiate fresh and stored emulsions. The discrimination on PC-1 was due to alcohols (1-pentanol, 1-heptanol, 1-octanol and 1-octen-3-ol), ketones (3-octen-2-one, 6-methyl-5-heptene-2-one, 2-heptanone and 2-octanone) and furans (2-ethyl furan and 2-propyl

furan), which were positively loaded on PC-1. PC-2 was able to distinguish the samples stored at 14 and 28 days by aldehydes, such as propanal, pentanal, hexanal, heptanal, octanal, 2-pentenal and 2,4-heptadienal, which clustered together to the bottom of the loading plot that were associated with higher concentrations at Day 14.

The fresh control emulsions had eight volatile compounds, pentanal, hexanal, heptanal, nonanal, 2,4-heptadienal isomer 2, 1-penten-3-ol, 3,5-octadien-2-one isomers 1 and 2. In addition to the compounds that appeared in the controls, the fresh emulsions with bulk and instantised GMO had additional compounds, including 2-heptenal, 1-pentanol, 1-heptanol, 1-octanol and 1-octen-3-ol. The number of volatiles increased to 35 compounds in the control emulsions after 28 days of storage, while the emulsions with GMO had 36 compounds. None of the furan compounds were detected in the fresh emulsions.

(a) Scores plot



(b) Loadings plot

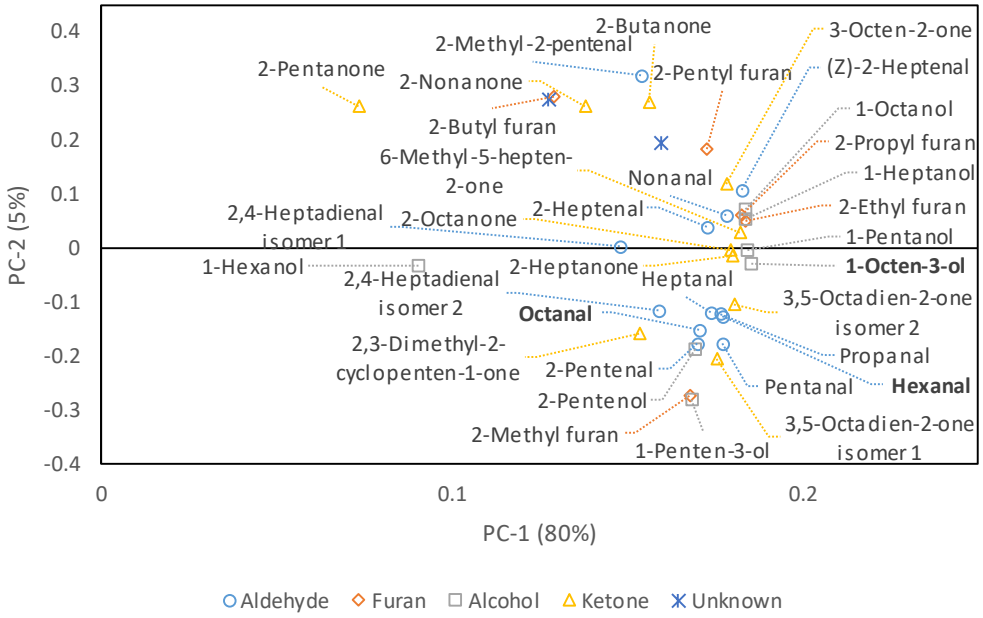


Figure 6.5 Principal component analysis (a) scores plot of sample formulations for model emulsions and (b) loadings plot of volatile compounds at various storage times. Each sample in the score plot has six replicate points (2 batches x 3 replicates). Hexanal, octanal and 1-octen-3-ol (bold text) were selected as oxidation markers.

Three compounds, namely hexanal, octanal and 1-octen-3-ol, were selected as oxidation markers to explain oxidative stability of the emulsions. These compounds have been used as lipid oxidation markers in **Chapter 3** and past literature (Berton et al., 2012; Damerau et al., 2014; Vandamme et al., 2016). Hexanal and 1-octen-3-ol are formed by oxidation of linoleic acid, while octanal is derived from oleic acid (Choe & Min, 2006; Frankel, 1985; Genot et al., 2003). Calibration curves for hexanal, octanal and 1-octen-3-ol in the range of 0-900 mg/mL were used for semi-quantification. **Table 6.4** shows the concentration of the calibrated compounds at various storage times. The concentration of hexanal in the 28-day stored emulsions was outside of the calibration curve and was determined by extrapolating the calibration curve beyond 900 mg/mL with the assumption that the response continued to be linear.

All fresh emulsions showed a comparable concentration of hexanal and octanal, while 1-octen-3-ol was only detected in the emulsions with bulk GMO. This observation indicated that canola oil and GMO used to prepare the emulsions were fresh with very little oxidation since no additional compounds were detected. All three oxidation markers increased with increasing storage time, which aligned with the PCA results, where compounds were positively loaded on PC-1 (**Figure 6.5**). The oxidation markers in the 28-day aged emulsions did not show any effect of the emulsifier system on oxidative stability. **Table 6.5** shows an additional 12 selected lipid oxidation compounds in the 28-day aged emulsions derived from oleic, linoleic and linolenic acids (Genot et al., 2003) that had high loadings on PC-1. Four compounds, propanal, 2,4-heptadienal isomer 2, 3-octen-2-one and 3,5-octadien-2-one isomer 1 indicated that bulk GMO had significantly higher concentration of these compounds than the controls ($p<0.05$). All selected compounds, except propanal, indicated that there is no difference between instantised GMO and the control. This observation was positive because it indicated that instantised GMO at low concentration did not affect the oxidative stability of protein-stabilised emulsions. Previous research (Fomuso, Corredig, & Akoh, 2002; N. Kim & Choe, 2012) has reported that higher monoglyceride content (around 1%) could enhance oxidative stability in emulsions. It is hypothesised that a higher monoglyceride concentration forms a more compact and tighter packing of emulsifier at the oil-water interface, and therefore the interface acts as a barrier to oxygen diffusion into the oil droplets reducing the rate of oxidation.

There was no influence of emulsion droplet size on lipid oxidation, which agrees with findings by Osborn and Akoh (2004) and Dimakou, Kiokias, Tsaprouni, and Oreopoulou (2007). All emulsions also had the same zeta potential, which indicated that the emulsions had similar protein composition in the aqueous phase and at the emulsion interface. The research findings on the effect of protein composition on physical stability in **Chapter 4** hypothesised that the formation of a compact multilayer interface comprised of sodium caseinate and whey proteins could improve oxidative stability in lipid emulsions. In this study, the emulsions with bulk and instantised GMO had the same oxidative stability as control. The same protein composition at the interface indicated by the zeta potential could have a bigger influence on oxidative stability compared to GMO at low concentration and mask any effect by the GMO.

Table 6.4 Lipid oxidation markers for fresh and 28-day stored (45 °C) emulsions as a function of emulsifier system.

Retention index	Volatile compound	Day	Concentration (µg/L)					Source of lipid oxidation*
			bGMO	*GMO+DE10*	*GMO+DE18*	*DE10*	*DE18*	
1082	Hexanal	0	25.0 ± 8.4^{a}	17.7 ± 7.1^{a}	21.9 ± 6.1^{a}	16.2 ± 8.8^{a}	18.7 ± 6.0^{a}	n-6 linoleic acid
		14	567.8 ± 118.8^{bc}	490.7 ± 102.7^{abc}	670.1 ± 294.3^{c}	294.8 ± 121.1^{a}	361.9 ± 20.8^{ab}	
		28	945.0 ± 364.6^{a}	819.7 ± 309.6^{a}	902.4 ± 266.7^{a}	739.5 ± 262.5^{a}	726.8 ± 143.8^{a}	
1292	Octanal	0	n.d.	n.d.	n.d.	n.d.	n.d.	n-9 oleic acid
		14	180.8 ± 52.4^{a}	141.0 ± 18.6^{a}	226.0 ± 145.4^{a}	145.2 ± 24.7^{a}	166.2 ± 82.4^{a}	
		28	380.3 ± 111.3^{a}	292.0 ± 68.5^{a}	297.7 ± 66.5^{a}	232.1 ± 77.6^{a}	273.4 ± 68.6^{a}	
1441	1-Octen-3-ol	0	2.1 ± 0.1	n.d.	n.d.	n.d.	n.d.	n-6 linoleic acid
		14	28.7 ± 4.2^{a}	26.1 ± 2.9^{a}	29.5 ± 8.1^{a}	19.4 ± 1.5^{a}	20.0 ± 1.9^{a}	
		28	60.3 ± 15.8^{a}	49.8 ± 8.7^{a}	54.7 ± 14.9^{a}	43.4 ± 18.4^{a}	38.9 ± 5.6^{a}	

Values are means concentration (µg/L) ± standard deviations of 6 measurements (2 batches x 3 replicates). Different letters in the same row indicate statistically significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test.
n.d. = not detected
*(Genot et al., 2003)

Table 6.5 Lipid oxidation compounds in 28-day stored (45 °C) emulsions as a function of emulsifier system.

Retention index	Compound	TIC peak area (million au)				
		bGMO	*GMO+DE10*	*GMO+DE18*	*DE10*	*DE18*
794	Propanal	33.11 ± 6.62^{c}	28.12 ± 6.23^{bc}	30.96 ± 7.61^{c}	18.28 ± 4.98^{ab}	16.22 ± 2.54^{a}
956	2-Ethyl furan	171.70 ± 41.12^{a}	129.93 ± 34.55^{a}	137.76 ± 41.87^{a}	132.89 ± 79.60^{a}	101.67 ± 22.18^{a}
982	Pentanal	56.61 ± 13.02^{a}	48.06 ± 13.51^{a}	43.86 ± 11.09^{a}	46.29 ± 20.04^{a}	42.55 ± 7.87^{a}
1232	2-Pentyl furan	85.45 ± 31.15^{a}	55.89 ± 17.00^{a}	60.65 ± 16.57^{a}	60.20 ± 58.76^{a}	38.99 ± 7.59^{a}
1278	(Z)-2-Heptenal	14.51 ± 4.85^{a}	11.11 ± 2.71^{a}	12.76 ± 4.90^{a}	9.55 ± 4.06^{a}	8.99 ± 1.85^{a}
1397	Nonanal	38.51 ± 12.78^{a}	30.27 ± 7.12^{a}	31.51 ± 9.50^{a}	25.94 ± 17.65^{a}	21.06 ± 7.23^{a}
1414	3-Octen-2-one	52.50 ± 21.47^{b}	38.44 ± 8.94^{ab}	43.51 ± 17.97^{ab}	23.13 ± 9.97^{a}	21.48 ± 5.39^{a}
1448	1-Heptanol	55.12 ± 17.99^{a}	38.75 ± 7.59^{a}	44.75 ± 15.39^{a}	34.80 ± 21.37^{a}	30.01 ± 8.11^{a}
1505	2,4-Heptadienal isomer 2	14.27 ± 5.21^{b}	9.44 ± 1.57^{ab}	10.36 ± 3.00^{ab}	6.73 ± 3.59^{a}	5.25 ± 0.80^{a}
1527	3,5-Octadien-2-one isomer 1	94.63 ± 22.08^{b}	77.37 ± 10.67^{ab}	85.60 ± 19.21^{ab}	66.08 ± 14.70^{a}	59.94 ± 5.82^{a}
1551	1-Octanol	29.96 ± 10.32^{a}	21.77 ± 4.74^{a}	25.05 ± 8.87^{a}	19.16 ± 12.92^{a}	16.78 ± 4.37^{a}
1581	3,5-Octadien-2-one isomer 2	338.93 ± 54.61^{a}	294.74 ± 32.67^{a}	315.95 ± 59.86^{a}	280.03 ± 41.43^{a}	264.48 ± 25.33^{a}

Values are means peak area (million au) ± standard deviations of 6 measurements (2 batches x 3 replicates). Different letters in the same row indicate statistically significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test.

6.4 Conclusions

This study evaluated the effect of instantised GMO powders on physicochemical properties, creaming stability and oxidative stability in protein-stabilised emulsions. Emulsions with instantised GMO showed smaller mean droplet sizes and narrower size distributions than control emulsions (no GMO), which was similar to the emulsions with bulk GMO. The small droplet size with narrow distribution resulted in greater stability against creaming. Maltodextrin in the emulsions did not show any effect on droplet size distribution and creaming stability. All fresh emulsions showed a similar volatile profile after microfluidisation and changed significantly over time during storage. The emulsions after 28 days of storage were not affected by GMO. This finding reaffirmed that GMO plays a role in improving droplet size distribution that enhanced creaming stability in the emulsions. The smaller droplet size of the emulsions did not have any detrimental effect on oxidative stability. Instantised GMO powder retained all the functionality of bulk GMO and showed additional advantages such as stable emulsion properties after reconstitution, direct dispersion in aqueous formulation, ease of handling and a longer shelf life compared to bulk GMO. This demonstrates the potential of this instantised GMO ingredient to function as secondary emulsifying and stabilising agents that improve creaming stability of protein-stabilised emulsions during storage.

Chapter 7

Effect of Bulk and Instantised Glycerol Monooleate Concentrations on Physicochemical Properties, Creaming Stability and Volatile Profile of a Milk Coffee Emulsion

7.1 Introduction

Milk coffee beverages in the ready-to-drink format (canned or bottled) are gaining a lot of interest, demonstrated by the development, innovation and consumption of these products. Milk coffee beverages are oil-in-water emulsions prepared with milk and coffee extracts, having a pH range from 5 to 7 (Hayakawa et al., 2012). Coffee beverages at a low pH of around 5.5 present numerous challenges during formulation as they undergo adverse changes in emulsion stability during storage. One of the issues in coffee emulsions that needs to be addressed is the resistance of oil droplets against creaming during storage. A cream layer consists of oil droplets with no physical and chemical interactions that can easily redistribute upon agitation. Other issues to address in coffee emulsions are flocculation and aggregation, which are due to the low pH (Ogawa & Cho, 2015; Scott et al., 2003). Flocculation means the association of the oil droplets to form flocs and aggregation refers to the solid particles sticking together.

Milk proteins and low-molecular-weight emulsifiers are surfactants that stabilise oil droplets in milk coffee beverages. Milk proteins such as casein and whey proteins are relatively large biopolymers containing both charged and hydrophobic regions that adsorb to the oil-water interface and provide steric and electrostatic forces to stabilise oil droplets in the aqueous phase (Bos & van Vliet, 2001). Due to the acidity of coffee extracts, adding coffee lowers the pH in milk and cause milk proteins, i.e. casein, to precipitate at pH 4.6 (isoelectric point of casein). As the emulsion pH reduces from neutral pH to pH 4.6, the net negative charge decreases, which reduces casein solubility in water. Low-molecular-weight emulsifiers, such as monoglycerides, are usually added to the emulsion to increase its physical stability (Fredrick et al., 2013; McClements, 2004).

Previous work on different monoglyceride compositions in model emulsions (**Chapter 3**) showed that glycerol monooleate (GMO) formed small oil droplets with a narrow size distribution resulting in improved stability against creaming. GMO is an unsaturated and oil-soluble monoglyceride that has poor dispersibility in water, and is thus inappropriate for many food formulations. In **Chapter 5**, GMO was successfully transformed into a dry powder format using spray-drying encapsulation with appropriate wall materials to form an instantised powder with good dispersibility in water. Model emulsions prepared with instantised GMO powders also showed the same effect on droplet size and improved

creaming stability (**Chapter 6**). GMO is prone to oxidation due to unsaturation of its fatty acids, which cause rancidity and off-flavours. Headspace solid-phase microextraction (HS-SPME) with gas chromatography-mass spectrometry (GC-MS) has been used extensively to study lipid oxidative stability by measuring the volatile secondary oxidation products (Damerau et al., 2014) and evolution of volatile flavour compounds (Caprioli et al., 2012; Fabre, Aubry, & Guichard, 2002; Kühn et al., 2006) during storage.

Chapter 5 characterised the physicochemical properties and oxidative stability of the instantised GMO powders produced using spray-drying with different formulations. Further investigation of the instantised GMO powders was carried out to understand its effect on physicochemical properties, creaming stability and oxidative stability in protein-stabilised emulsions (**Chapter 6**). This study aims to understand the effect of the concentrations of bulk GMO and instantised GMO powders on physicochemical properties, creaming stability, oxidative stability and flavour compounds in an application of a model milk coffee emulsion.

7.2 Materials and Methods

7.2.1 Materials

Glycerol monooleate (Radiamuls MG 2905K) (containing at least 90% monoglyceride content) was a gift from Oleon (Klang, Malaysia). Sodium caseinate and whey protein concentrate 80% (WPC) were provided by Tatua Co-operative Dairy Company Ltd. (Morrinsville, New Zealand). Maltodextrin with dextrose equivalent (DE) value of 10 was purchased from Hawkins Watts Ltd. (Auckland, New Zealand). Refined canola oil, freeze-dried coffee granules (Moccona classic medium roast) and caster sugar were purchased from the local supermarket. Sodium azide was purchased from Sigma-Aldrich (St. Louis, MO, USA). The instantised GMO powder was prepared using spray-drying as described previously (**Section 5.2.2**). The GMO powder was formulated with 33.6% GMO and 15% canola oil as core materials, 47% maltodextrin (DE 10), 3% sodium caseinate and 1.4% sodium stearate as wall materials. The powder had 2.1% moisture, 96% solubility in water and 74% dispersibility in water. Coffee granules were used as an acidic component for a slightly acidic beverage application and the application rate at 2% level was to get sufficient volatile compound concentrations for headspace volatile analysis using gas chromatography-mass spectrometry.

7.2.2 Preparation of model coffee emulsions

Eight model coffee emulsions with two types of GMO-based emulsifier systems (bulk GMO (bGMO) and instantised GMO powder (iGMO)) at three levels of GMO concentration (0.03%, 0.1% and 0.2%) and two controls (Control 1 with no GMO; Control 2 with maltodextrin and no GMO) were prepared by microfluidisation (**Table 7.1**). Each sample formulation was prepared in duplicate. Samples were prepared within two days in a randomised block design. Water-phase ingredients consisted of sodium caseinate (0.8% w/w), whey protein concentrate (0.2% w/w), caster sugar (6% w/w), freeze-dried coffee granules (2% w/w) and sodium azide (0.03% w/w), reconstituted in Milli-Q water at 50 °C. Instantised GMO powder (0.09%, 0.3% or 0.6% w/w) or maltodextrin (0.13%, 0.23% or 0.28% w/w) was added to the water-phase when required in the formulation. This model coffee formulation represents the composition range of ready-to-drink coffee milk beverages available commercially. The pH of this emulsion is near to the isoelectric point of casein, which is the major cause affecting physical stability of coffee emulsions. The concentration of coffee granules in the formulation was determined by piloting the concentration of volatile compounds to achieve optimal headspace extraction and detection by gas chromatograph-mass spectrometry. Sodium azide was used to protect against effects of microbial growth during creaming, emulsion stability and volatile profile analyses. The water-phase was continuously agitated using a high-shear homogeniser, Ultra-Turrax (IKA-Werke GmbH and Co. KG, Stufen, Germany), at 10,000 rpm and was simultaneously heated to 75 °C. GMO (0.03%, 0.1% or 0.2% w/w), when required, was added to canola oil (4% w/w) at 60 °C to form the oil-phase. The oil-phase was mixed with the water-phase solution, and was homogenised for three min at 75 °C to form a coarse emulsion. The coarse emulsion was passed through a microfluidiser (Microfluidics Corporation, Massachusetts, USA) using a single pass at 55 MPa and 65 °C. The microfluidiser was pre-heated by circulating water at 70 °C for 2 min. All coffee emulsions were stored with lids on either in 100 mL bottles, clear test tubes (16 mm OD x 150 mm height) and 20 mL headspace vials with Teflon-lined septa for emulsion stability, creaming index and oxidative stability, respectively. The physical and oxidative stability of the emulsion droplets was evaluated under accelerated conditions at 45 °C for 1-28 days.

Table 7.1 Formulation table for preparing model coffee emulsion samples.

Sample	**Bulk GMO (bGMO)**				**Instantised GMO (iGMO)**			
GMO level (%)	***Control 1***	***0.03%***	***0.1%***	***0.2%***	***Control 2***	***0.03%***	***0.1%***	***0.2%***
(a) Oil phase								
Canola oil	4.0%	4.0%	4.0%	4.0%	4.0%	4.0%	4.0%	4.0%
Glycerol monooleate (GMO)	-	0.03%	0.10%	0.20%	-	-	-	-
(b) Water phase								
Caster sugar	6.0%	6.0%	6.0%	6.0%	6.0%	6.0%	6.0%	6.0%
Sodium caseinate	0.80%	0.80%	0.80%	0.80%	0.80%	0.80%	0.80%	0.80%
Whey protein concentrate	0.20%	0.20%	0.20%	0.20%	0.20%	0.20%	0.20%	0.20%
Sodium azide	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%
Maltodextrin	-	-	-	-	0.28%	0.23%	0.13%	-
Instantised GMO powder	-	-	-	-	-	0.09%	0.30%	0.60%
Freeze-dried coffee granules	2.0%	2.0%	2.0%	2.0%	2.0%	2.0%	2.0%	2.0%
Milli-Q water	87.0%	86.9%	86.9%	86.8%	86.7%	86.7%	86.5%	86.4%

7.2.3 Droplet size and polydispersity index of model coffee emulsions

Droplet size and size-range distributions of the model emulsions were measured by dynamic light scattering using a Malvern Zetasizer Nano S (Malvern Instruments Ltd., Malvern, Worcestershire, UK). The measurements were carried out using the procedures described previously in **Section 3.2.3**. Samples were diluted at 1:1000 with a 6% sucrose solution to keep the sample matrix and osmolarity constant during analysis. The diluted sample was analysed for 60 s at 25 °C with duplicate measurements carried out for each sample. The average droplet size was expressed as the intensity-weighted mean diameter (Z-average), and the degree of non-uniformity of a droplet size distribution was presented as the polydispersity index.

7.2.4 Zeta potential

Zeta potential of the model emulsions was determined using the Malvern Zetasizer Nano S. The procedure was described previously in **Section 3.2.4**. Samples were diluted at 1:20 with a 6% sucrose solution. The diluted sample was analysed at 25 °C with duplicate measurements carried out for each sample.

7.2.5 Viscosity

Viscosity of the model emulsions was performed using a double gap geometry (measuring cup DG42 RO and rotor DG43 DIN53544 Ti) and 11.5 mL sample volume at 20 °C with a Rheostress 1 (Haake, Karlsruhe, Germany) rheometer coupled with a Haake DC-30 circulating bath. The procedure was described previously in **Section 4.2.5**. Measurements were carried out in duplicate for each sample at the shear rate range of 1 to 500 s^{-1}.

7.2.6 pH

The pH of model emulsions was tested at room temperature using a pH209 (HANNA Instruments, Woonsocket, RI, USA) pH meter.

7.2.7 Creaming index

The creaming index of the emulsions was obtained using a visual observation method as described previously in **Section 3.2.7**. Creaming measurements were made on the emulsions in clear test tubes sealed with screw cap lids, with extra precautions taken not

to disrupt the cream layer. Creaming index was defined as the ratio of cream height over total height of emulsion upon standing (McClements, 2007).

7.2.8 Emulsion stability

All model emulsions were stored in 100 mL bottles with screw caps and subjected to analysis on droplet size, polydispersity index, zeta potential, viscosity and pH. The bottles were agitated until the cream layer was well redispersed in the emulsion before conducting any analysis at each time interval during the 28-day ageing period.

7.2.9 Morphology of the emulsion droplets by optical microscope

The morphology of the emulsions after 28 days of ageing were observed with a light microscope at room temperature. Samples were visualised using a Ceti Magnum trinocular microscope (Medline Scientific Ltd., Oxon, UK) and images were taken by using an attached XCAM 5.0 MP camera (Medline Scientific Ltd., Oxon, UK).

7.2.10 Headspace solid-phase microextraction (HS-SPME)

The extraction of volatiles in the samples were carried out using the procedures described previously in **Section 4.2.9**. All emulsions were pre-weighed (5 g) into 20 mL headspace vials, and the vial was and then capped with a Teflon-lined silicone rubber septum. Samples were stored in the sealed headspace vials at 45 °C for 1-28 days and removed at specified time intervals. Samples were then stored in a -20 °C freezer until all samples with different storage times were collected before volatile analysis. All vials were covered with aluminium foil to prevent light-induced chemical reactions. 3-Octanone was used as an internal standard, which was prepared by dissolving 1% v/v of 3-octanone in methanol and then diluting it to 5 ppm using Milli-Q water. An aliquot (100 µL) of the internal standard solution was added to each frozen sample to achieve the target concentration of 3-octanone (0.1 ppm in the sample) within a week before executing the HS-SPME procedure. This concentration was determined during piloting to ensure that the peak area of the internal standard was similar to the peak area of major compounds in the fresh coffee emulsions. The extraction of volatiles was carried out with a PAL RSI 85 multipurpose sampler (CTC Analytics, Bern, Switzerland) at 45 °C. The samples were equilibrated at 45 °C for 5 min, before the SPME fibre (50/30 µm divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS)) (Supelco, Bellefonte, PA, USA), was exposed for 40 min. After the extraction, the SPME fibre was injected into the gas

chromatograph inlet for desorption and analysis (see **Section 7.2.11**). Six HS-SPME analyses were performed on each emulsion formulation (2 emulsion batches x 3 analytical replicates per batch).

7.2.11 GC-MS analysis

The analyses of headspace volatiles in the samples were carried out as described previously in **Section 4.2.10** with some modifications using an Agilent GC 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 5975B VL MSD quadrupole mass spectrometer (Agilent Technologies, Wilmington, DE, USA). SPME desorption was carried out at 240 °C for 2 min in splitless mode, followed by 3 min in split mode. Following sample desorption, the SPME fibre was conditioned for 2 min at 270 °C with a purge flow of 50.0 mL min^{-1}. Pure helium gas was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The chromatographic separation was performed on a 60 m x 0.32 mm i.d. x 0.5 μm Zebron ZB-WAX column (Phenomenex, Torrance, CA, USA). The oven temperature was held for 5 min at 40 °C, increased at a rate of 5 °C min^{-1} until 190 °C, then subsequently increased at a rate of 10 °C min^{-1} to 240 °C and held for 5 min. The transfer line to the MS was set to 230 °C and the quadrupole was set to 150 °C. The mass spectrometer was operated at a scan speed of 5.1 scans s^{-1} and mass spectra were recorded in the range of 29-300 m/z. Carryover between GC runs was assessed using 2 blank samples (an empty vial and a vial containing deionised water only) after every 24 samples. A randomised block of 24 samples was organised by replication and storage time to run on each day and the analyses were performed over a period of 6 days.

The Agilent Enhanced MSD Chemstation software (version F.01.01.2317, Agilent Technologies Inc., USA) was used to convert the total ion count (TIC) chromatogram and data from the mass spectrometer to netCDF format. Data mining was carried out using PARADISe (PARAFAC2 based Deconvolution and Identification System) software, version 3.9 (Johnsen, Skou, Khakimov, & Bro, 2017). Data were first aligned using the built-in function in the software, and then the volatile compounds were manually selected for each time interval of interest along the chromatogram for deconvolution by the software. A total of 161 intervals, each with at least one peak, were selected. Modelling options were set to a maximum of 7 compounds per interval and 2000 maximum iterations per interval. The PARAFAC2 models created for each interval

were optimised for the number of compounds according to this order: the highest model fitting, the highest core consistency, lowest residuals, and avoiding model overfitting (Johnsen et al., 2017). The volatile compounds were identified by comparing the elution profiles to the National Institute of Standards and Technology (NIST14) database, supported by retention index (RI). Linear retention indices were calculated using a C9-C17 n-alkane series as reference compounds using the following equation:

$$\mathrm{RI}(x) = (100 \times z) + \frac{\mathrm{RT}(x) - \mathrm{RT}(z)}{\mathrm{RT}(z+1) - \mathrm{RT}(z)} \qquad \textbf{Equation 7.1}$$

Where RI(x) is the retention index of the unknown compound x, z is the number of carbon atoms of the n-alkane eluted before the unknown compound x, z+1 is the number of carbon atoms of the n-alkane eluted after the unknown compound x, RT(x) is the retention time of the unknown compound x, RT(z) is the retention time of the n-alkane eluted before the unknown compound x, RT(z+1) is the retention time of the n-alkane eluted after the unknown compound x.

The data matrix generated by PARADISe was saved in a format that can be opened by Microsoft Excel for future data transformations. The deconvoluted peak areas provided by PARADISe were normalised by dividing the values by the area of 3-octanone (IS) to correct the differences between extraction batches and reported as % relative area.

7.2.12 Statistical analysis

IBM SPSS Statistics Version 23 (IBM Corporation, Armonk, New York, USA) was used for statistical comparison of the results. One-way analysis of variance (ANOVA) was carried out to determine significant differences between the results, followed by Tukey's post hoc test for pairwise comparison. Independent t-tests were carried out to compare the results of bulk GMO and instantised GMO at the same concentration. All tests were performed at the 95% confidence level. Principal component analysis (PCA) was carried out on all volatile compounds that changed significantly over time using The Unscrambler X Version 10.5 (CAMO, Oslo, Norway) and all variables in the PCA were standardised (1/standard deviation).

7.3 Results and Discussion

7.3.1 Droplet size and polydispersity index of model coffee emulsions

Table 7.2 shows the droplet size and polydispersity index of fresh model coffee emulsions prepared with bulk or instantised GMO at various concentrations. Control 1 (no GMO, no maltodextrin) and Control 2 (no GMO, with maltodextrin) emulsions formed oil droplets with an average droplet size of 300-320 nm. The average droplet size of emulsions prepared with bulk GMO significantly reduced in size as the GMO concentration was increased ($p<0.05$). The droplet sizes formed were in this order: Control > 0.03% and 0.1% bGMO > 0.2% bGMO. The same trend was observed in the emulsions prepared with iGMO. This finding agrees with previous work on oil-in-water emulsions that showed a reduction of droplet size with increasing emulsifier concentration (Berton-Carabin et al., 2014; Donnelly et al., 1998; Sun et al., 2007). The increased GMO concentration in the emulsions means an increase of total surface-active molecules that can participate at the oil-water interface. GMO being a small-molecule emulsifier can rapidly adsorb to the interface due to the smaller sizes compared to milk proteins. Milk proteins have a larger molecular size and undergo structural changes during homogenisation which tend to adsorb preferentially to the interface.

Table 7.2 Z-average droplet size and polydispersity index of fresh model coffee emulsions prepared with different GMO concentrations.

GMO level (%)	Droplet size (nm)		Polydispersity index	
	bGMO	*iGMO*	*bGMO*	*iGMO*
Control*	313.7 ± 4.0^{cA}	306.6 ± 16.1^{cA}	0.276 ± 0.002^{bA}	0.249 ± 0.019^{bA}
0.03	292.8 ± 5.8^{bB}	281.1 ± 5.4^{bA}	0.247 ± 0.015^{aB}	0.222 ± 0.006^{bA}
0.1	284.2 ± 4.0^{bB}	266.8 ± 4.9^{bA}	0.249 ± 0.007^{aB}	0.229 ± 0.015^{bA}
0.2	267.3 ± 2.3^{aB}	233.0 ± 3.5^{aA}	0.248 ± 0.008^{aB}	0.184 ± 0.015^{aA}
F-value	83.7	46.5	9.4	13.7
p-value	<0.001	<0.001	<0.01	<0.001

Values are means ± standard deviations of 4 measurements (2 batches x 2 replicates). Different small letters in the same column indicate statistically significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test. Different capital letters in the same row for the same measurement indicate statistically significant differences by independent t-test ($p<0.05$).

*Two control samples were prepared for comparison to bGMO (Control 1; no GMO, no maltodextrin) and iGMO (Control 2; no GMO, with maltodextrin) samples.

Pairwise comparisons (independent t-test) between the emulsions with bGMO and iGMO for the same GMO concentration showed that iGMO formed emulsions with significantly smaller oil droplet sizes than bGMO ($p<0.05$). This result could be due to the better emulsification properties by the iGMO, as it is already in the emulsified form compared to bGMO. The emulsions with bGMO need higher energy, i.e. higher pressure or increased number of passes during microfluidisation, to form the same oil droplet sizes as emulsions with iGMO. The amount of canola oil, sodium caseinate and sodium stearate in the iGMO were very low and unlikely to contribute to the observed variations. Maltodextrin also did not influence the oil droplet size of the emulsions as there was no significant different ($p>0.05$) between the controls with and without maltodextrin (Control 1 vs Control 2).

Polydispersity index of fresh emulsions prepared with bGMO was approximately 10% lower than control. The increased concentration of the bGMO did not affect the polydispersity index of the emulsions. For the emulsions prepared with iGMO, 0.2% iGMO emulsion showed a significantly lower polydispersity index than the control and emulsions with lower iGMO concentrations. Pairwise comparisons between emulsions with bGMO and iGMO showed that iGMO formed more uniform droplet size than bGMO.

The volume-weighted size distribution (**Figure 7.1a** and **Figure 7.1b**) shows that all fresh coffee emulsions had a monomodal droplet size distribution. The emulsions prepared with increasing GMO concentration showed a reduction of emulsion droplet size. This effect is due to the ability of GMO to interact with milk proteins at the interface and form droplets that are more compact. The emulsions prepared with instantised GMO (**Figure 7.1b**) showed a lower number of large droplets around 5 µm compared to bulk GMO (**Figure 7.1a**), which may be affected by the maltodextrin in the formulations with instantised GMO. Maltodextrin is frequently used as a stabiliser in food emulsions to improve long-term stability (Hogan, McNamee, O'Riordan, et al., 2001; Klinkesorn, Sophanodora, Chinachoti, & McClements, 2004).

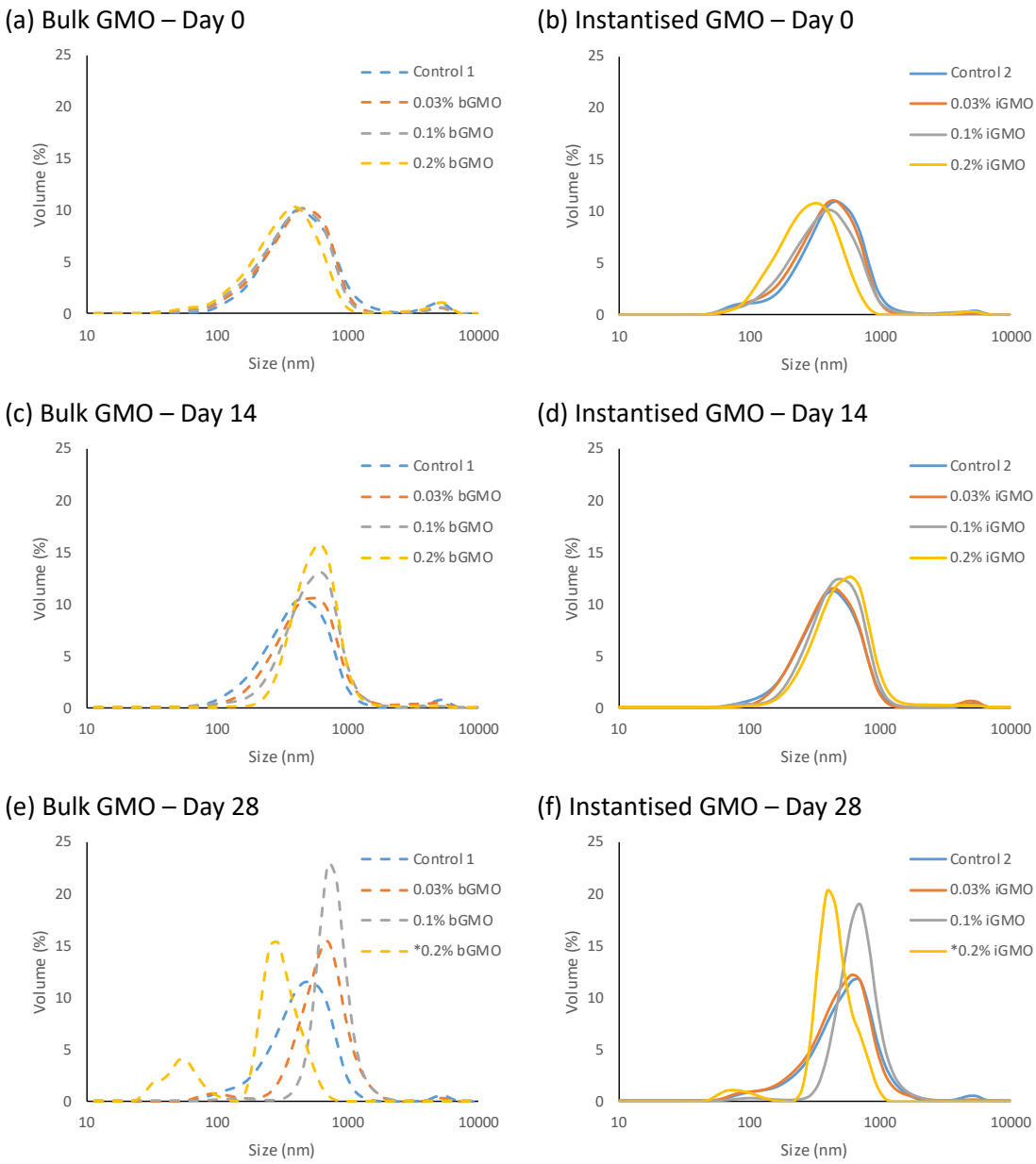


Figure 7.1 Volume-weighted size distribution of coffee emulsions prepared with different concentrations of bulk GMO and instantised GMO at various ageing times.

***The dynamic light scattering results for 0.2% bGMO and 0.2% iGMO samples after 28 days of ageing contained an artefact from sediment particles present in the samples that exceed the upper size limitation of this technique.**

Figure 7.2 shows the droplet size of the coffee emulsions prepared with different concentrations of bulk and instantised GMO over 28 days of ageing at 45 °C. No emulsions, except samples 0.2% bGMO and 0.2% iGMO at 28 days of ageing, showed any protein precipitation. These two samples with protein precipitation at 28 days of ageing could not be measured correctly by dynamic light scattering (**Figure 7.1e** and **Figure 7.1f**) and hence are not reported in **Figure 7.2**. Both control emulsions did not show any significant change in droplet sizes over 28 days of ageing at 45 °C ($p>0.05$). The droplet sizes for all emulsions prepared with bulk or instantised GMO, except 0.03% iGMO, changed significantly over ageing time. Among the emulsions prepared with bulk GMO, the 0.2% bGMO sample had the highest rate of droplet growth until 14 days of ageing and formed visible aggregates in the sample around 21 days of ageing. Sample 0.2% iGMO showed the same droplet growth pattern as 0.2% bGMO, but visible aggregates only appeared in the sample around 28 days of ageing. The aggregates in the 0.2% iGMO when observed using a microscope (**Figure 7.3d**) revealed the presence of flocculated oil droplets. The oil droplets in the control and 0.03% iGMO samples at 28 days of ageing were mainly small, with only a few larger droplets (**Figure 7.3a** and **Figure 7.3b**). This observation is consistent with the average droplet size and measured size distribution of these emulsions. The appearance of more large droplets in the 0.1% iGMO sample is most likely due to the flocculation and coalescence (**Figure 7.3c**).

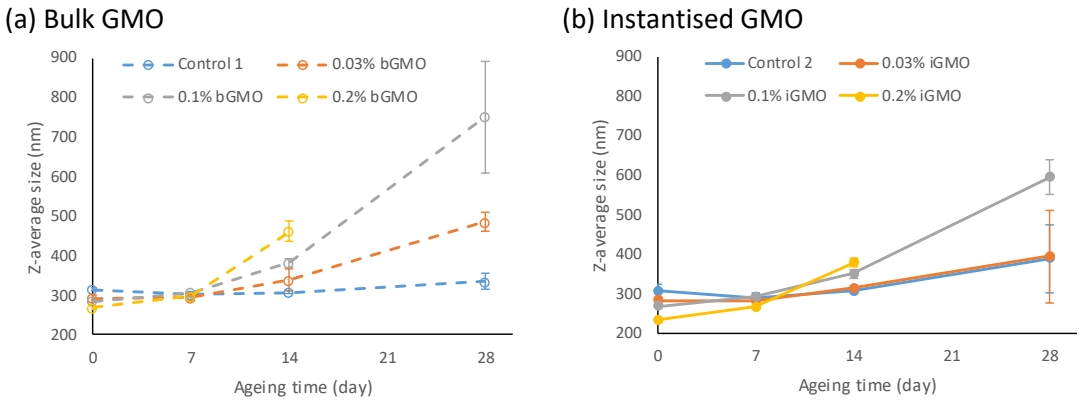


Figure 7.2 Average droplet size of coffee emulsions prepared with different concentrations of (a) bulk GMO and (b) instantised GMO at different ageing times.

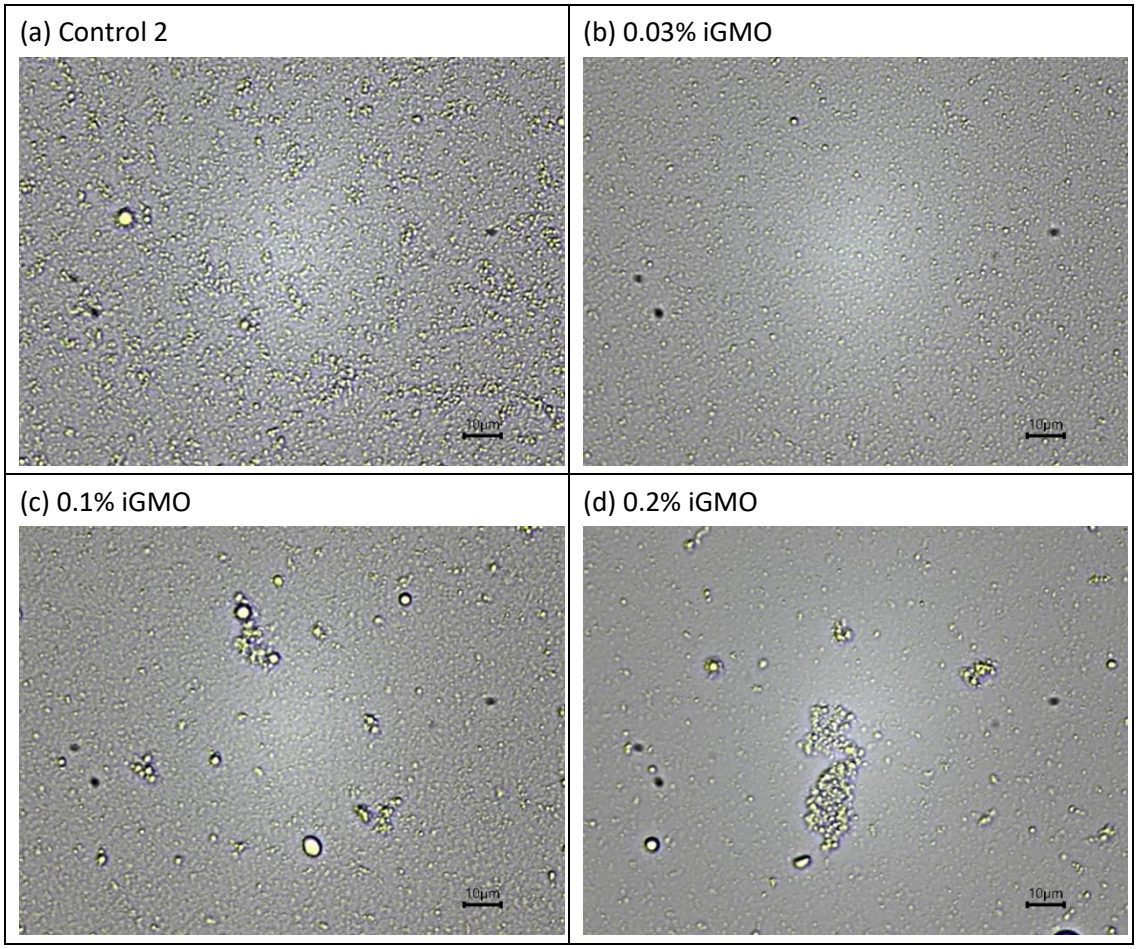


Figure 7.3 Morphologies of the emulsion droplets in the control and emulsions with different concentrations of instantised GMO at 28 days of ageing at 45 °C (400x magnification).

7.3.2 Zeta potential of model coffee emulsions

Table 7.3 shows the zeta potentials of the coffee emulsions prepared with different concentrations of bulk and instantised GMO over 28 days of ageing at 45 °C. All fresh emulsions had a zeta potential between -31 and -34 mV. In order to simplify the discussion of the results, zeta potential refers to the magnitude, and its negative sign is ignored because all the emulsions have negative values. Hence, an increase in zeta potential refers to the increase in the negativity of the zeta potential. The zeta potential provides information on the repulsive forces at the oil-water interface, which can explain droplet stability in the emulsions (Tcholakova et al., 2006).

Both control emulsions had the same zeta potential over 28 days of ageing at 45 °C ($p>0.05$). All emulsions prepared with various GMO concentrations showed a significant

change in zeta potential ($p<0.05$), however, these emulsions only had a small reduction in zeta potential (<4 mV) over 28 days of ageing. Among the fresh coffee emulsions (**Table 7.3a**), only the emulsions with 0.2% bGMO had significantly ($p<0.05$) lower zeta potential than the control; however, zeta potential for the emulsions with different iGMO (**Table 7.3b**) were the same as control ($p>0.05$). All emulsions still had high repulsive forces (>30 mV) between oil droplets during ageing, except samples 0.2% bGMO and 0.2% iGMO. According to Ross and Morrison (1988), emulsions with zeta potential more negative than -30 mV are stable against flocculation. Both emulsions with 0.2% GMO showed a reduction in zeta potential (<30 mV) after 14 days of ageing, and this could explain the appearance of visible sediment due to protein aggregation in the coffee emulsion.

Table 7.3 Zeta potential of model coffee emulsions prepared with different concentrations of (a) bulk GMO and (b) instantised GMO at various ageing time.

(a) Bulk GMO

GMO level (%)	**Zeta potential (mV)**			
	Day 0	*Day 7*	*Day 14*	*Day 28*
Control	-33.1 ± 0.3^{ab}	-32.8 ± 0.3^{ab}	-32.4 ± 0.4^{a}	-32.8 ± 0.1^{a}
0.03	-33.8 ± 0.7^{a}	-33.2 ± 0.4^{a}	-31.7 ± 0.9^{ab}	-31.8 ± 0.4^{a}
0.10	-32.2 ± 0.9^{bc}	-32.4 ± 0.3^{bc}	-30.9 ± 0.1^{b}	-30.6 ± 0.4^{b}
0.20	-31.4 ± 0.7^{c}	-30.5 ± 0.3^{c}	-29.0 ± 0.4^{c}	-27.6 ± 0.8^{c}
F-value	9.3	52.4	32.6	78.3
p-value	<0.01	<0.001	<0.001	<0.001

(b) Instantised GMO

GMO level (%)	**Zeta potential (mV)**			
	Day 0	*Day 7*	*Day 14*	*Day 28*
Control	-32.7 ± 0.6^{a}	-32.9 ± 0.6^{a}	-32.2 ± 0.2^{a}	-32.8 ± 0.1^{a}
0.03	-32.8 ± 0.2^{a}	-33.1 ± 0.5^{a}	-31.8 ± 0.6^{a}	-31.8 ± 0.4^{a}
0.10	-32.4 ± 0.5^{a}	-31.7 ± 0.4^{b}	-31.1 ± 0.9^{a}	-30.6 ± 0.4^{a}
0.20	-31.8 ± 1.0^{a}	-31.1 ± 0.5^{b}	-29.2 ± 0.7^{b}	-27.6 ± 0.8^{b}
F-value	1.8	15.5	15.9	13.9
p-value	0.20	<0.001	<0.001	<0.001

Values are means ± standard deviations of four measurements. Different letters in the same column indicate statistically significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test.

7.3.3 Viscosity of model coffee emulsions

All eight coffee emulsions prepared with bulk and instantised GMO, including two controls, showed a linear relationship between shear stress and shear rate (**Figure 7.4**), which indicated that all emulsions observed the flow behaviour of a Newtonian fluid. All the experimental data best fitted a Newtonian model (R^2>0.99), hence emulsion viscosity was reported as the adjusted value according to the Newtonian model.

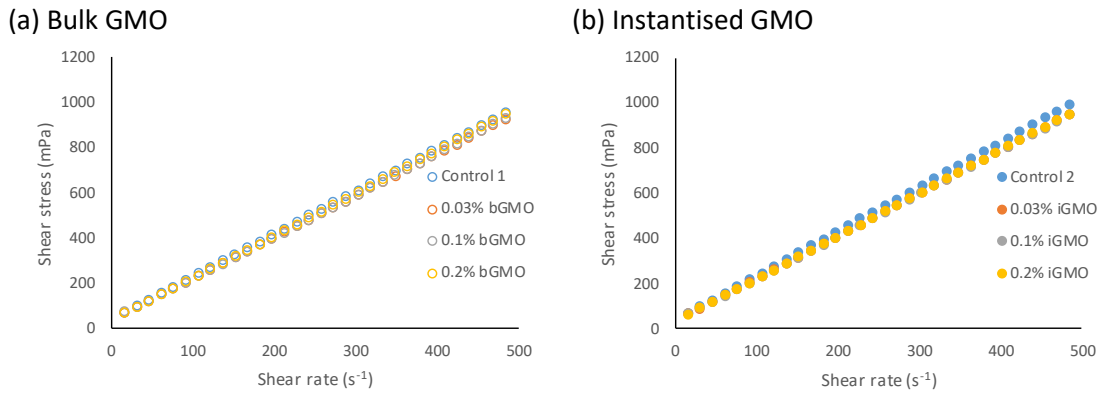


Figure 7.4 Rheological properties of model coffee emulsions prepared with different concentrations of (a) bulk GMO and (b) instantised GMO.

Figure 7.5 shows the viscosity of coffee emulsions prepared with different GMO concentrations over 28 days of ageing at 45 °C. The fresh emulsions prepared with various concentrations of GMO were significantly different in viscosity ($p<0.05$). However, these differences are considered minimal because all the emulsions had viscosities in the range of 1.9 to 2.2 mPa s until 14 days of ageing. The viscosities of the 28-day aged emulsions were significantly different ($p<0.05$) for the samples with different concentrations of both bulk and instantised GMO. The viscosities of the 28-day aged emulsions with bulk GMO were in this order: Control 1 < 0.03% bGMO and 0.1% bGMO < 0.2% bGMO. Among the emulsions with instantised GMO, sample 0.2% iGMO had the highest viscosity ($p<0.05$). Both samples 0.2% bGMO and 0.2% iGMO had at least 35% higher viscosity than its respective control and increased more than 60% from its respective initial value. The increased viscosity in 0.2% bGMO and 0.2% iGMO samples was due to flocculation of oil droplets as observed in the sediment particles. McClements (2007) and Quemada and Berli (2002) have reported that flocculation of oil droplets increases emulsion viscosity.

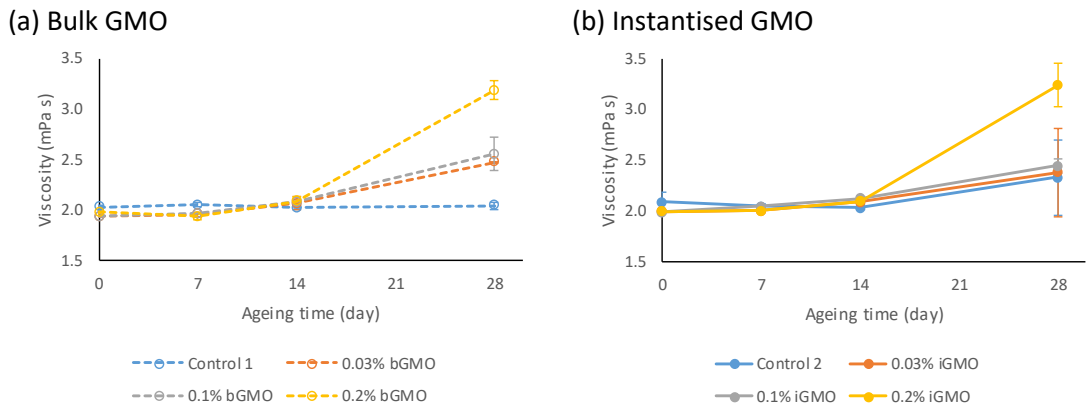


Figure 7.5 Viscosity of model coffee emulsions prepared with different concentrations of (a) bulk GMO and (b) instantised GMO at various ageing times. Points represent means viscosity and the error bars are the standard deviations (n=4).

7.3.4 pH of model coffee emulsions

The pH of all fresh emulsions was similar (pH 5.3-5.4), and were measured to decrease slightly (<0.2) over 28 days of ageing. The inclusion of bulk and instantised GMO at up to 0.2% level did not affect the pH of any model emulsions. This finding was expected because GMO is a non-ionic emulsifier that does not change the pH of the solution. The pH of these emulsions was close to the isoelectric point of casein (pH 4.6), where casein solubilisation is reduced due to structural changes that exposes more hydrophobic groups (Perugini et al., 2018). W. Chen et al. (2019) reported that low-molecular-weight emulsifiers can displace protein from the interface when the surface-active properties of protein are reduced. The partially displaced casein from the interface had hydrophobic regions that have strong attractive forces to another oil droplets, causing protein aggregate induced flocculation and eventually forming visible sediment particles in the emulsions.

7.3.5 Creaming index of model coffee emulsions

Figure 7.6 shows the creaming index of the coffee emulsions prepared with different concentrations of bulk and instantised GMO over 28 days of ageing at 45 °C. A thin layer of cream started to develop in all emulsions containing bulk GMO after 2 days of ageing at 45 °C and the cream layer developed at a slower rate than the control. In the emulsions prepared with instantised GMO, the lowest creaming rate was observed in the emulsions

with 0.2% GMO level. This observation showed that resistance against creaming improved when instantised GMO concentration increased in the emulsions. Both control emulsions showed a creaming index of 5.1-5.2% at 28 days, which is higher than other formulations (1.9-3.5% creaming index). This observation was the same as previous findings (**Chapter 3** and **Chapter 6**), where the incorporation of GMO in the protein-stabilised emulsion showed reduced creaming.

The droplet size of the coffee emulsions prepared with GMO increased during ageing time. The emulsions had a larger droplet size than control around 14 days of ageing, but did not show a higher creaming rate in the samples. The increased oil droplet size could be due to the flocculation of oil droplets through inter-droplet hydrophobic interactions of the protein adsorbed at the interface (Shao & Tang, 2014; Tang & Liu, 2013). The sediment particles in the 0.2% bGMO and 0.2% iGMO samples contained flocculated oil droplets, which inferred that the oil droplets were highly attractive to each other. As sediment moves in the opposite direction of the oil droplets or cream, this explains why the creaming stability of the emulsions was not affected by the increased droplet size due to sedimenting protein aggregates that have higher densities than water. The aggregation of proteins in the emulsions is hypothesised to be due to destabilisation of the milk proteins during storage, particularly the caseins that could undergo structural changes over time at a pH close to the isoelectric point. This hypothesis could explain the observation of the sedimenting protein aggregates (heavy phase) that contain flocculated oil droplets. Therefore, it is important to note that a low concentration of GMO (≤0.03%) can successfully improve droplet size and enhance creaming stability in coffee milk emulsions without causing any protein aggregated oil droplets.

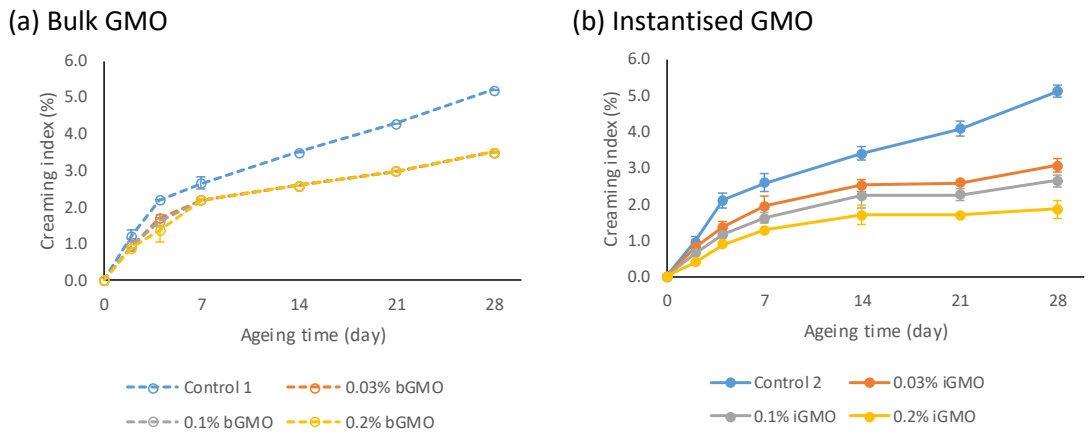


Figure 7.6 Creaming index of model coffee emulsions prepared with different concentrations of (a) bulk GMO and (b) instantised GMO at various ageing times. Points represent means creaming index and the error bars are the standard deviations (n=6).

7.3.6 Oxidative stability and volatile flavour compounds of model coffee emulsions measured using volatile analysis

The formation of volatile compounds was monitored in all coffee emulsions with different concentrations of bulk and instantised GMO at various ageing times using headspace SPME with GC-MS as a tool to evaluate changes in secondary oxidation products and volatile flavour compounds associated with coffee during storage. Analysis of the volatile compounds identified 77 compounds in the emulsions, from seven chemical classes, namely aldehyde, furans, alcohols, ketones, pyrazines, pyrroles, and other miscellaneous compounds (phenols, thiazole, toluene, acids, pyridines).

It is well known that specific target compounds that increase in concentration during storage could be used to explain lipid oxidation of an emulsion system (Berton et al., 2012; Faraji et al., 2004). However, it becomes more challenging to explain the changes of coffee flavour during storage, as there are compounds with increasing concentrations due to the Maillard reaction, or with decreasing concentrations due to structural decomposition, oxidation, loss or binding to proteins. As this chapter aims to distinguish the effect of types and concentrations of emulsifiers (bulk GMO vs instantised powder) on the volatile composition, only compounds that changed significantly during storage were considered. A total of 68 compounds changed significantly over time ($p<0.05$) and were further analysed using principal component analysis (PCA) to investigate

multidimensional relationships in the dataset. **Figure 7.7** shows the scores and loadings plot of PCA for coffee emulsions at various storage times. The full list of coded compounds together with their identified compound name and retention index data is presented in **Appendix E.1**. The first principal component (PC-1) explained 56% of the variation compared to 25% on PC-2. Storage time at 45 °C was the main cause of variation in the PCA model, differentiating fresh and stored emulsions into three clusters according to the storage time, namely Day 0, 14 and 28. The fresh emulsions were negatively loaded on PC-1, whereas all the stored emulsions (Day 14 and 28) were in the positively loaded region (right hand side). Compounds detected in reconstituted coffee in water (data not shown) such as pyrazines, pyrroles, furans (2-(methoxymethyl)furan, 2-furfural methyl sulphide, 2-furanmethyl acetate and furfural) and ketones (2,3-pentanedione, 1-acetyloxy-2-propanone and 2-methyl-2-cyclopenten-1-one) were located on the left of PC-1, associated with higher concentrations in the fresh samples (**Figure 7.7**, loadings plot). The compounds derived from lipid oxidation, such as aldehydes (propanal, pentanal, hexanal and octanal), ketones (3,5-octadien-2-one and (E,E)-3,5-octadien-2-one) and alcohols (1-octen-3-ol and 1-octanol) and Maillard reaction products such as furans (2-ethylfuran and 2-pentylfuran), were positively correlated to the stored samples. PC-2 discriminated the emulsion samples according to the formulation and GMO concentrations, where the samples with low GMO concentration (control and 0.03% GMO) were positioned in the negative region, with 0.1% and 0.2% samples positioned in the positive region (top of the loadings plot). The eight compounds (2-furanmethyl acetate, 1-(2-furanylmethyl)-1H-pyrrole, 2-furfuryl methyl sulphide, 1-methyl-1H-pyrrole, pyrrole, 2,3-pentanedione, 1-acetyloxy-2-propanone and 2-methyl-3-pentanone) clustered together in the top left corner of the loadings plot and distinguished 0.2% GMO from the rest of the GMO concentration in the fresh emulsions (Day 0). The compounds (2-pentylfuran, 2-butylfuran and toluene) in the top right corner of the loadings plot showed a positive correlation to the stored emulsions with 0.1% and 0.2% GMO concentration. The cluster of pyrazines from coffee was negatively loaded on PC-2 and showed higher concentrations in the control and 0.03% GMO samples at 14 and 28 days of storage.

Three compounds that significantly increased over time, namely 2-pentylfuran (C28), hexanal (C21), and octanal (C34), were selected as oxidation markers to examine oxidative stability of the emulsions during storage at 45 °C as each of them represented

a different cluster in the positive region of PC-1 (**Figure 7.8a-c**). These compounds have been commonly used as lipid oxidation markers in past literature (Berton et al., 2012; Böttcher et al., 2015; Damerau et al., 2014; Vandamme et al., 2016). Hexanal and 2-pentylfuran are the secondary lipid oxidation products of linoleic acid, while octanal is derived from oleic acid (Choe & Min, 2006; Frankel, 1985; Genot et al., 2003). All three oxidation markers increased during storage, which aligned with the PCA results and reaffirmed the occurrence of lipid oxidation during accelerated ageing. Both hexanal and octanal indicated that higher oxidation rates occurred in the control and 0.03% GMO samples, while the concentration of 2-pentylfuran increases with GMO concentrations. The contradictory results demonstrated by 2-pentylfuran may indicate that there were more complex interactions occurred in the emulsion to form this compound and other compounds in the same cluster. The same phenomenon was observed in both bulk and instantised GMO. As most of the oxidation markers are located in the bottom right of the PCA loadings plot (close to hexanal and octanal), it appears that the oxidative stability of emulsions increases as the GMO concentration increased.

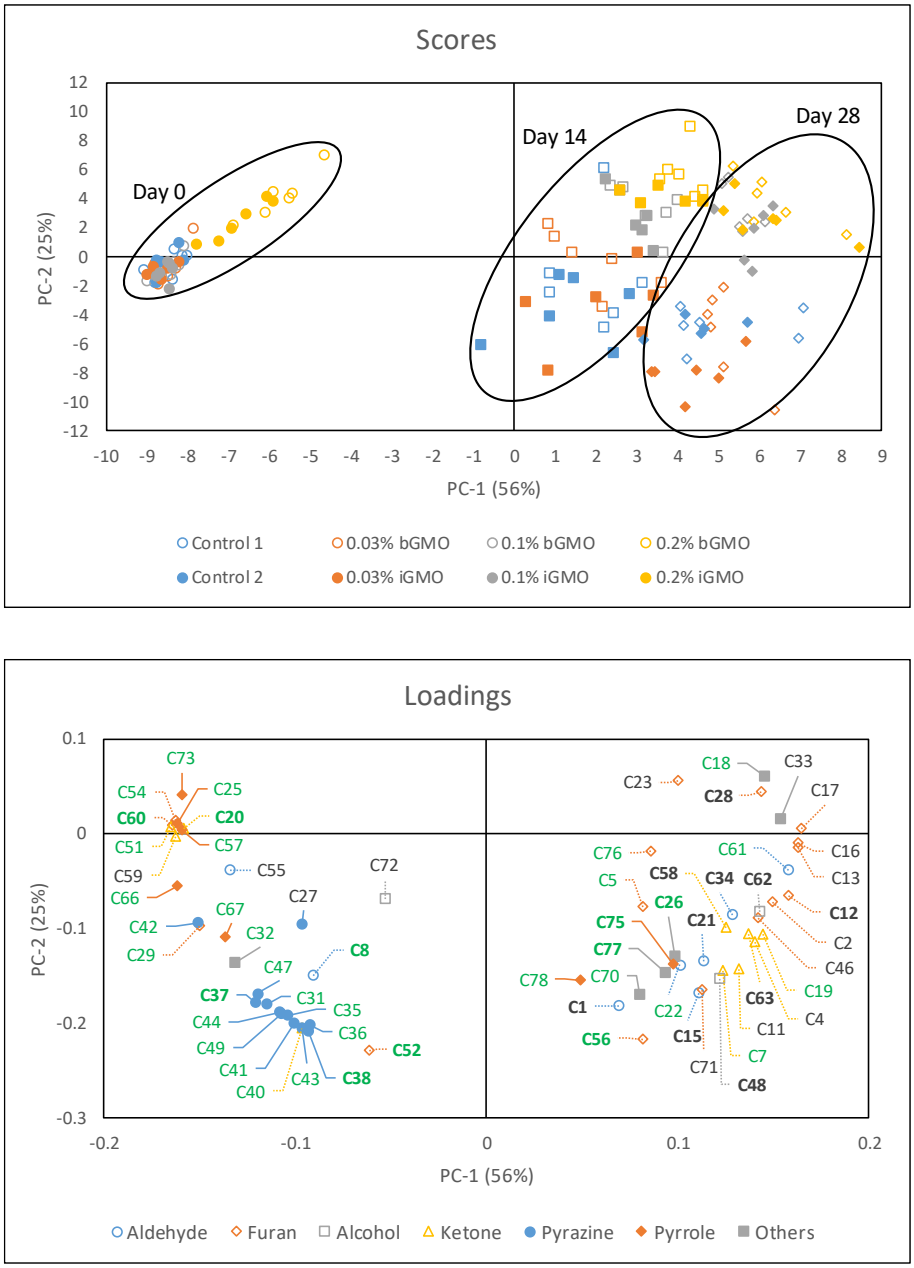


Figure 7.7 Principal component analysis: (a) scores plot of sample formulations and (b) loadings plot of volatile compounds for model coffee emulsions at various storage time. Each sample in the score plot has six replicate points (2 batches x 3 reps), except Control 2 stored for 28 days has five replicate points. Compounds selected as oxidation markers were bolded, i.e. C12: 2-Ethylfuran, C28: 2-Pentylfuran, C62: 1-Octanol, C48: 1-Octen-3-ol, C1: Propanal, C15: Pentanal, C21: Hexanal, C34: Octanal, C58: 3,5-Octadien-2-one and C63: (E,E)-3,5-Octadien-2-one. Volatile flavour compounds associated with coffee were in green colour font and those selected for further analysis were bolded, i.e. C20: 2,3-Pentanedione, C60: 2-Furanmethyl acetate, C8: 2-Methylbutanal, C52: Furfural, C37: Ethylpyrazine, C38: 2,3-Dimethylpyrazine, C26: Pyridine, C56: 2-Acetylfuran, C77: Phenol, C75: 1-(1H-Pyrrol-2-yl)ethanone.

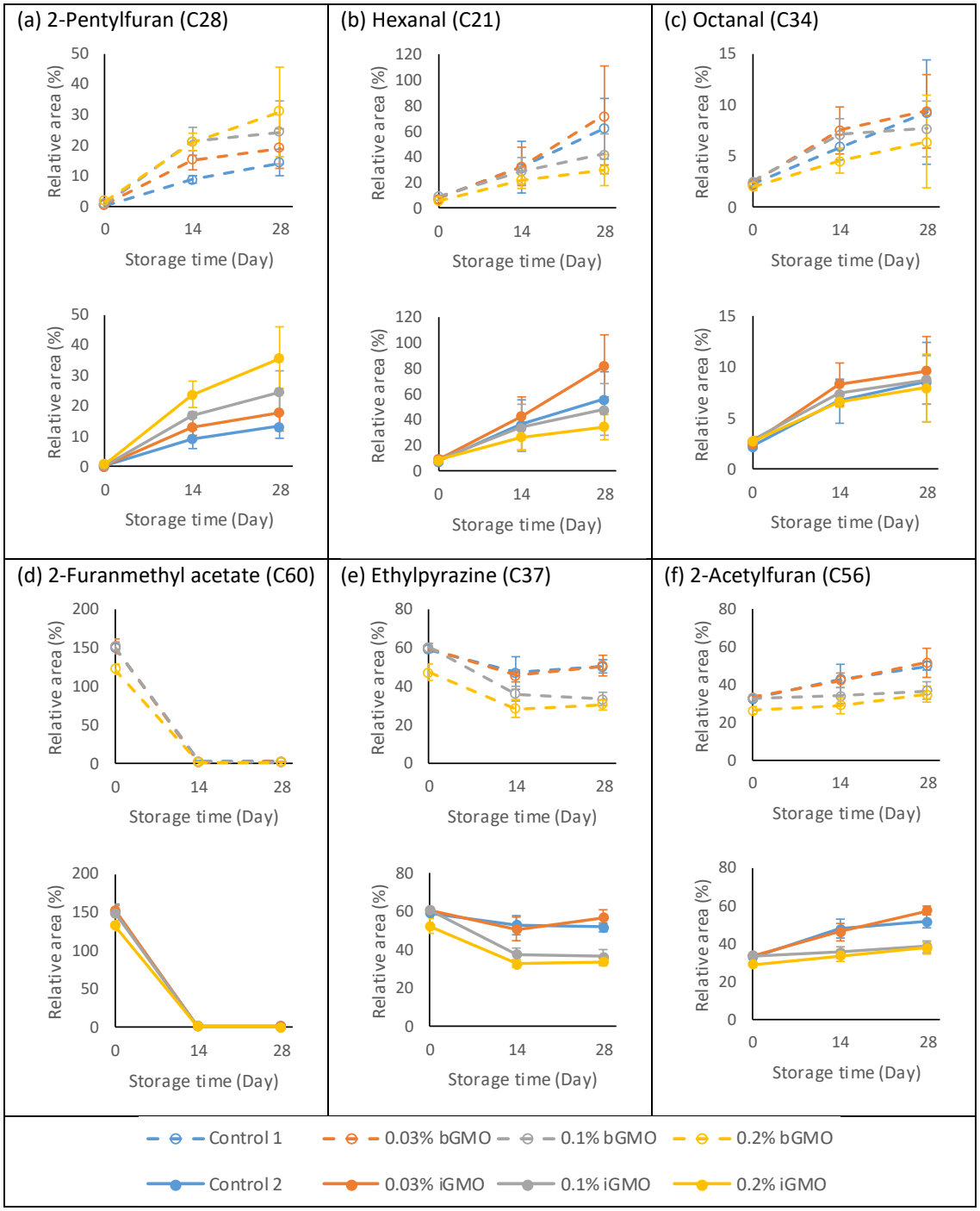


Figure 7.8 Lipid oxidation markers (a-c) and volatile flavour compounds associated with coffee (d-f) at various storage time at 45 °C. Each point represents the mean value and the error bar represents the standard deviations (2 batches x 3 reps), except Control 2 stored for 28 days has five replicate points.

Table 7.4 shows the relative peak areas of ten selected lipid oxidation compounds in the emulsion samples at 28 days of storage. These compounds were secondary lipid oxidation products derived from oleic, linoleic and linolenic acids (Genot et al., 2003) and had high positive loadings on PC-1. Only 2-pentylfuran had significantly higher concentration present in 0.2% bGMO and 0.2% iGMO than their respective control ($p<0.05$). The rest of the oxidation compound markers indicated that controls had either the same or higher concentration than 0.2% GMO samples. These findings agreed with past research (Fomuso et al., 2002; N. Kim & Choe, 2012) that monoglycerides at higher concentration form a compact and thick interface that is less permeable to oxygen diffusion, resulting in improved oxidative stability. All oxidation markers, except pentanal, showed that the respective control and 0.03% GMO samples had the same concentration ($p>0.05$). This observation was positive because it indicated that incorporation of bulk and instantised GMO at low concentration (0.03%) do not alter the oxidative stability of coffee emulsions.

Three volatile flavour compounds associated with coffee (**Figure 7.8d-f**), namely 2-furanmethyl acetate (C60), ethylpyrazine (C37) and 2-acetylfuran (C56), were selected to see the effect of GMO concentration on these compounds in the headspace during storage at 45 °C. 2-Furanmethyl acetate was positioned at the top left quadrant of the PCA loadings plot and showed a rapid reduction over storage time. Ethylpyrazine in the bottom left quadrant of the PCA loadings plot showed that this compound (along with other pyrazine compounds) reduced over time and that a significantly higher reduction occurred for the samples with 0.1% and 0.2% GMO concentration. 2-Acetylfuran at the bottom right quadrant of the PCA loadings plot showed an increase in 2-acetylfuran concentration during storage and this compound was formed at a slower rate with increasing GMO concentration. These three selected compounds are representative compounds that show other compounds located close to them in the loadings plot behave in the same way. Pyridine (C26), 1-(1H-pyrrol-2-yl)ethanone (C75) and phenol (C77) were compounds clustered together with 2-acetylfuran and showed similar behaviour as 2-acetylfuran during storage.

Table 7.5 shows ten selected volatile compounds in the emulsions at 28 days of storage. These compounds have been previously identified as odorants in coffee and associated with specific flavour attributes (Andueza et al., 2003; Grosch, 1998; Maeztu et al., 2001).

These compounds represented three different regions across the PCA loadings plot. The compounds in the top left and bottom right regions of the PCA loadings plot showed that controls had either the same or higher concentration of the compounds than 0.2% GMO samples. All compounds in the bottom left quadrant of the PCA loadings plot showed controls had a higher concentration of these compounds compared to samples with 0.2% GMO. Controls and 0.03% bGMO samples had the same concentration for all selected compounds associated with coffee at 28 days. Controls also had the same concentration of these selected flavour compounds as 0.03% iGMO samples, except for furfural, 2,3-dimethylpyrazine, 2-acetylfuran and phenol, which were higher in iGMO samples.

The emulsion prepared with 0.03% bGMO had the same concentration of lipid oxidation markers and selected coffee associated flavour compounds as the control. The same selected volatile compounds, except pentanal, furfural and 2,3-dimethylpyrazine, were at the same concentration in the emulsion prepared with 0.03% iGMO and the control. These results indicated that there were minimal changes to the volatile profile of the emulsions when GMO was added at a low concentration. All oxidation markers, except 2-pentylfuran, showed a lower concentration in the 0.2% GMO emulsions compared to controls, and indicated that GMO at higher concentration increased the oxidative stability of the emulsion. However, the emulsions prepared with 0.2% GMO also had lower concentrations of the selected coffee flavour compounds than controls. This phenomenon could be due to the formation of a compact multilayer interface that protects the oil droplet from oxidation by reducing the oxygen diffusion to the core oil, but may also influence partitioning and release of coffee flavour compounds. The thicker oil-water interface was also found to enhance the oxidative stability of emulsions by other researchers (Donnelly et al., 1998; McClements & Decker, 2000). Protein aggregate induced flocculation of oil droplets in the 0.2% GMO samples may have also contributed to the lower oxidation rate as the flocs moved away from the oxygen source.

Table 7.4 Lipid oxidation compounds in 28-day stored (45 °C) emulsions at various GMO concentration.

Retention index	Compounds		Type	GMO level (%)				Source of lipid oxidation*
				Control	*0.03*	*0.1*	*0.2*	
955	C12	2-Ethylfuran	Bulk	18.56 ± 2.85^{B}	18.39 ± 2.88^{B}	15.44 ± 2.19^{AB}	13.49 ± 1.20^{A}	n-3 linolenic acid
			Instantised	16.73 ± 1.81^{AB}	17.80 ± 2.26^{B}	17.08 ± 1.78^{B}	13.57 ± 1.97^{A}	
1228	C28	2-Pentylfuran	Bulk	14.31 ± 4.55^{A}	18.95 ± 6.67^{AB}	24.19 ± 10.35^{AB}	30.96 ± 14.82^{B}	n-6 linoleic acid
			Instantised	13.36 ± 4.09^{A}	18.02 ± 6.48^{A}	24.77 ± 6.67^{AB}	35.85 ± 10.13^{B}	
1549	C62	1-Octanol	Bulk	4.00 ± 1.45^{A}	4.21 ± 1.42^{A}	3.71 ± 0.92^{A}	3.44 ± 1.52^{A}	n-9 oleic acid
			Instantised	3.92 ± 1.32^{A}	4.69 ± 1.08^{A}	4.00 ± 0.88^{A}	3.85 ± 1.21^{A}	
1440	C48	1-Octen-3-ol	Bulk	6.86 ± 0.88^{A}	6.84 ± 1.81^{A}	5.22 ± 0.70^{A}	5.11 ± 1.33^{A}	n-6 linoleic acid
			Instantised	6.89 ± 0.93^{BC}	8.34 ± 1.10^{C}	5.88 ± 0.70^{AB}	5.26 ± 0.82^{A}	
794	C1	Propanal	Bulk	3.37 ± 0.36^{B}	3.35 ± 0.73^{B}	1.85 ± 0.52^{A}	1.65 ± 0.23^{A}	n-3 linolenic acid
			Instantised	3.12 ± 0.69^{B}	3.13 ± 0.87^{B}	2.24 ± 0.52^{AB}	1.77 ± 0.27^{A}	
981	C15	Pentanal	Bulk	9.52 ± 1.23^{B}	9.78 ± 2.11^{B}	5.85 ± 0.93^{A}	4.60 ± 0.80^{A}	n-6 linoleic acid
			Instantised	8.52 ± 0.56^{C}	10.16 ± 1.13^{D}	6.88 ± 0.91^{B}	5.16 ± 0.57^{A}	
1081	C21	Hexanal	Bulk	62.23 ± 24.04^{AB}	72.47 ± 38.30^{B}	42.27 ± 15.56^{AB}	29.93 ± 12.33^{A}	n-6 linoleic acid
			Instantised	56.14 ± 21.40^{AB}	82.13 ± 24.31^{B}	48.06 ± 20.54^{A}	34.54 ± 9.74^{A}	
1290	C34	Octanal	Bulk	9.27 ± 5.13^{A}	9.40 ± 3.60^{A}	7.68 ± 2.74^{A}	6.42 ± 4.50^{A}	n-9 oleic acid
			Instantised	8.58 ± 3.88^{A}	9.69 ± 3.31^{A}	8.80 ± 2.37^{A}	8.00 ± 3.27^{A}	
1524	C58	3,5-Octadien-2-one	Bulk	13.08 ± 1.42^{A}	13.24 ± 3.47^{A}	10.69 ± 2.63^{A}	9.62 ± 2.23^{A}	n-3 linolenic acid
			Instantised	12.78 ± 2.40^{A}	13.75 ± 3.26^{A}	10.65 ± 2.05^{A}	10.76 ± 2.21^{A}	
1578	C63	(E,E)-3,5-Octadien-2-one	Bulk	16.72 ± 1.44^{B}	16.80 ± 4.22^{B}	12.98 ± 3.12^{AB}	10.69 ± 2.46^{A}	n-3 linolenic acid
			Instantised	16.97 ± 2.52^{AB}	17.29 ± 3.31^{B}	13.08 ± 2.31^{AB}	12.54 ± 2.69^{A}	

Values are means relative peak area (%) ± standard deviations of 6 measurements (2 batches x 3 replicates), except Control 2 has five replicate points. Different letters in the same row indicate statistically significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test.
*Genot et al. (2003)

Table 7.5 Selected flavour compounds associated with coffee in 28-day stored (45 °C) emulsions at various GMO concentration.

Retention index	Compounds		Type	GMO level (%)			
				Control	*0.03*	*0.1*	*0.2*
1052	C20	2,3-Pentanedione	Bulk	0.33 ± 0.11^{B}	0.28 ± 0.08^{AB}	0.20 ± 0.06^{A}	0.22 ± 0.06^{AB}
			Instantised	0.30 ± 0.09^{A}	0.36 ± 0.14^{A}	0.23 ± 0.08^{A}	0.26 ± 0.05^{A}
1535	C60	2-Furanmethyl acetate	Bulk	1.83 ± 0.22^{B}	1.82 ± 0.23^{B}	1.40 ± 0.11^{A}	1.35 ± 0.12^{A}
			Instantised	1.87 ± 0.17^{B}	1.96 ± 0.16^{B}	1.44 ± 0.11^{A}	1.44 ± 0.15^{A}
915	C8	2-Methylbutanal	Bulk	52.98 ± 7.89^{B}	48.88 ± 11.14^{B}	28.70 ± 7.43^{A}	30.46 ± 3.70^{A}
			Instantised	48.38 ± 7.69^{B}	46.16 ± 8.61^{B}	34.58 ± 6.57^{A}	29.74 ± 3.34^{A}
1470	C52	Furfural	Bulk	219.51 ± 16.71^{B}	224.22 ± 24.89^{B}	162.51 ± 18.75^{A}	153.83 ± 17.71^{A}
			Instantised	225.05 ± 12.29^{B}	253.95 ± 21.78^{C}	167.92 ± 18.39^{A}	166.79 ± 9.71^{A}
1338	C37	Ethylpyrazine	Bulk	50.32 ± 3.17^{B}	50.69 ± 5.21^{B}	33.44 ± 3.50^{A}	30.58 ± 2.66^{A}
			Instantised	52.01 ± 2.19^{B}	57.04 ± 3.82^{B}	36.47 ± 3.60^{A}	33.72 ± 1.88^{A}
1351	C38	2,3-Dimethylpyrazine	Bulk	13.92 ± 0.82^{B}	14.19 ± 2.03^{B}	9.34 ± 0.75^{A}	8.80 ± 1.18^{A}
			Instantised	14.23 ± 0.67^{B}	16.32 ± 0.98^{C}	10.69 ± 1.11^{A}	10.06 ± 0.85^{A}
1181	C26	Pyridine	Bulk	128.58 ± 37.28^{B}	128.18 ± 17.21^{B}	58.04 ± 26.49^{A}	56.11 ± 28.74^{A}
			Instantised	137.39 ± 31.13^{AB}	166.63 ± 26.59^{B}	80.19 ± 43.02^{A}	78.71 ± 42.43^{A}
1513	C56	2-Acetylfuran	Bulk	49.85 ± 2.38^{B}	51.92 ± 7.71^{B}	36.85 ± 4.52^{A}	35.02 ± 4.30^{A}
			Instantised	51.96 ± 3.13^{B}	57.77 ± 2.68^{C}	38.54 ± 3.06^{A}	38.04 ± 3.05^{A}
1980	C75	1-(1H-Pyrrol-2-yl)ethanone	Bulk	16.74 ± 3.56^{AB}	18.27 ± 2.54^{B}	13.97 ± 1.33^{A}	14.21 ± 2.13^{AB}
			Instantised	16.91 ± 1.86^{AB}	18.85 ± 2.67^{B}	13.84 ± 1.64^{A}	14.25 ± 2.49^{A}
2015	C77	Phenol	Bulk	9.80 ± 1.07^{A}	10.90 ± 1.07^{A}	9.12 ± 0.67^{A}	10.01 ± 1.27^{A}
			Instantised	9.82 ± 0.56^{A}	11.39 ± 0.96^{B}	9.03 ± 0.39^{A}	10.34 ± 1.26^{AB}

Values are means relative peak area (%) ± standard deviations of 6 measurements (2 batches x 3 replicates), except Control 2 has five replicate points. Different letters in the same row indicate statistically significant differences ($p<0.05$) by Tukey post-hoc multiple comparison test.

7.4 Conclusions

This study evaluated the effect of bulk and instantised GMO concentrations on physicochemical properties, creaming stability and volatile profile of coffee emulsions and the changes that occurred during storage. Increasing the concentration of instantised GMO in the fresh emulsions resulted in smaller average droplet sizes and narrower size distributions than the control (no GMO). The same effect on droplet size distribution was observed in the emulsions prepared with bulk GMO. The emulsions with smaller droplet size displayed greater stability against creaming. At the higher concentrations of GMO (0.1% and 0.2%) in the emulsions, droplet size increased during ageing time due to flocculation and coalescence. At 0.2% GMO level, the emulsions formed insoluble particles that consisted of protein aggregates with oil droplets when observed using an optical microscope, and the emulsion droplets had lower repulsive forces and higher emulsion viscosity. The volatile profile of all fresh emulsions changed significantly during storage ($p<0.05$). The emulsions with 0.2% GMO at 28 days of storage generally had lower concentrations of oxidation markers and coffee flavour compounds when compared to their respective control emulsions. This study reaffirmed the findings in **Chapter 3** and **Chapter 6** that GMO improves oil droplet size distribution thus enhancing creaming stability in the emulsions. However, GMO had to be used sparingly in the coffee emulsions because of lower pH near to the isoelectric point of casein and the hydrophobicity of casein at the interface increasing during storage, resulting in the formation of visible sediment particles. Emulsions with 0.03% iGMO had stable droplet size, zeta potential and viscosity over ageing time and contained a very similar volatile profile with control emulsions after 28 days of storage, but improved creaming stability of the emulsions. Although 0.03% bGMO samples showed the same volatile profile as the control, the bulk liquid nature of the GMO means that it is not in a suitable form for many food formulations. This research shows that instantised GMO at 0.03% is effective as an emulsifying and stability agent that improves creaming stability and causes minimal change to the volatile profile of the emulsions during storage. As the odour thresholds for the compounds contributing to rancidity and coffee aroma were not determined in this chapter, the effect of the new formulation could be investigated by performing a consumer acceptance test, but this was beyond the scope of this thesis. A coffee emulsion with the desired emulsion properties and stability can be achieved by using an optimum concentration for GMO.

Chapter 8

General Discussion, Future Outlook and Conclusions

8.1 General discussion

Protein-stabilised oil-in-water emulsions include a broad range of food products, including ready-to-drink (RTD) milk coffee beverages. Due to the thermodynamically unstable nature of emulsions, they are prone to creaming, flocculation and coalescence during storage. The physical stability of emulsions depends on the surfactant composition, including low-molecular-weight emulsifiers and proteins, and their interactions at the oil-water interface of the emulsion. Mono- and diglycerides are commonly used to improve emulsion quality and stability during storage, and have been well-studied in many food formulations such as ice-cream, whipped cream and coffee creamers (Dickinson & Tanai, 1992). However, in the available literature, there is a lack of systematic research using commercial mono- and diglycerides to enhance stability of emulsions. There is also very little information published on the encapsulation of emulsifiers as a way to improve the dispersion properties of oil-soluble emulsifiers in the aqueous phase. In this thesis, model emulsions were employed instead of complex food systems to investigate the effect of the emulsifier system on emulsion quality and stability.

This project had two main objectives. The first objective was to determine the effect of mono- and diglycerides and milk protein composition on the physical stability of protein-stabilised oil-in-water emulsions. The experimental evidence showed that glycerol monooleate (GMO), an unsaturated emulsifier with high monoglyceride content (>90%), formed small oil droplets with a narrow size distribution that reduced the extent of creaming. The second objective was to develop a spray-dried encapsulated emulsifier system and to characterise after reconstitution the physicochemical properties of this novel ingredient and emulsion stability. The experimental results demonstrated the feasibility to prepare a powdered encapsulated emulsifier system that has excellent dispersion in water and forms stable emulsion droplets after reconstitution. In order to achieve these main objectives, five experimental studies were conducted according to the experimental design outlined in **Figure 8.1**. This thesis had a linear structure where each chapter built upon the findings of the previous chapter.

Figure 8.1 Schematic overview of the experimental study design. MDG: Mono- and diglycerides, Sat: Saturated, Unsat: Unsaturated, MG: Monoglyceride, Na stearate: Sodium stearate, Cas: Sodium caseinate, WPC: Whey protein concentrate, MD: Maltodextrin, DE: Dextrose equivalent, GMO: Glycerol monooleate

The first specific objective of this thesis (**Chapter 3**) was to investigate the effect of different mono- and diglyceride compositions (unsaturation of fatty acids; monoglyceride content; and sodium stearate content) on physical properties and creaming stability in protein-stabilised emulsions. The experimental evidence demonstrated that unsaturated mono- and diglycerides formed a narrow droplet size range distribution with the lowest amount of creaming when compared to the saturated mono- and diglycerides and the control. A high monoglyceride content was also more effective in achieving small droplet sizes and narrow size distributions than low monoglyceride content. Samples with different sodium stearate content in the unsaturated mono- and diglycerides formed emulsions with the same droplet size and creaming stability. In contrast, in the emulsions prepared with saturated mono- and diglycerides, the sample with high monoglyceride content with 3% sodium stearate formed emulsions with better creaming stability than the other emulsions. The sample with unsaturated mono- and diglycerides with high monoglyceride content (primarily glycerol monooleate (GMO)) showed the best resistance to creaming of the emulsions evaluated. A stable droplet size and large negative surface charge (based on zeta potential) were maintained during storage showing that oil droplets did not coalesce or flocculate. The proteins adsorbed at the oil-water interface provided a large negative surface charge that led to electrostatic stabilisation of the emulsion droplets provided, which prevented oil droplets coming together (Dickinson, 1997; McClements, 2007).

A limitation of **Chapter 3** was the high stability of the model emulsions at room temperature and low oil content (1.1%) resulting in low levels of creaming after 28 days of storage and a lack in discrimination between samples with a cream layer that was almost the same for all emulsions. As the creaming stability is determined by measuring the height of the cream layer, the minimal creaming resulted in a cream layer that was difficult to accurately measure. Therefore, in subsequent chapters, pilot tests were carried out to reformulate the model emulsion and storage conditions to accelerate the creaming without affecting the characteristics of the emulsion droplets during storage. The emulsions prepared with 1.1% hydrogenated coconut oil (model emulsion for **Chapter 3**) were compared against two model emulsions reformulated with 4% hydrogenated coconut oil or 4% canola oil, which was a more typical fat content of milk and commercial milk coffee beverages. The emulsions were compared in term of physical properties (droplet size and viscosity) and creaming stability at different storage

conditions (4 °C, 25 °C and 45 °C). The experimental findings demonstrated that both emulsions with 4% oil content formed at least twice the amount of cream than the emulsion with 1.1% oil and the thickest cream layer was observed at the storage temperature of 45 °C. All emulsions with different formulations had similar oil droplet size distributions and viscosities to that of a ready-to-drink coffee beverage, and creaming instability were accelerated by the increase in oil content and storage temperature. Hence the emulsion with 4% canola oil was used as a model emulsion for further investigation in **Chapter 4**, **Chapter 6** and **Chapter 7**. Canola oil was selected instead of hydrogenated coconut oil because it has a higher level of unsaturated fatty acids (mostly oleic acid) and is more prone to lipid oxidation, in order to investigate the effect of emulsion systems on chemical stability and resistance to oxidation.

Chapter 4 investigated the effect of milk protein composition (ratio of sodium caseinate to whey protein concentrate (WPC) was varied) on physicochemical properties, creaming stability and oxidative stability of protein-stabilised emulsions prepared with GMO. The increased proportion of sodium caseinate in the milk protein composition resulted in emulsions with smaller oil droplet size, large negative surface charge (higher repulsive forces between droplets) and higher viscosity. All emulsions, regardless of milk protein compositions, formed a thin layer of cream and the ratio of sodium caseinate and WPC did not affect creaming stability during storage. The most exciting finding was the effect of protein compositions on oxidative stability during storage. The emulsions prepared with mixtures of sodium caseinate and WPC exhibited greater oxidative stability during storage when compared to the emulsions prepared with only sodium caseinate or WPC. This finding may be due to the mixed proteins forming a thicker layer at the oil-water interface (Donnelly et al., 1998; Haahr & Jacobsen, 2008; McClements & Decker, 2000) and the antioxidant effect of the unadsorbed protein in the aqueous phase (Faraji et al., 2004).

GMO demonstrated a positive effect on creaming stability in protein-stabilised emulsions (**Chapter 3**). Nevertheless, the oil-soluble GMO has poor dispersion in water and is not suitable for direct application in many food formulations. It was postulated that encapsulation of GMO by spray-drying could transform this emulsifier into an instantised powder with good dispersibility in water enabling it to be used as a novel, functional ingredient. **Chapter 5** investigated the feasibility to encapsulate GMO by spray-drying

and successfully demonstrated instantised GMO powders could be formed that dispersed well in water. This chapter also evaluated the influence of monoglyceride level and maltodextrin type on emulsion properties, particle properties and the oxidative stability of the GMO powder. Monoglyceride level demonstrated a larger effect on the emulsion and particle properties compared to maltodextrin type. Increasing monoglyceride level in the formulations formed emulsions with higher viscosities and larger oil droplets, resulting in dried particles with higher surface oil that negatively affected the reconstitution properties of the dried particles (larger droplet growth after reconstitution and lower dispersibility). Though all GMO powders showed greater oxidative stability than bulk GMO, and three samples, namely LowMG-DE10, HighMG-DE10 and HighMG-DE18, demonstrated excellent oxidative stability.

Chapter 6 investigated the effect of two instantised GMO powders developed in **Chapter 5** on physicochemical properties, creaming stability and oxidative stability in model protein-stabilised emulsions. Both GMO powders formed oil droplets that had the same physicochemical properties and creaming stability as bulk GMO, where all emulsions prepared with GMO had smaller droplet sizes, narrower size distributions and greater creaming stability than control emulsions (no GMO). The emulsions prepared with GMO powders had the same concentrations of selected volatile compounds as the samples prepared with bulk GMO and the control (no GMO), demonstrating a similar oxidative stability. The smaller mean droplet size of the emulsions did not cause any detrimental effect on oxidative stability. These findings showed that GMO powder had the same functionality as bulk GMO in a model protein-stabilised emulsion, but with improved functionality and dispersibility.

One instantised GMO powder developed in **Chapter 5** was evaluated against bulk GMO at different concentrations on the physicochemical properties, creaming stability and volatile profile in a milk coffee emulsion containing 4% canola oil, 2% coffee extracts, 0.8% sodium caseinate, 0.2% whey protein concentrate, and 6% caster sugar (**Chapter 7**). This chapter demonstrated the application of the instantised GMO powder in a real coffee application to gain more understanding of the performance of the new ingredient in a product. This was important to evaluate as the addition of coffee extracts will lower the pH of the emulsions, which is known to decrease emulsion stability during storage. Canola oil was used as the oil-phase instead of milk so that bulk GMO could be

incorporated in the emulsions and allowed for direct comparison between bulk GMO and GMO powder on their effect on emulsion properties and stability. The increase in GMO level (regardless of the type of GMO) in the fresh emulsions improved emulsion stability against creaming by forming smaller droplet sizes and narrower size distributions. This result agreed with previous findings in **Chapter 3** and **Chapter 6**, where the emulsions prepared with GMO formed smaller oil droplets and displayed reducing creaming than control samples (no GMO). However, in contrast to previous findings, the emulsions prepared with 0.1% and 0.2% GMO were not stable during storage when a lower pH was used to prepare the emulsions and resulted in the growth of the oil droplet size and the presence of sediment. These emulsions at 28 days of storage were visualised by optical microscope, and the presence of flocculated oil droplets could be observed. The visible sediment particles in the emulsions with 0.2% GMO were likely associated with protein-aggregation induced flocculation, where the zeta potential was less negative than the critical theoretical level (-30 mV) and reduced solubility of the caseinate. The volatile profile of the emulsions changed significantly over time, and the emulsions with 0.2% GMO at 28 days of storage had a lower concentration of volatile compounds associated with lipid oxidation and coffee than control emulsions. These findings agreed with previous research (Fomuso et al., 2002; N. Kim & Choe, 2012) that high concentration of monoglycerides forms an interface that is less permeable to oxygen diffusion, resulting in improved oxidative stability. The emulsions prepared with 0.03% GMO had greater creaming stability than the controls and demonstrated stable droplet size, zeta potential and viscosity with a very similar volatile profile compared to the controls. The increase of emulsifier concentration is associated with the formation of smaller droplet size and improved creaming stability in oil-in-water emulsion (Berton-Carabin et al., 2014; Donnelly et al., 1998; Sun et al., 2007). However, the emulsion droplet size was not stable during storage in slightly acidic emulsions, where the oil droplets flocced together due to inter-droplet hydrophobic interactions of the protein adsorbed at the interface, thus resulting in an increased apparent droplet size (Shao & Tang, 2014; Tang & Liu, 2013) and an increased emulsion viscosity (McClements, 2007; Quemada & Berli, 2002).

In summary, the results from this thesis provide new insights into the relationship between emulsion properties and volatile profile using different emulsifier systems and their behaviour in neutral-pH (model emulsion systems) and slightly acidic emulsions in

a coffee beverage application. The knowledge from this study can be used as a platform for designing food ingredients and formulations with improved quality and functionality.

8.2 Limitations and future recommendations

This thesis has shown the interactions between mono- and diglycerides and milk proteins at the oil-water interface of a protein-stabilised emulsion, and how these interactions affect its physicochemical properties, physical stability and oxidative stability. These findings indicated that the ingredients at the interface had a significant role affecting the properties and stability of the emulsions. The main limitations of this study are that all emulsions were very stable, and only a thin cream layer was formed in all studied systems, making it difficult to accurately measure the amount of creaming that had formed in the emulsions. There was also a lack of sophisticated instruments available to measure creaming stability more precisely in this study, which could be explored in future research. An analytical technique that can accelerate creaming by centrifuging the sample at variable centrifugation speed, temperature and time, integrated with sectional droplet profiling by measuring the light scattering in different regions of the sample, could provide valuable information on creaming stability with more precision (McClements, 2007; Mengual et al., 1999). Some example approaches which could be used to increase the accuracy of the creaming measurements are using "turbiscan" (Jukkola, Partanen, Xiang, Heino, & Rojas, 2019) or "lumifuge" (Liang et al., 2016) instruments.

The current study has also shown that oil-soluble liquid GMO can be spray-dried to form a powder with good dispersibility in water. In this study, only one set of spray-drying parameters were used to gauge the feasibility of spray-drying to transform oil-soluble GMO into instantised powder comparing four different formulations. In future, experiments should be carried out to investigate the effect of spray-drying parameters such as feed rate, outlet temperature and homogenisation pressure on the dried particles to optimise the ingredient properties. A full optimisation study using a response surface methodology could be used to optimise the spray-drying parameters. Further research might be aimed to understand the possible underlying mechanism and effect of spray-drying parameters on the particle properties and process yield. Detailed characterisation of the instantised GMO powder should also be evaluated such as particle size, stability under different storage conditions, stickiness and flow properties, the thickness of the

wall material and morphology of the powder by scanning electron microscopy or confocal laser scanning microscopy (Bakry et al., 2016). It would be interesting to understand how lipid oxidation is affected by the morphology and properties of the dried particles.

The changes in the volatile composition have shown that volatile compounds can be used as markers representing lipid oxidation during storage for emulsions and the powdered ingredient. Changes in the volatile profile may also affect the perception of flavour in the ready-to-drink beverage, but further investigations to understand how this is related to sensory perception of consumers and product quality is required.

In all principal component analysis (PCA) model (Chapters 5-7), multiple variables (formulation, storage time) are being considered. As PCA is an unsupervised technique, it has the advantage of not being biased by pre-determined categories. Therefore, we believe that PCA was appropriate for the thesis objectives. However, one limitation of this technique is that PCA transforms the data set into a new dimension without considering their labels. This could result in the principal component (PC) with the highest variation not providing the highest separation for the samples. An alternative technique could be partial least squares discriminant analysis (PLS-DA) that required pre-determined categories (Aprea et al., 2006).

The evaluation of the volatile profile was carried out without an internal standard (**Chapters 4-6**) due to the interference of the available standards with target analytes. The introduction of an appropriate internal standard in the coffee emulsions (**Chapter 7**) showed tremendous improvement in the results leading to a more apparent differentiation among the samples. The oxidative stability using industry standardised chemical methods used in the food industry such as peroxide value and p-anisidine value should also be investigated to compare the results against volatile analysis.

8.3 Conclusions

Incorporating GMO into the milk coffee beverages ranging from slightly acidic to neutral pH has many advantages. The addition of GMO in emulsions forms uniformly distributed and fine droplet sizes that enhance their emulsion stability during storage (Dickinson & Tanai, 1992) (**Chapter 3**) with no increase in terms of oxidation-induced volatile compounds during storage (Dimakou et al., 2007; Osborn & Akoh, 2004) (**Chapter 6**).

Any effect of GMO on lipid oxidation was most likely masked by the formation of a thick, multilayer interface by the adsorption of both casein and whey proteins at the oil-water interface (Donnelly et al., 1998; Haahr & Jacobsen, 2008; McClements & Decker, 2000) or the antioxidant effects of the unadsorbed protein in the aqueous phase (Faraji et al., 2004) (**Chapter 4**). When coffee was added to the emulsion, the emulsion system became less stable as a result of the slightly acidic pH that is near to the isoelectric point of casein, thus reducing the solubility of casein in water. In this emulsion, GMO has to be used at very low concentrations (0.03%) to achieve uniformly distributed emulsion droplets that improve creaming stability with minimal change to the volatile profile (**Chapter 7**). This knowledge on the mono- and diglyceride and milk protein compositions are useful to food technologists to develop a coffee emulsion beverage with a uniform droplet suspension that has excellent creaming and oxidative stability.

The successful preparation of an instantised GMO powder by spray-drying (**Chapter 5**) is a breakthrough in ingredient science to produce a novel ingredient that will be of value to the food and beverage industry. The developed GMO powder demonstrated that it formed an emulsion with uniform oil droplets, exhibiting the same creaming stability and oxidative stability as bulk GMO in model emulsion systems (**Chapter 6**). The concentration of GMO was not critical when applied in neutral-pH emulsions, but concentrations at 0.1% caused protein aggregate induced flocculation in a slightly acidic milk coffee emulsion (**Chapter 7**).

Further development may be required to determine optimum dose rates, depending on specific applications. The instantised GMO powder will give better control over the dosage to ensure the desired functionality is obtained. This demonstrated the critical understanding of the interaction between emulsifiers (emulsifier composition and concentrations) and proteins at the oil-water interface and how they are affected by pH.

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Appendix A

Supplementary data for Chapter 3

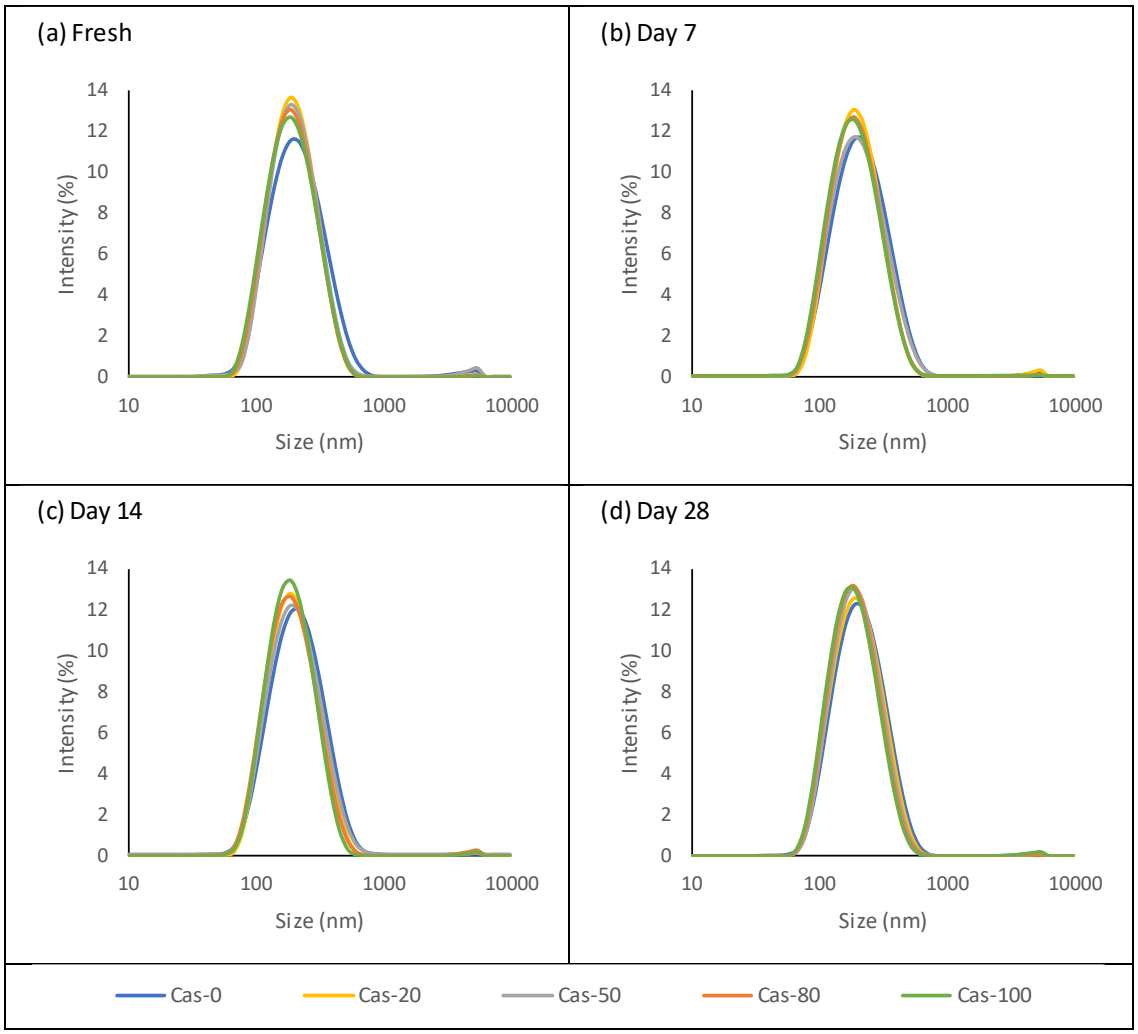
A.1 ANOVA table for (a) droplet size and (b) polydispersity index of emulsions at different ageing time.

Ageing time (day)		Sum of Squares	df	Mean Square	F	Sig.
(a) Droplet size						
0	Between Groups	155.200	2	77.600	1.060	0.355
	Within Groups	3293.354	45	73.186		
	Total	3448.555	47			
7	Between Groups	152.311	2	76.156	1.197	0.312
	Within Groups	2863.081	45	63.624		
	Total	3015.393	47			
14	Between Groups	131.458	2	65.729	1.177	0.317
	Within Groups	2512.479	45	55.833		
	Total	2643.937	47			
28	Between Groups	138.333	2	69.166	1.116	0.337
	Within Groups	2789.964	45	61.999		
	Total	2928.297	47			
(b) Polydispersity index						
0	Between Groups	0.000	2	0.000	0.770	0.469
	Within Groups	0.006	45	0.000		
	Total	0.006	47			
7	Between Groups	0.000	2	0.000	1.519	0.230
	Within Groups	0.007	45	0.000		
	Total	0.008	47			
14	Between Groups	0.000	2	0.000	0.295	0.746
	Within Groups	0.011	45	0.000		
	Total	0.011	47			
28	Between Groups	0.000	2	0.000	0.850	0.434
	Within Groups	0.010	45	0.000		
	Total	0.011	47			

Appendix B

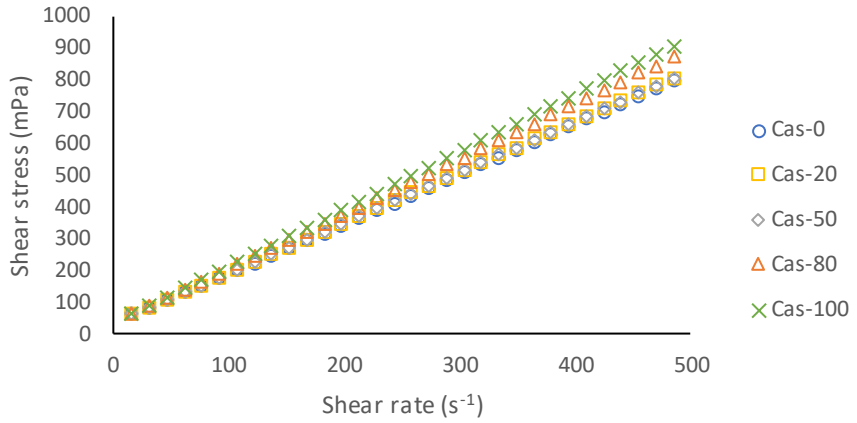
Supplementary data for Chapter 4

B.1 Intensity-weighted size distribution of the emulsions at different ageing time.

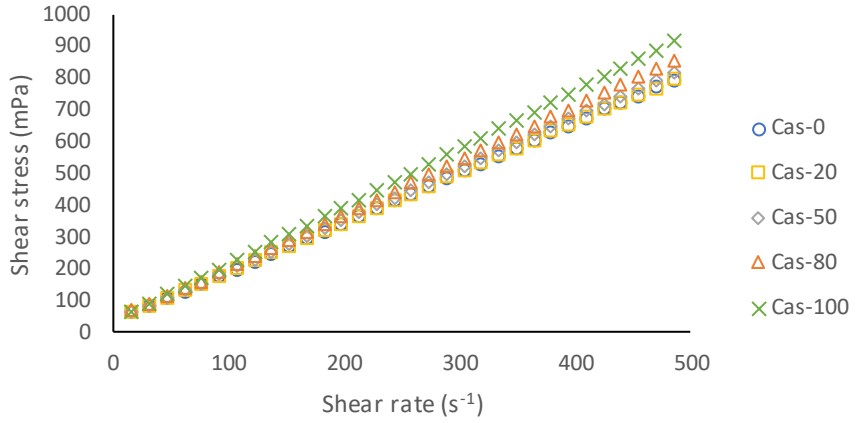


B.2 Rheological properties of emulsions prepared with different milk protein compositions at different ageing time.

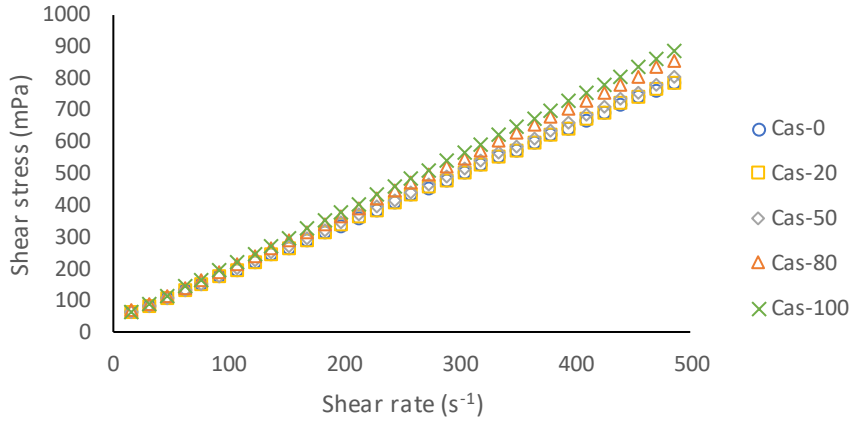
(a) Day 7



(b) Day 14



(c) Day 28



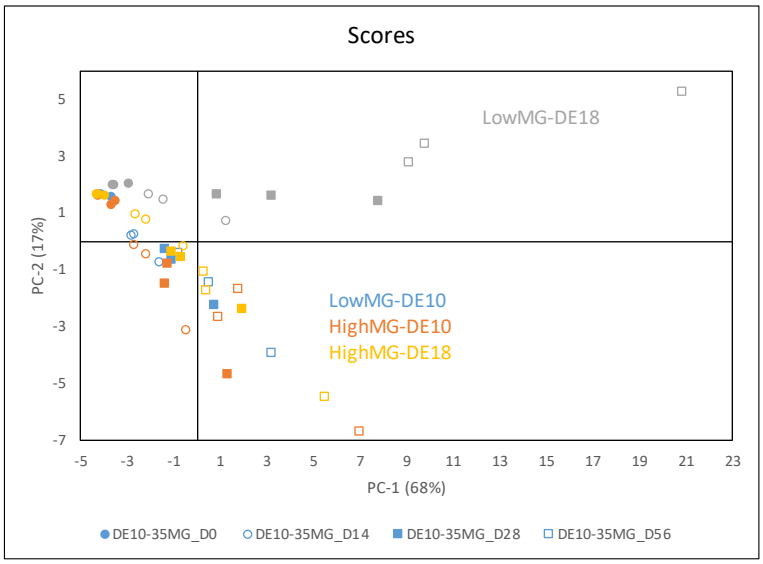
B.3 ANOVA for hexanal among the emulsions with different milk protein compositions at 28 days.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.483×10^{16}	4	1.121×10^{16}	0.864	0.499
Within Groups	3.241×10^{17}	25	1.296×10^{16}		
Total	3.689×10^{17}	29			

Appendix C

Supplementary data for Chapter 5

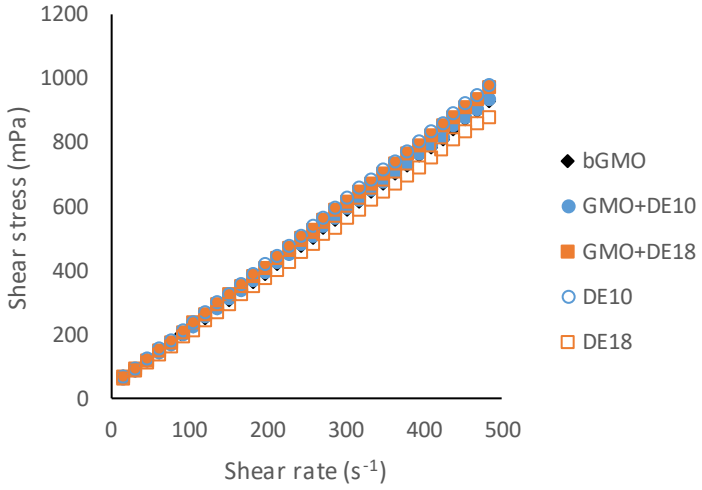
C.1 PCA scores plot for instantised GMO powders at the various storage time at 45 °C.



Appendix D

Supplementary data for Chapter 6

D.1 Rheological properties of fresh emulsions prepared with different milk protein compositions.



Appendix E

Supplementary data for Chapter 7

E.1 List of volatile compounds that changed significantly ($p<0.05$) over 28 days of storage at 45 °C.

RI	Code	Compound name	RI	Code	Compound name
794	C1	Propanal	1379	C40	2-Methyl-2-cyclopenten-1-one
801	C2	Furan	1389	C41	2-Ethyl-6-methylpyrazine
816	C4	Acetone	1395	C42	2-Ethyl-5-methylpyrazine
871	C5	2-Methylfuran	1407	C43	2-Ethyl-3-methylpyrazine
903	C7	2-Butanone	1424	C44	2-Propylpyrazine
915	C8	2-Methylbutanal	1432	C46	3-Furaldehyde
931	C11	3-Methyl-2-butanone	1439	C47	2,6-Diethylpyrazine
955	C12	2-Ethylfuran	1440	C48	1-Octen-3-ol
955	C13	2,5-Dimethylfuran	1448	C49	3-Ethyl-2,5-dimethylpyrazine
981	C15	Pentanal	1460	C51	1-Acetyloxy-2-propanone
1033	C16	2-Ethyl-5-methylfuran	1470	C52	Furfural
1033	C17	2-Propylfuran	1495	C54	2-Furfuryl methyl sulphide
1043	C18	Toluene	1502	C55	(E,E)-2,4-Heptadienal
1052	C19	3-Hexanone	1513	C56	2-Acetylfuran
1058	C20	2,3-Pentanedione	1518	C57	Pyrrole
1081	C21	Hexanal	1524	C58	3,5-Octadien-2-one
1096	C22	(E)-2-Methyl-2-butenal	1525	C59	2-Methyl-3-pentanone
1128	C23	2-Butylfuran	1535	C60	2-Furanmethyl acetate
1141	C25	1-Methyl-1H-pyrrole	1539	C61	Benzaldehyde
1181	C26	Pyridine	1549	C62	1-Octanol
1212	C27	Pyrazine	1578	C63	(E,E)-3,5-Octadien-2-one
1228	C28	2-Pentylfuran	1625	C66	1-Ethyl-1H-pyrrole-2-carboxaldehyde
1238	C29	2-(Methoxymethyl)furan	1637	C67	1-Methyl-1H-pyrrole-2-carboxaldehyde
1269	C31	Methylpyrazine	1676	C70	3-Methylbutanoic acid
1283	C32	4-Methylthiazole	1715	C71	5-Methyl-2-furanmethanol
1287	C33	2,7-Dimethyloxepine	1747	C72	2-[(Z)-(9-octadecenyloxy)]ethanol
1290	C34	Octanal	1841	C73	1-(2-Furanylmethyl)-1H-pyrrole
1326	C35	2,5-Dimethylpyrazine	1980	C75	1-(1H-Pyrrol-2-yl)ethanone
1331	C36	2,6-Dimethylpyrazine	1990	C76	2,2'-[oxybis(methylene)]bisfuran
1338	C37	Ethylpyrazine	2015	C77	Phenol
1351	C38	2,3-Dimethylpyrazine	2075	C78	1H-Pyrrole-2-carboxaldehyde