Egg White Foam

A thesis presented in partial fulfilment of the requirements

for the degree of Master of Food Technology

at Massey University, Auckland, New Zealand

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2013

Abstract

Egg white is extensively utilized as a functional food material in food processing due to the multiple functional roles of egg white proteins such as foaming, gelling and emulsifying properties. The foaming property of egg white has been widely studied using different methods. In this research, two different foaming methods were used to prepare egg white foams by a whipping method using a standard mix beater and a sparging method using a whipped cream dispenser (pressurized dispenser). Egg white is also commercially available in several different physical forms, such as fresh egg white liquid, frozen fresh egg white liquid (EWL) and spray dried egg white powder (EWP). In this study, EWL and EWP solutions were used to compare their foaming ability and foam stability. Various factors affecting on the formation and stability of egg white foam were investigated to understand their impact on the functional properties of egg white as foaming agents under specific conditions, including whipping time and speed, shaking time, temperature, pH, type and ionic strength of salts, thermal treatment and addition of some ingredients (e.g. sugar and hydrocolloids). All foams produced were analysed on the basis of two different parameters of foam properties, such as foamability after preparation and foam stability with time after foam preparation. Foam stability was also analysed by two different aspects, foam volume stability against foam collapse and foam liquid stability against liquid drainage. Another objective of this study was to investigate the application of cooking egg white foam in a microwave oven after the foam preparation with an aim of developing a prototype of value added new products derived from egg white foam. The microbiological stability of egg white was also measured to determine the shelf stability of non-pasteurised and pasteurised egg white solutions with and without added ingredients against microbial growth. Overall the results obtained in this study provide significant insights into the impact of various factors affecting the formation and stability of egg white foam and the potential application of microwave cooking of egg white foam for applications in various food industries.

Keywords: Egg white foam, foamability, foam stability, whipped cream dispenser, microwave oven, microbial stability

Acknowledgements

My deepest and most heartfelt thanks go to several people for their assistance in this research:

Dr Sung Je Lee for his guidance and knowledge in order to complete this research. Especially for his patience and instructions on the construction of my research

Associate Professor Marie Wong for her encouragement and advising me the correct direction to complete this research successfully. Also I would like to thank her for her instructions, knowledge and inspirational suggestions on the structure of my research.

To Mr Peter Johnson for his financial support to complete this research and his guidance.

My parents for their financial and moral support. Also I would like to thank them for their encouragement in completing this research.

Also, I would like to thank the King of Saudi Arabia, Abdullah bin Abdul-Aziz, who gave me the opportunity to complete this degree under his scholarship.

Also, I would like to thank my husband for his support in keeping me focused on my research and helping me managing my time, especially, given we are both studying towards a master's degree. Also my child for being patient and helping me to focus on my studies.

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Chapter 1 Introduction

Egg white is extensively utilized as a functional food material in many food products due to the multiple properties of egg white proteins, such as foaming (whippability), gelling (coagulation) and emulsifying properties (Bergquist, 2000; Alleoni, 2006). Egg white is also well-known as an excellent source of dietary protein in human nutrition owing to its high bioavailability and high content of essential amino acids (Bergquist, 2000; Lomakina & Mikova, 2006). The biological value of egg white protein is known to be highest among all food proteins as egg white proteins are readily absorbed, broken down and efficiently utilised in the human body (Stadelman & Cotterill, 1995).

As a foaming agent, when egg white is whipped, egg white proteins are able to rapidly adsorb on the air-water interface and form a cohesive viscoelastic film around air bubbles through their intermolecular interactions (Lomakina & Mikova, 2006). The foaming ability of egg white can be attributed to the chemical structure and physical properties of egg white proteins. Some of important egg white proteins that are responsible for conferring the high foaming ability include ovalbumin, ovomucin and ovoglobulins (Darmodaran et al., 1998). These proteins are globular proteins containing free sulfhydryl (SH) groups and disulfide bonds as well as non-polar groups clustered and packed in the interior of the protein molecules. They are readily undergo denaturation at the air-water interface, called “interfacial denaturation”, when mechanical shear forces are applied to egg white. As a result, the proteins unfold their conformational structures and expose reactive amino acids, causing protein aggregation at the interface that leads to form a rigid interfacial film around bubbles which promotes foam stabilisation (Kinsella, 1981).

Foam can be produced by various methods, such as whipping, gas sparging and shaking. The whipping method uses mechanical forces to generate foams using electric mix beaters, blenders, shear mixers and whiskers. This method is the most commonly used to produce foams from aqueous protein solutions. The sparging method involves injection of gas, such as nitrogen, carbon dioxide, into solution. Shaking is relatively as simple method to create foams by shaking bottles containing solution.

Experimental techniques used to assess and characterise the properties of foam include

several measurements associated with foaming ability and foam stability. Foaming ability, also referred to as foamability, is related to the volume of air that is incorporated into solution. Foam stability is related to the properties of interfacial films surrounding air bubbles, in terms of their strength and viscoelastic properties. Foam stability is normally determined by measuring the amount of foam volume being decreased over time due to bubble coalescence, disproportionation and/or collapse and the rate of liquid drainage from foam over time after foam preparation. These two parameters of foam stability are also measured by monitoring the length of time required for half of the foam mass to collapse or the foam liquid to drain.

The efficiency of egg white proteins as functional proteins can be adversely or favourably affected by changes in their physicochemical properties due to various factors, including physical factors and environmental and chemical conditions. Physical factors include thermal treatment, high pressure and mechanical shear forces. Environmental and chemical conditions include pH, ionic strength, temperature, type and concentration of protein and presence of other components. Examples of ingredients that can have an effect on the foamability and foam stability of egg white foam include salt, sugar and hydrocolloids which are generally formulated in aerated food products. It is important to take into account various factors that can affect the formation and properties of foams when egg white foams are used and applied in complex food systems.

The objectives of this project were to i) investigate the foamability and foam stability of egg white foams prepared from two different types of egg whites, such as fresh egg white liquid and spray-dried egg white powder, by using two different methods, such as whipping and gas sparging method, ii) determine a variety of factors that can affect the properties and stability of egg white foam, iii) determine the impact of cooking egg white foams in a microwave oven and iv) analyse the microbial stability of egg white liquid before and after heat treatment.

As a whipping method, a standard mix beater was used in this study. As a sparging method, a whipped cream dispenser with NO_2 gas charger was utilised to explore its efficiency in producing egg white foam and determine the properties of egg white foam produced by this device. No studies have been reported on the foamability and stability of egg white foams produced by using the whipped cream dispenser. Also, research studies on the application of microwave cooking of egg white foam have been very

limited and no literature information is currently available. The development of microwavable egg white foams can have a significant potential to generate a new market for egg white.

Chapter 2 Literature Review

2.1 Nutritional composition of eggs

Eggs are classified as an ideal food for providing dietary requirements with a high content of protein (12%), fat (12%), minerals (e.g. iron, phosphorus and potassium) and vitamins (e.g. vitamins A, D, E, riboflavin (B2), niacin (B3)). However, there is no vitamin C in eggs. The composition of eggs (i.e. egg white, egg yolk and whole egg excluding the egg shell) is shown in Table 2.1. Egg yolk is comprised of about 50% water, 15-17% protein, 35% fat, <1% carbohydrate and 1.1% ash (minerals). Almost all of the fat in egg is exclusively found in egg yolk. Egg yolk is a good source of phospholipids and unsaturated fatty acids but is also high in cholesterol as such whole egg has a negative aspect in terms of its nutritional value.

Egg white, also referred to as albumen, contains about 89% water, 10% protein, <1% carbohydrate and 0.5% ash but its lipid content is almost negligible less than 0.01%. As can be seen in Table 2.1, the main constituent of egg white is protein, excluding water, thereby making the portion of egg white as a rich source of food proteins. Egg white is also high in vitamin B12 (riboflavin). However, the content of carbohydrates in both egg white and egg yolk is very low but they exist in a free and combined form with proteins. An example of free carbohydrate in egg white is glucose (0.4%) whereas the combined form is present as glycoprotein that contains mannose and galactose (0.5%) (Stadelman & Cotterill, 1995). The amounts of other minor nutrients (e.g. minerals and vitamins) in egg yolk and egg white are presented in Table 2.2.

Table 2.1 Composition of egg white, egg yolk and whole egg excluding the shell.

Nutritional Compositions (g)	Egg White (100g)	Egg Yolk (100g)	Whole Egg (100g)
Water	88.6	49	74.4
Protein	9.7-10.6	15.7-16.6	12.8-13.4
Carbohydrate	0.4-0.9	0.2-1.0	0.3-1.0
Fat	0.1	34.5	11.9
Ash	0.5-0.6	1.1	0.8-1.0

Sources: Hui (2007) and Huopalahti et al. (2007)

Table 2.2 Vitamin and mineral compositions of egg white, egg yolk and whole egg.

Elements (mg)	Egg white 100g	Egg yolk 100g	Whole egg 100g
Minerals			
Iron	0.1	4.8	1.7
Calcium	8	133	50
Potassium	140	100	125
Sodium	155	50	120
Magnesium	10	15	12
Phosphorus	18	530	193
Sulphur	163	165	164
Zinc	0.12	3.9	1.4
Copper	0.02	0.14	0.06
Vitamins			
Ascorbic Acid	0	0	0
Vitamin A	0	450	150
Vitamin B2	430	480	447
Folic Acid	12	140	56
Niacin	90	60	79

Source: Huopalahti et al. (2007)

2.2 Nutritional value of egg proteins

Proteins are nitrogen-containing substances consisting of amino acids. They are the major structural roles in muscle and other tissues for the human body. The importance of proteins is also associated with their functions to serve as an energy source, catalyse and regulate metabolic reactions (e.g. enzymes and hormones), transport and store ions and minerals (e.g. transferring, haemoglobin and myoglobin) and enhance the immune systems (e.g. antibodies and antibiotics) (Bilsborough & Mann, 2006). The main dietary sources of proteins are meat, eggs, fish and milk (Bilsborough & Mann, 2006) (Table 2.3). Recently, the recommended dietary allowances (RDAs) of proteins (i.e. daily protein intake) have been adjusted considering some variation among individuals of the same age and gender (Table 2.4). The protein requirements for athletic populations have also been the subject of much scientific debate. Both strength/power and endurance athletes require a large consumption of protein than the general population (Berdanier, 2000; Bilsborough & Mann, 2006).

Table 2.3 The content of protein in major foods in the human diet.

Food	Protein (g)
Milk	8
Cheddar cheese	25.4
Egg	12.2
Apple	0.18
Fish, cod, poached	20.9
Beef, pot roast	25.9
Liver, pan fried	27.1

Source: Yildiz (2009)

Table 2.4 The recommended dietary allowances of proteins for healthy people.

	Age and category	Male proteins (g/per day)	Female proteins (g/per day)
Infant	To 5 months old	13	13
	5 months - 1 year	14	14
Children	1-3	16	16
	4-6	24	24
	7-10	28	28
	11-14	45	46
	15-18	59	44
Adult	19-24	58	46
	25-50	63	50
	51+	63	50
Pregnant	-	-	60
Athletes	General	64	52
Athletes	Strength trained athletes	112-144 (80 kg individual)	
	Endurance trained athletes	96-112 (80 kg individual)	
	gym-goers, active people and body builders	150 to 400 g	

Sources: Berdanier (2000), Bilsborough & Mann (2006), Phillips et al. (2007) and Yildiz (2009)

2.3 Digestibility and quality of egg proteins

Eggs provide a unique, well balanced source of nutrients for people of all ages. The efficiency of protein in eggs is comparable with milk, meat, poultry and fish (Stadelman & Cotterill, 1995). The efficiency of a protein is correlated with its digestibility and quality. The term 'protein digestibility' refers to the utilisation of proteins in the body which is important in evaluating the quality of proteins as a nutritional source of amino acids (Hoffman & Falvo, 2004). Thus, the amount of proteins required from the diet depends mainly on the digestibility of proteins and the availability of essential amino acids (Friedman, 1996).

Generally, the digestibility of vegetable proteins is lower than that of animal proteins (Young & Pellett, 1994) as shown in Table 2.5. Isotope techniques were used to evaluate the digestibility of egg white proteins in raw and cooked eggs (Evenepoel et al., 1998). Raw egg white protein is generally considered to be less digestible than heat-treated egg white protein, with 51.35% and 90.9%, respectively. The low protein digestibility of raw egg white may be related partly to ovomucoid, acting as a trypsin inhibitor, which is one of the minor egg white proteins present. The high digestibility of heated egg white protein is due to the structural changes of protein molecules induced by denaturation which facilitates the access of digestive enzymes to the peptide bonds (Evenepoel et al., 1998).

Furthermore, protein quality is determined by the availability of amino acids (Hoffman & Falvo, 2004). Generally, there are 20 amino acids which are classified as i) dietary essential, which cannot be synthesized in the human body, ii) dietary non-essential, which can be synthesized in the human body and iii) conditionally essential amino acids, such as cysteine and tyrosine, which become essential only under specific physiological conditions (Boland et al., 2013). Among the 20 amino acids, there are nine dietary essential amino acids, including histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Boland et al., 2013). These amino acids are essential for the human body growth and metabolism. The absence of any of these essential amino acids will compromise the ability of tissue to grow, repair or maintain (Bilsborough & Mann, 2006). Non-essential amino acids can be produced by the body, including glycine, arginine, alanine, cysteine, aspartic acid, glutamic acid, asparagine, serine, tyrosine and proline (Boland et al., 2012).

Table 2.5 Digestibility of food proteins from different sources.

Proteins sources (100g)	True digestibility (%)
Egg	97
Milk, Cheese	95
Fish, Meat	94
Maize	85
Whole wheat	86
Beans	78

Source: Young and Pellett (1994)

Essential amino acids have also been classified according to their chemical structure. These include aromatic amino acids, sulphur-containing amino acids and hydrophilic amino acids (Langel et al., 2011). A group of amino acids referred to as sulphur-containing amino acids includes cysteine and methionine. The body requires 25 mg of these amino acids per day. For some hydrophilic essential amino acids, such as lysine, histidine and threonine, the body requires 51 mg, 18 mg and 27 mg of these amino acids per day, respectively (Langel et al., 2011). Generally, all dietary animal protein sources are considered to be 'complete proteins' due to the presence of all of the essential amino acids. However, proteins from vegetable sources are incomplete and are lacking or very low in one or two essential amino acids such as lysine and methionine (Pellett, 1990; Friedman, 1996; Hoffman & Falvo, 2004; Yildiz, 2009). Soy protein is considered as one of the best plant proteins but it contains low proportion of the essential sulphur-containing amino acid (e.g. methionine) (Yildiz, 2009). In comparison, egg white protein is considered to have the best amino acid profiles for human nutrition (Tables 2.6 and 2.7).

Table 2.6 The content of essential amino acids in egg white, egg yolk and whole egg.

Amino acids (mg)	Egg white (100g)	Egg yolk (100g)	Whole egg (100g)
Histidine	-	-	-
Isoleucine	240	410	290
Leucine	560	870	660
Lysine	880	1,390	1,040
Methionine + cystine	660	1,170	820
Phenylalanine + tyrosine	670	660	640
Threonine	1,020	1,420	1,150
Tryptophan	470	850	590
Valine	170	240	190

Source: Huopalahti et al. (2007)

Table 2.7 Percentage of amino acids in egg white protein.

Amino acids	Egg white protein (w/w %)
Histidine	2.2
Methionine	3.6
Cystine	2.5
Tyrosine	2.7
Aspartic acid	8.9
Glutamic acid	13.5
Serine	7.3
Phenylalanine	6.0

Source: Yildiz (2009)

2.4 Microbial safety

Eggs can cause some food-borne illnesses due to the contamination and presence of pathogens in and on raw eggs, with *Salmonella* being the most common pathogen of concern. Microbial contamination of egg occurs from the surrounding environment or systematically from the infected chickens (Monfort et al., 2012). Environmental contamination can occur when infected chickens in commercial egg-laying chicken houses deposit faeces which come into contact with the shell of laid eggs. *Salmonella* can enter the egg white (albumen) through the small fissures or pores in the shell. Systemic contamination occurs when chickens consume faeces of infected chickens during feeding, so the infection occurs systemically in the chicken (Monfort et al., 2012). For instance, *Salmonella enteritidis* has the ability to enter the blood stream of the chicken and pass trans-ovarially into the interior of the egg, leading to yolk

contamination, which can easily transfer to the egg white.

Egg possesses two defensive mechanisms to ward off bacterial invasion structurally and chemically (Macherey et al., 2011). The structure of egg shell plays an important role in protecting the content of egg against microbial invasion as the main defense mechanism through three different structural components, namely, cuticle layer, shell and shell membrane. The cuticle layer covers the pore canals on the egg shell. Therefore, it prevents bacteria from penetrating through the pore canals into the interior of the egg. The other structural defense mean is the egg shell itself. The ability of egg shell to protect is affected by various factors such as the shell thickness and the condition of the shell. A thicker shell has a higher ability to resist microbial invasion. The egg shell membrane also plays a major part of the egg's defensive mechanism (Macherey et al., 2011).

On the other hand, the chemical defence mechanisms of eggs are mainly located in the egg white either through its compositions or its physical properties. Egg white contains several proteins that possess different chemical features. For example, ovomucoid is a trypsin inhibitor, lysozyme has bacteriolytic effect, ovoinhibitor inhibits bacterial and fungal serine proteases and ovomacroglobulin inhibits viral hemagglutination (Stadelman & Cotterill, 1995; Macherey et al., 2011). The physical property of egg white includes its viscosity which provides some protection from microbial invasion by preventing invading bacteria from moving freely through the egg to the yolk (Stadelman & Cotterill, 1995; Macherey et al., 2011).

2.5 Pasteurisation of egg

Many methods have been used to control the growth of *Salmonella* in liquid egg and egg products; one is heat pasteurisation (Meszaros et al., 2006). Traditional thermal treatments used in the U.S.A to pasteurise whole eggs use a temperature of 60°C maintained for less than 3.5 min. In Britain, this temperature is insufficient to ensure a satisfactory product, thus the requirement temperature is 64°C for 2.5 minutes (Stadelman & Cotterill, 1995; Unluturk et al., 2008; Monfort et al., 2012). Heating up the liquid whole egg ensures food safety by reducing the number of *Salmonella* serotypes from 5 to 9 Log_{10} CFU/ml (Monfort et al., 2012). However, some microorganisms showed resistance to the higher temperature and they can survive.

Examples are *Bacillus, Pseudomonas, Proteus*, *Listeria monocytogenes* and *Escherichia coli*.

In addition, even though the heat pasteurisation is efficient to control *Salmonella* growth, it was observed that this method may affect the functional properties of egg components (Stadelman & Cotterill, 1995; Badr, 2006; Unluturk et al., 2008). To overcome these limitations of traditional heat pasteurisation, alternative non-thermal methods have been introduced and classified as safe methods. Alternative pasteurisation methods include: i) pulsed electric field (PEF), UV radiation, ultrasonic treatment, ionizing radiation treatment and high hydrostatic pressure processing (HHP) for liquid egg products, ii) washing, rapid chilling for shell egg, and iii) heat-plus-vacuum process, heat-plus-hydrogen peroxide process and heat treatment of dried egg white (Stadelman & Cotterill, 1995; Meszaros et al., 2006; Monfort et al., 2012).

An example of these alternative pasteurisation methods used for shell eggs is irradiation treatment, which has the ability to destroy harmful pathogens along with extending the shelf life of foodstuffs (Badr, 2006). The Food and Drug Administration (FDA) of the U.S.A (2000) approved doses of ionizing radiation up to 3 kGy, which is sufficient to reduce the level of *Salmonella* in shell eggs to safe levels (Meszaros et al., 2006). In recent years, the use of HHP at refrigeration, ambient or moderate heating temperature has been applied in the food industry to eliminate the growth of microorganisms, thus improving microbiological safety and extending shelf life (San Martin et al., 2002; Monfort et al., 2012). For liquid whole eggs, the required pressure to reduce microorganisms such as *Escherichia coli* and *Salmonella enteritidis* is between 300 and 450 MPa for 5-15 min (Monfort et al., 2012). The main disadvantage of using the HHP process on egg products is because this process can cause protein denaturation, aggregation and coagulation at pressures over 300 MPa. Thus, for liquid whole eggs, changes in the physicochemical properties of egg white proteins induced by HHP must be considered (Monfort et al., 2012).

A combination of HHP treatment and temperature has shown to have the ability to inactivate microorganisms. Generally, high pressure processing at room temperature destroys harmful microorganisms whereas it was found to be more effective when it is applied at higher temperatures (Yuste et al., 2001). Specifically, temperatures in the range of 50 to 70°C significantly improve the effect of pressure-induced microbial inactivation. A combination of high pressure with other physical treatments, such as

high or low temperatures, ultraviolet and ionizing radiation, pulsed electric field and ultrasound, or chemical treatments, such as the addition of bacteriocins (nisin and pediocin), lysozyme and other preservatives, acidificants and antioxidants, can increase the harmful effect on microorganisms (Yuste et al., 2001).

2.5.1 Pasteurisation methods of egg white

Generally, to overcome the contamination of egg white, pasteurisation has been widely used. According to Stadelman and Cotterill (1995), there are several methods for egg white pasteurisation. For most efficient treatment, pH values, thermal temperature and time should be considered. The lactic acid-aluminium sulphate process depends on using a temperature range of 60 to 62°C for 3.5 to 4 min at near natural pH of egg white (around pH 9) (Stadelman & Cotterill, 1995). A heat-plus-vacuum process involves pulling a vacuum on the egg white for 17 to 20 min. Then, the egg white is heated to 57°C for 3.5 min. Similarly, the heat-plus-hydrogen peroxide process also has a significant effect on the microbial inhibition. In this process, hydrogen peroxide, which is known as a bactericidal agent, is added to egg white solution and heated to 52°C or 54°C for 3.5 min to produce a safe pasteurised egg white which also has good foaming ability (Stadelman & Cotterill, 1995). In the case of dried egg white, the temperatures of 50, 60 or 70°C are applied as heat treatment to eliminate *Salmonellae* without affecting the functional properties of egg white (Stadelman & Cotterill, 1995).

Another non-thermal process for pasteurisation of egg white is ultraviolet (UV) light. The UV system inactivates both gram-positive and gram-negative bacteria at room temperature (Geveke, 2008). The effect of UV on the inactivation of *Escherichia coli* O157:H7 in egg white was widely investigated. The number of *Escherichia coli* was decreased by 1.63±0.10 log units at a temperature of 30°C. However, *Listeria innocua* was not affected by the UV treatment. *Listeria innocua* was decreased by 2.5 log units (Geveke, 2008). The UV pasteurisation of egg white may be an alternative method to thermal processing and other non-thermal processing. UV treatment uses temperatures between 30 and 50°C, which are below the coagulation temperature of egg white proteins. The other advantage of using this process is that it's highly accepted in the food industry (Geveke, 2008).

2.6 Structure and composition of egg

The egg is composed of shell, shell membranes, air cell, pore canals, chalazae, egg white (albumen) and egg yolk (Figure 2.1).

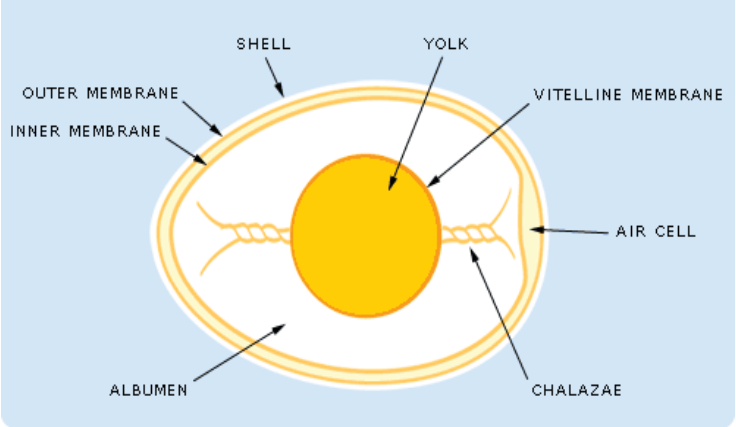

Figure 2.1 Structure of egg.
Source: from Hui (2007)

2.6.1 Egg shell

The egg shell comprises 9 to 12 % of the total egg weight. It is made up of calcium carbonate (94%), proteins (4%), magnesium carbonate (1%) and calcium phosphate (1%) (Stadelman & Cotterill, 1995). The carbonates of egg shell are embedded and sealed by a layer of fibrous proteins which consist mainly of keratin. There are also a number of pores (7,000-17,000 pore canals) in the egg shell for gas exchange. These pores permit the exit of carbon dioxide and moisture from the egg, and they also permit bacterial penetration (Stadelman & Cotterill, 1995; Hui, 2007).

The other components of the egg shell are the outer and the inner shell membranes which are composed of protein-polysaccharide fibres and are semi-permeable. These shell membranes are located beneath the shell and separate the egg content (egg white and yolk) from the egg shell. The inner membrane is thinner than the outer membrane and is made of keratin and collagen-like proteins, also working as a defence means against microorganisms (Hui, 2007). These two membranes consist of different types of proteins that have a high content of arginine, glutamic acid, methionine, histidine, cystine and proline, while the level of glycine is low (Stadelman & Cotterill, 1995).

Additionally an air cell is located at the blunt end of the egg. The size of air cell is small immediately after laying but it increases during cooling after laying due to contraction of the contents of egg and also during storage by losing some moisture and carbon dioxide (Hui, 2007). The other minor component of the egg is chalazae, which is a cord-like strand connected to the thick egg white and its role is holding the egg yolk in the centre of the egg (Hui, 2007).

2.6.2 Egg yolk

The yolk is located inside the egg white and is supported by the chalazae (Figure 2.1) and makes up 36% of the total egg weight (Huopalahti et al., 2007). It consists of latebra, germinal disk and two concentric layers of light and dark. The egg yolk composed of all these components are covered by the yolk membrane called vitelline. The vitelline membrane is also surrounded by the chalaziferous layer of albumen and is made up of two layers (Stadelman & Cotterill, 1995).

Regarding the composition of egg yolk, it comprises water, lipids, and proteins. Lipids make up about 65% of the yolk dry matter (Huopalahti et al., 2007). The major lipid components in egg yolk are triglycerides (66.2%), phospholipids (29.6%) and cholesterol (4.2%). The types of fatty acids present in egg yolk are saturated (30-35%) monounsaturated (40-45%) and polyunsaturated fatty acids (20-25%) (Huopalahti et al., 2007). In egg yolk, proteins exist in high density lipoproteins (HDL, nearly 16%), and low density lipoproteins (LDL, about 68%), 10% globular proteins (livetins), 4% phosphoprotein (phosvitin) and 2% minor proteins.

The yolk can be separated into granules and plasma using high speed centrifugation. The granules are an insoluble fraction that makes up about 19-23% of the total solids in the yolk (Table 2.8). However, the plasma accounts for the major portion of the yolk as a soluble fraction, about 78% of the total liquid yolk (Table 2.8) (Stadelman & Cotterill, 1995; Bergquist, 2000). It is important to notice that the egg yolk colour is due to the presence of fat-soluble carotenoids (xanthophylls) in the lipid portion of lipoprotein which are yellow pigments (Stadelman & Cotterill, 1995).

Table 2.8 Compositions of two fractions (granules and plasma) of egg yolk.

Components	Yolk (dry matter)	Lipids	Proteins
	%	%	%
Yolk (total)	100	64	32
Plasma	78	73	25
LDL	66	88	10
Livetins	10	-	96
Granules	22	31	64
HDL	16	25	75
Phosvitin	4	-	95
LDL	2	88	10

Source: Huopalahti et al. (2007)

2.6.3 Egg white

The white fraction of hen egg is called egg white (albumen). It is an aqueous solution that has a gel-like structure. The gel structure of egg white is due to the high content of ovomucin which is an egg white protein with fibrous conformational structure. This protein is strongly responsible for the mucous nature of the egg white (Drakos & Kiosseoglou, 2006). The egg white represents about 63% of the whole egg, and consists of two layers, thick (outer layer) and thin albumen layers (the inner layer). The outer thick albumen is surrounded by a membrane called vitelline (yolk membrane) as described earlier. Two strands of chalazaes, which anchor the yolk in place in the centre of egg white, originate from the thick albumen (Stadelman & Cotteill, 1995; Hui, 2007). The main difference between these albumens is their viscosity, which is much higher in the thick albumen, due to its high content of ovomucin protein (Hui, 2007). The other difference between the two albumen layers is their protein composition. The protein composition of egg white is explained in more detail in the following section.

2.7 Physicochemical properties of egg white proteins

As shown in Table 2.1, egg white contains about 9.7-10.6% protein. Most of egg white proteins are classified as globular proteins. Five major egg white proteins are ovalbumin, conalbumin, ovomucoid, ovomucin and lysozyme (Alleoni, 2006). The other minor egg white proteins are ovoglobulins G2 and G3, avidin, ovoinhibitor,

cystatin, ovoglycoprotein, ovomaroglobulin and ovoflavoprotein (Alleoni, 2006). The major differences between egg white proteins are summarised in Table 2.9.

Table 2.9 Physicochemical properties of egg white proteins.

	Proteins	Protein %	MW[a] (kDa)	pI	T_d (^oC)[b]	Characteristics
Major proteins	Ovalbumin	54	44.5	4.5	71.5-84	Glycophosphoprotein, gelling and foaming properties
	Conalbumin (ovotranferrin)	13	77.7-80	6.0-6.6	57.3	Binds to metal ions and antimicrobial protein
	Ovomucoid	11	28.0	3.9-4.3	77	Trypsin inhibitor, heat stable in acidic conditions, allergic reactions to egg white
	Ovomucin	3.5	110	4.0-4.5	-	Glycosulphiprotein, heat resistance, gel-like nature, good foaming agent
	Lysozyme	3.4	14.3-14.6	10.7	81.5	Antimicrobial protein
Minor protein	Ovoglobulins	8	30-49	5.5-5.8	92.5	Foaming agent
	Avidin	0.5	68.3	9.5-10	85	Biotin binding protein, antibacterial function
	Ovoinhibitor	1.5	50	5.1-5.2	69-72	Serine protease inhibitor
	Cystatin	0.05	12	5.1	-	Protease inhibitor
	Ovoglycoprotein	1	24- 24.4	3.9	69-72	
	Ovomacroglobulin	0.5	760-900	4.5-4.7	69-72	
	Ovoflavoprotein (flavoprotein)	0.8	32-35	4.0-4.1	69-72	Vitamin chelating protein, e.g. riboflavin (vitamin B2)

[a] Molecular weight
[b] Denaturation temperature
Sources: Stevens (1991), Stadelman & Cotterill (1995), Campbell et al. (2003) & Alleoni (2006)

2.7.1 Major egg white proteins

2.7.1.1 Ovalbumin

Ovalbumin is the major protein in egg white and it comprises 54% of the total egg white proteins (Table 2.9). Ovalbumin is a globular phosphoglycoprotein with a molecular weight of 44.5 kDa and an isoelectric point of 4.5 (Hui, 2007). Of its complete sequence

of 385 amino acids, half are hydrophobic (Alleoni, 2006; Hui, 2007). Ovalbumin has six cysteine residues and two of them are involved in the formation of a disulphide bond. This means that ovalbumin contains four free sulfhydryl groups (Huopalahti et al., 2007). Ovalbumin is easily denatured when exposed to an interface of air and water or oil and water and it readily coagulates when heated (Hui, 2007; Hoppe, 2010). The denaturation temperature of ovalbumin is between 71.5 and 84°C (Stadelman & Cotterill, 1995; Campbell et al., 2003).

2.7.1.2 Conalbumin

Conalbumin also referred to as ovotransferin is a glycoprotein, and it accounts for 13% of the egg white protein content (Table 2.9). It has a molecular weight of 77.7-80.0 kDa with an isoelectric point between pH 6.0 and 6.6 (Alleoni, 2006). It has 15 disulphide bonds of which six are located in the N-terminal domain and nine in the C-terminal domain of conalbumin, thus providing the protein with high stability (Stevens, 1991). However, it is more easily denatured by heat in its native biological state than ovalbumin. Its denaturation temperature is 57.3°C (Campbell et al. 2003). Conalbumin is also considered as an antimicrobial protein. It has the ability to bind metal ions (e.g. iron and copper) and it is able to bind to two atoms of Fe^{3+}. Thus, the function of conalbumin is to transport irons. However, this binding is pH dependent (Stevens, 1991). When it binds to metal ions, it shows high resistance to denaturation by heat, pressure, proteolytic enzymes and denaturant agents (Hui, 2007).

2.7.1.3 Ovomucoid

Ovomucoid is a glycoprotein and makes up about 11% of the total egg white proteins. It consists of 185 amino acids and has a molecular weight of 28 kDa. Its isoelectric point is between pH 3.9 and 4.3 (Stevens, 1991; Campbell et al., 2003). Ovomucoild is highly stable to heat denaturation in acid solutions, compared to other egg white proteins, due to its high level of disulphide linkages (9 disulphide bonds). For example, it can be kept at 100°C under acidic conditions for a long period without any observed changes in its physical or chemical properties (Hui, 2007). Ovomucoid may play an important role in the pathogenesis of allergic reactions to egg white (Alleoni, 2006).

2.7.1.4 Ovomucin

Ovomucin is a glycosulphiprotein with a large molecular weight of around 110 kDa (Table 2.9). It makes up 3.5% of the egg white protein and it is also present in the chalazaes and in the outer layer of the vitelline membrane. It contains sulphate esters and a large number of cystine (i.e. disulphide bonds), and has a significant content of sialic acid about 50% which is responsible for its low isoelectric point (Campbell et al., 2003; Alleoni, 2006; Hiidenhovi, 2007). The isoelectric point of ovomucin ranges from pH 4.0 to 4.5 (Campbell et al., 2003). According to Stadelman and Cotterill (1995), ovomucin is resistant to heat alterations and can be heated at 90° for 2 hr at pH 7.1 and at pH 9.4 without any changes in viscosity. There are two different forms of ovomucin; a soluble form present in both thick and thin albumen, and an insoluble ovomucin which is located only in the thick albumen, which is the major contributor to the gel-like nature of egg white (Stadelman & Cotterill, 1995; Alleoni, 2006).

2.7.1.5 Lysozyme

Lysozyme is a glycoprotein, representing about 3.4% of the egg white's proteins (Table 2.9). It contains a single polypeptide chain with 129 residues of amino acids linked by four disulphide bonds. Its molecular weight ranges from 14.3 to 14.6 kDa, and its isoelectric point is pH 10.7 (Alleoni, 2006). It is commonly used as a naturally occurring bacteriolytic enzyme, being used as food preservative (Hui, 2007). Lysozyme is more heat resistance then other egg white proteins, but denatured at 81.5°C (Campbell et al., 2003).

2.7.2 Minor albumen proteins

Ovoglobulin is found in egg white as two different chemical forms. Initially, ovoglobulin had been classified into three types, ovoglobulin G1, G2 and G3, but later, ovoglobulin G1 was identified as a lysozyme. Together ovoglobulin G2 and G3 make up about 0.4% of the total egg white proteins (Table 2.9). Their molecular weights range from 30 and 49 kDa and the isoelectric points are pH 5.5 and 5.8, respectively, for G2 and G3 (Alleoni, 2006). Two forms have been separated by starch-gel electrophoresis or ion-exchange chromatography to study the effect of temperature (Stadelman & Cotterill,

1995). It was demonstrated that G3 was more resistant to heat denaturation than G2 (92.5°C).

Avidin represents only about 0.5% of egg white proteins, with a molecular weight of 68.3 kDa and isoelectric point of pH 9.5-10 (Campbell et al., 2003) (Table 2.9). Avidin is a glycoprotein consisting of four subunits, each having a single vitamin binding site. It is a vitamin binding protein, especially effective with vitamin B7 (biotin), also known as vitamin H. In total it can bind to four biotin molecules (Stevens, 1991). The avidin-biotin complex is resistant to denaturation compared to avidin in its biological state (native protein). It has been illustrated that avidin denatures at 85°C, but the complex is stable even when it is heated at 100°C (Stadelman & Cotterill, 1995).

Ovoinhibitor is a serine protease inhibitor, making up 1.5% of the egg white proteins. It has a large molecular weight of 50 kDa and an isoelectric point of pH 5.1-5.2 (Table 2.9). The denaturation temperature is reported to be between 69 and 72°C (Campbell et al., 2003). It is able to inhibit trypsin, chymotrypsin and fungal and bacterial proteases.

Cystatin is found in egg white in small quantities (0.05%) with a molecular weight of 12.000Da and isoelectric point of pH 5.1 (Table 2.9). It inhibits a number of cysteine proteinases such as ficin, papain, cathepsin B and dipeptidyl peptidase (Stevens, 1991). There are two forms of cystatin, referred to as A and B, having ionic strength values of 6.5 and 5.6, respectively. They are immunologically identical and do not contain carbohydrate (Stevens, 1991).

Ovoglycoprotein is a glycoprotein, which contributes to 1% of the egg white proteins. It has a molecular weight of nearly 24 kDa and an isoelectric point of pH 3.9 (Table 2.9).

The other glycoprotein present in egg white is **ovomacroglobulin**, making up 0.5% of the total egg white proteins. It is composed of four subunits attached by disulphide bonds. Its molecular weight is between 760 and 900 kDa and its isoelectric point is pH 4.5-4.7 (Table 2.9) (Stadelman & Cotterill, 1995; Campbell et al., 2003).

Ovoflavoprotein is another minor egg white protein that has the ability to bind to vitamin B2 (riboflavin) and represents 0.8% of the total egg white proteins. The isoelectric point of this protein is low at pH 4.0-4.1 and this is related to its high content of phosphate groups, acidic amino acids and sialic acid. It has a molecular weight

between 32 and 35 kDa (Table 2.9) (Stevens, 1991; Campbell et al., 2003). It contains nine disulphide bridges which increase its resistance to heat, which plays a key role in stabilizing the structure of many egg white proteins (Langel et al., 2011). The denaturation temperatures for ovoflavoprotein, ovomacroglobulin and ovoglycoprotein are between 69°C and 72°C (Stadelman & Cotterill, 1995).

2.8 Applications of egg components

2.8.1 Food and non-food applications of eggs

Eggs have been used widely in food and non-food applications. Examples for food applications are the use of egg products such as liquid, frozen and dried eggs as ingredients in a variety of different formulated food products, including cake mixes, salad dressing, noodles, mayonnaise, confectionary products, hard cooked egg rolls, prepared omelette, egg crust pizza, fruit juice egg drinks and liquid low fat egg products (McKee, 2000). Egg white proteins are also used in industrial applications. For instance, lysozyme is added in the production of oral health care products, including toothpaste, mouthwash and chewing gum, to protect against periodontitis-causing bacteria, and to prevent infections in the oral mucosa (Kovacs-Noian et al., 2005).

The majority of non-food uses of eggs are as pet foods for dogs and cats, shampoos and fertilizers (Stadelman & Cotterill, 1995). In addition, proteins from eggs are considered to be the most important and commercially available film-forming agents (Huopalahti et al., 2007). Egg white is the most significant source of proteins for the formation of films and coatings. This is due to the high content of sulphydryl (SH) amino acids in some egg white proteins such as ovalbumin, and also due to the presence of many disulphide (S-S) bonds in some egg white proteins, such as ovotransferrin, ovomucoid and lysozyme (Huopalahti et al., 2007).

Egg white proteins have also been used widely in biomedicine and in industrial applications (Kovacs-Nolan et al., 2005; Huopalahti et al., 2007). In biological matters, eggs play an important role in the production of bacterial culture media (Stadelman & Cotterill, 1995). Egg yolk components have been recognized as valuable bio-chemicals for the vaccine production in the pharmaceutical industry (Huopalahti et al., 2007).

2.8.2 Food safety applications of egg white proteins

One example of these applications is the use of lysozyme and ovalbumin in food safety. Lysozyme has the ability to lyse the cell wall of certain gram-positive bacteria such as *Staphylococcus aureus* (Mine et al., 2004). In addition, lysozyme performs several biological functions including an antiviral effect on certain viruses, preventing and controlling several viral skin infections, including herpes simplex and chicken pox (Mine et al., 2004; Kovacs-Nolan et al., 2005). Moreover, some peptides produced by the digestion of ovalbumin were found to be active against *Bacillus subtilus, Escherichia coli*, *Bordetella bronchiseptica*, and *Serratia marcescens* (Kovacs-Nolan et al., 2005).

2.9 Functional properties of egg white

2.9.1 Coagulation (gelation) property

Egg white proteins have an important functional property which is heat-induced gelation. A protein gel is defined as a three-dimensional cross-linked network of protein molecules entrapping a large amount of water in the gel network (Hui, 2007). Its water content can be very high up to 95-98%. There are many food applications of protein gels, such as custards, yogurts, cheeses and frankfurters. Gelation may be induced by a variety of different methods, such as temperature, ionic strength and pH or by adding denaturing or cross-linking agents (McClements, 1999). Egg white gel is normally produced by heat treatment. The gel formed is irreversible, which means when egg white gel is cooled to room temperature, it remains as a white gel (McClements, 1999).

Protein gel networks are classified into two types, stranded and particulate, based on their overall appearance and microstructure. Generally, these networks can be differentiated because stranded gels formed from one of the milk proteins such as β-lactoglobulin are more translucent, hold more water and more elastic, while particulate gels formed from egg white proteins are opaque and have lower water-holding ability (Li et al., 1999). An intermediate structure between stranded and particulate networks is known as a mixed network (Li et al., 1999).

Egg white is considered as a good gelling agent in many food products. Among different egg white proteins, the major proteins involved in heat-induced gelation are

ovalbumin, conalbumin, ovomucoid and lysozyme (Dickinson, 2003). Egg white is preferred over egg yolk for its gelling properties. The reason for that is related to a trace amount of lipids in egg white which makes it more stable (Hui, 2007). Egg white protein solution has a low gelation temperature, between 56 and 70°C, compared to whey protein solution at 80°C (Li et al., 1999). Thermal stability of egg white proteins differ due to several features, conalbumin is less stable against heat treatment due to the absence of disulphide bonds, whereas lysozyme has the high thermal stability because it has four disulphide cross-linkages (Dickinson, 2003).

2.9.1.1 Gel network formation

The gelation process of egg white is normally induced by thermal treatment to a critical temperature of egg white, resulting in several rheological changes such as an increase in the viscosity of egg white solution and then the formation of gel (McClements, 1999). The gelation temperature ranges for egg white proteins between 60 and 70°C, depending on the protein type because each protein of egg white has different denaturation temperatures (Table 2.9) (Alleoni, 2006). The formation of egg white protein gels by thermal treatment involves a two-step process; first denaturation of proteins and then aggregation of denatured proteins (Croguennec et al., 2002). First, heat treatment at high temperatures (e.g. 85-90°C) induces the denaturation of proteins, causing the destabilisation of proteins and altering the conformational structure of protein molecules, which lead to the exposure of reactive (free and disulphide groups) and hydrophobic amino acids from the interior of protein molecules. Secondly, denatured proteins start to interact and aggregate gradually or rapidly, depending on type of food proteins, through intermolecular and sulphydryl-disulphide interchanges and hydrophobic interactions to foam gels. A rapid aggregation of protein molecules upon denaturation results in the formation of a type of particulate gels. The particulate gels tend be opaque in appearance and have less water holding capacity than the stranded gels (fibrous gels) which normally form during cooling after the heat treatment (i.e. the speed of protein aggregation is slower than the speed of denaturation). As a result of low water holding capacity, the particulate gels with disordered, random, irregular morphological structures are prone to syneresis, due to large pore sizes formed between these gels (McClements, 1999; Croguennec et al., 2002; Alleoni, 2006). The

formation of a particulate gel is determined based on the properties of proteins in terms of their physicochemical properties which in turn also determine a difference in the speed (rate) between denaturation and aggregation of proteins. Egg white tends to form a particulate gel as the gelation (aggregation) of egg white proteins occurs rapidly upon heat treatment (i.e. denaturation).

2.9.2 Emulsification property

In general, there are two types of food dispersions; i) dispersions where a liquid phase is dispersed in another liquid phase, such as oil-in-water or water-in-oil emulsions and ii) dispersions where air (gas) bubbles are dispersed in an aqueous medium such as foam (Damodaran, 2005). In oil-in-water (O/W) emulsions, oil is dispersed as small droplets in an aqueous solution. The aqueous phase is referred to as the continuous or external phase while the dispersed oil is called the internal or discontinuous phase (Damodaran, 2005; Hui, 2007). In the case of water-in-oil (W/O) emulsions, oil is the continuous phase and water is the dispersed phase. In both O/W and W/O emulsions, the dispersed phase is distributed in the form of micron and submicron-size droplets and the dispersed droplets are covered and stabilised by a layer of surface active agents (e.g. protein or carbohydrate emulsifiers and small molecular surfactants). This kind of emulsion system is also often called a colloidal dispersion (Damodaran, 2005). Egg yolk and milk are examples of natural emulsions. Other examples are manufactured and processed foods, such as ice cream, chocolate and mayonnaise (Stadelman & Cotterill, 1995; Hui, 2007). Emulsions are also important in many bakery products where fats and oils are added (Bergquist, 2000).

The emulsifying ability of eggs has been attributed to the constituent of phospholipids in egg yolk but other components such as egg white proteins also have emulsifying properties. It has been indicated that when lipids, including phospholipids, are extracted from whole egg, the remaining liquid portion (serum) containing egg white proteins also has good emulsifying properties (Bergquist, 2000). The emulsion properties of egg white has been improved by using several methods, such as dry heating which leads to partial denaturation of proteins and Maillard reaction which forms protein-sugar or polysaccharide conjugates (Hui, 2007). Among many different chemical and enzymatic modifications of egg white proteins to improve their functionality, the Maillard reaction

is one of the most promising methods for food applications (Kato et al., 1993). The effect of the Maillard reaction on the emulsifying ability of egg white was extensively studied (Kato et al., 1993; Campbell et al., 2003; Hui, 2007). For example, it has been reported that the emulsifying properties of ovalbumin, which accounts for about 54% of the egg white proteins, was improved by conjugation with glucuronic acid at 50°C, through the Maillard reaction (Campbell et al., 2003). The enhancement of emulsifying properties of ovalbumin was through an induction of structural modification. The structural modification includes a conversion of the cationic amino groups in ovalbumin to anionic residues, thus improving their functional properties (Campbell et al., 2003).

The effect of the Maillard reaction on the emulsifying ability of egg white was also examined by conjugating egg white proteins with polysaccharides, such as guar gum (galactomannan), followed by heating the conjugates formed (Campbell et al., 2003). It was shown that the treated egg white had a better emulsifying activity and emulsion stability than untreated egg white (without galactomannan) samples. The galactomannan conjugated egg white proteins also exhibited a better emulsifying activity compared to other commercial emulsifiers (Kato et al., 1993; Campbell et al., 2003). This is due to the formation of a covalent cross-linkage between amino groups in proteins and the reducing-end carbonyl group in polysaccharides. Therefore, proteins were converted into stable and soluble forms by binding with polysaccharides (Kato et al., 1993). It was concluded that the attachment of polysaccharides to the protein chain was essential for the enhancement of emulsifying properties of the conjugates. This is because the Maillard conjugates could “mimic” the character of egg-yolk lipoproteins, thus enhancing the emulsion properties of egg white proteins (Campbell et al., 2003).

2.9.2.1 Emulsion stability

During emulsification, the interfacial area between the continuous and dispersed phase increases, leading to an overall increase in the free energy of the system compared with its free energy before dispersion. This increase makes the emulsion system thermodynamically unstable. Damodaran (2005) defined the emulsion stability as the amount of oil that is separated from an emulsion under specific conditions of time, temperature and gravity. In order to stabilise the emulsion, the surface active agents in egg yolk or egg white (proteins), which are called emulsifiers, form a film around the

oil droplets and prevent their coalescence. The non-polar segments of emulsifiers are oriented to the oil phase while the polar segments are projected into the aqueous phase. As a result, the two phases are separated, minimising the interfacial tension and the free energy, and it becomes stable (Stadelman & Cotterill, 1995; Damodaran, 2005; Hui, 2007). Generally, an emulsion undergoes various processes causing destabilisation, including creaming, flocculation, coalescence and oiling-off. These processes may occur singly or in combination. The rate of these processes is affected by several physical factors (Damodaran, 2005; Hui, 2007).

Creaming refers to the tendency of oil droplets in an emulsion to rise to the top against gravity (Damodaran, 2005). The rate of creaming can be decreased by i) decreasing the particle size of emulsion droplets, thus reducing difference in density between the oil and water phases and ii) raising the viscosity of the continuous phase, for example by adding thickening agents such as hydrocolloids. Flocculation refers to a loose association of oil droplets. This is controlled by a net force of interaction among oil droplets, which depends on the magnitude of the force between the droplets and the oil volume fraction (Damodaran, 2005). Coalescence can be defined as a combination of two or more oil droplets to form a single large droplet. It involves rupture of the interfacial layer that covers dispersed oil droplets.

2.9.3 Foaming ability

In foam, the dispersed gaseous phase is surrounded by the liquid or solid continuous phase. In food systems, the continuous phase is mainly an aqueous phase, but this phase is converted to a solid-like phase by cooking (heating) or freezing (Murray & Ettelaie, 2004). Rodríguez Patino et al. (1995) defined foams as "thermodynamically unstable colloidal systems in which a gas is transiently maintained as a distinct dispersed phase in a liquid matrix". Examples of food foams include those that are prepared from protein solutions, such as angel food cake, confections and whipped toppings (Hui, 2007). Food foams are composed of gas (air), liquid (water) and surface active agents (proteins). Proteins display high viscosity, low density, high surface area and high surface energy. For the most favourable foam, proteins should be soluble in the liquid phase and be able to migrate and orientate rapidly to form an interfacial film around gas bubbles introduced into a system (Kinsella, 1981; Drakos & Kiosseogiou, 2006; Hui,

2007).

Foams have been classified into two types, spherical foams and polyhedral foams, depending on the amount of gas incorporated and the resulting bubbles shape. Spherical foam is formed when a low amount of gas has been induced to form foam, generating small bubbles. These bubbles have a spherical shape and are associated with high surface tension. An example of this type of foam is ice cream foam, which contains a certain amount of gas (e.g. 50%), depending on the extent of air injection applied, with the diameter of air bubbles between 20 and 60 μm. The other type is polyhedral foam, in which bubbles have a larger diameter and exhibit a polyhedral shape. In this type, the amount of gas is relatively large and the surface tension is low. For instance, beer foam has gas volume and bubble diameter of 75% and 500 μm, respectively (Eisner et al., 2007; Saint-James et al., 2012).

2.9.3.1 Mechanisms of foam formation

The formation of protein foam involves several principles and structural modifications of protein molecules as follows: i) the adsorption of protein at the air-water interface, ii) the unfolding and reorientation (rearrangement) of adsorbed proteins at the interface which directs the hydrophobic segments to water and the hydrophilic segments to the air bubbles and iii) the interaction of adsorbed proteins with the neighbouring protein molecules to form a dense cohesive film around the surface of foams by hydrogen bonding, electrostatic and hydrophobic interactions (Kinsella, 1981; Murray, 2007). Molecular interactions of proteins at the air-water interface depend on the properties of proteins and the dominant conditions of the solution, which in turn determine foam formation and stabilisation (Alleoni, 2006; Hui, 2007). This implies that during the foam formation, proteins are subjected to interfacial denaturation. However, it is important that surface-induced denaturation occurs to a limited extent in order to form an interfacial film with cohesiveness (viscosity), elasticity and rigidity (strength) since excessive denaturation leads to protein aggregation and coagulation, thus eventually destabilising the foam (Kinsella, 1981).

The ideal types of proteins suitable to form and stabilise foams have the chemical properties, such as a low molecular weight, high surface hydrophobicity, good solubility

and easy denaturation (Belitz et al., 2004). The protein adsorption at the air–water interface is promoted by the presence of some hydrophobic patches of non-polar amino acids on the surface of protein molecules. In most globular proteins, non-polar hydrophobic regions are buried in the interior of protein molecules and shielded from the aqueous phase by hydrophilic amino acids. Some factors which cause an exposure of hydrophobic regions from their interiors can increase the surface activity of proteins (Murray, 2007).

In the formation of foams, an energy-induced process is applied to generate air bubbles in the aqueous solution of proteins during which the protein molecules diffuse and adsorb to the air-water interface. If the protein molecules carry higher net charge and the solution has lower ionic strength, the electrostatic repulsive force between the adsorbed proteins is increased. As a consequence, the system energy increases, resulting in an increase in the surface pressure as such the adsorption of proteins is increased, thereby enhancing foamability (Foegeding et al., 2006; Hui, 2007; Wierenga & Gruppen, 2010). The other effect of higher electrostatic repulsion is on the shear viscosity of the solution which induces a larger average distance between the adsorbed proteins, leading to a lower shear viscosity (Wierenga & Gruppen, 2010).

2.10 Egg white foam

Egg white produces good protein foams due to several of its protein properties suitable for foaming, including the ability of egg white protein to rapidly adsorb on the air-water interface during whipping and the ability to form a cohesive viscoelastic film by intermolecular interactions between proteins (Lomakina & Mikova, 2006). The foaming ability of egg white comes from the structure and properties of its protein constituents. Egg white contains some globular proteins containing free sulphydryl groups and disulphide linkages and they can interact with each other via hydrophobic interactions and intermolecular sulphydryl-disulphide exchange reactions when they are denatured (Drakos & Kiosseogiou, 2006).

The major components of egg white proteins that determine the foaming ability and properties are globulins, lysozyme, ovomucoid and ovomucin (Damodaran et al., 1998). Globulins are the most surface active egg white proteins which contribute to the foaming ability (Lau & Dickinson, 2004). The significance of globulins is also related

to its high viscosity which delays liquid drainage from air bubbles. The excellent foaming properties of egg white are also attributable to the positively charged lysozyme. It plays a vital role in the formation and stability of egg white foams. During foaming, both the positively charged lysozyme and other negatively charged egg white proteins (mainly ovomucin) migrate to the air-water interface. At the interface, the positively charged lysozyme interacts electrostatically with the other negatively charged proteins, thus reducing electrostatic repulsive interactions at the protein film effectively and stabilising the foam (Damodaran et al., 1998; Lau & Dickinson, 2004; Hui, 2007).

Ovomucoid has the ability to retard liquid drainage with its high viscosity (Lau & Dickinson, 2004). Ovomucin is easily denatured, which can lead to the formation of a stable film around air bubbles in the foam (Belitz et al., 2004; Macherey et al., 2011). Over-whipping of egg white however causes over-solubilisation of ovomucin, lowering the elasticity of bubbles and facilitating the bubbles rupture (Macherey et al., 2011).

2.10.1 Foam structure

The foam consists of a lamella which is the wall of the air bubble. The lamella is thick and full of large amounts of fluid (Alleoni, 2006). Air bubbles are surrounded by a plateau border (Figure 2.2) (Muthukumaran et al., 2008). Air bubbles have a spherical shape with high internal pressure (Alleoni, 2006). The main function of proteins present in the lamella is to stabilise the air bubbles to prevent the coalescence phenomenon (Hui, 2007).

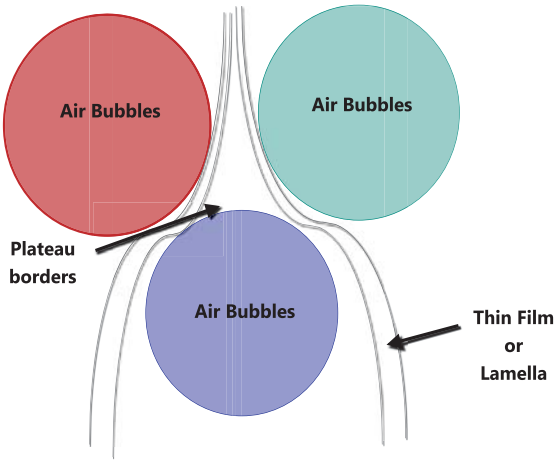

Figure 2.2 Foam structure,
Modified from Muthukumaran et al. (2008)

2.10.2 Foam production methods

There are several methods that can be used for foam formation, including sparging, whipping and shaking methods. The sparging method involves the injection of gas that can be either nitrogen or carbon dioxide (Hui, 2007). The nitrogen sparging is performed by using a foaming device which is made by fusing a fritted glass disk with a graduated glass cylinder, and the nitrogen gas is sparged into the column. In this way bubbles are directly formed (Wang & Wang, 2009). If carbon dioxide is used, it has to be dissolved in a liquid under pressure and then the pressure is released to form gas bubbles (Wang & Wang, 2009). The whipping method uses mechanical stress and shear forces to generate foams. This is the most frequently used method to generate foam from egg white protein solutions by using a blender or mix beater (Baniel et al., 1997; Wang & Wang, 2009). The other method used to generate foam is shaking which can be achieved by placing the egg white solution in a cylinder and then shaking (Chang et al., 1999).

Almost all researchers use a combination of foam generation methods to study foam characteristics. The main methods used were the whipping and sparging methods. These methods are suitable for measuring foam properties such as foamability and liquid drainage, however, the whipping method is preferred. Wang and Wang (2009) have evaluated the foaming properties of egg white liquid samples by using two methods. They concluded that the foaming properties including foam stability and foamability created by two methods were similar. However, some major differences regarding foamability and stability between samples were better observed by using a whipping method, but such differences were not revealed by the sparging method. They indicated that whipping is a better method in evaluating foaming in a variety of samples.

Baniel et al. (1997) also used egg white powder solution (> 90% protein) to compare their foam properties prepared using the whipping and sparging methods. The results showed that (i) the foam properties, including foamability, liquid drainage and foam density, produced by the two methods were similar with little difference, (ii) the foam density of egg white powder foams was higher when prepared using the whipping method than the sparging method, (iii) foam density was influenced by protein concentration only with the whipping method while protein concentration had no influence when the sparging method was used. Baniel et al. (1997) concluded that the

whipping method was more suitable for showing a difference between samples than the sparging method.

2.10.3 Foam characterization

Experimental techniques for investigating foam properties fall broadly into three categories; i) food quality measurements, which include rheology and texture, ii) foam properties including foamability and foam stability and iii) dispersed phase characteristics, including air content and bubble-size distribution (Lau & Dickinson, 2004). Table 2.10 shows some characterization methods used to analyse foam bubble and rheological properties of foam. The methods used for foam characterization that have been applied to study the foam rheological properties are the measurements of surface tension and density. Surface tension can be measured with a K12 tensiometer which has an automated contact angle geometer connected with a digital camera to capture the image of drop. Automated imaging analysis software calculates the surface tension every 2 seconds for a total of 600 seconds (Pernell et al., 2002; Nicorescu et al., 2011). Foam density has been shown to be determined by a density meter DMA 45 device (Kuropatwa et al., 2009; Miquelim et al., 2010; Yang & Foegeding, 2010).

The ability of egg white proteins to act as foaming agents is usually measured by foamability and foam stability. The foamability is related to the volume of air that is incorporated into the protein solution. A measurement of foam volume is expressed as foamability, foam capacity or overrun. Foaming capacity has been used to characterise a foam that shows the amount of interfacial area that can be produced by a particular protein. There are several quantitative methods used to express foaming capacity, most notably overrun (Macherey et al., 2011). Lau and Dickinson (2004) defined foam overrun as "the amount of additional air incorporated and expressed as the gas-to-liquid ratio on a percentage volume basis".

Foam stability is an indication of the film characteristics, such as the interfacial film strength and viscoelastic properties (Bovskova & Mikova, 2011). It is based on measurements taken over time, including the rate of liquid drainage and the rate of decrease in foam volume due to bubble coalescence, collapse and disproportion (Miquelim & Da Silva Lannes, 2009; Bovskova & Mikova, 2011). Foam stability is also

measured by recording the length of time required for half of the foam mass (liquid) to drain (Yang et al., 2009). Several other methods used to characterise the properties of foams is through an examination of dispersed phase characteristics, including an image analysis of bubbles' diameter by either using a microscope with a charge-coupled device (CCD) or confocal laser scanning microscopy (Pernell et al., 2002; Kampf et al., 2003; Raikos et al., 2007; Talansier et al., 2009; Yang & Foegeding, 2011).

Table 2.10 Some characterisation methods used to analyse foam bubble and rheological properties of foam.

Foam characterization	Methods	References
Bubbles size distribution	● Bubbles' structure examined microscopically using a Video Inspection Workstation MTV-6266.	Kampf et al. (2003)
	● Pictures of foam bubbles were taken using a CCD camera mounted on a Leica microscope.	Talansier et al. (2009)
Confocal laser scanning microscopy	● Microscopic images of the foams were obtained using a Leica DM-IRE2 confocal laser scanning microscope. The labelling dye was Fluorescein (isothiocyanate isomer I).	Raikos et al. (2007)
	● Confocal laser microscopic images of foams were taken on an inverted Leica DM IRBE confocal laser scanning microscope. Sodium fluorescein was added to the protein solutions.	Yang and Foegeding (2011)
	● Foams were imaged using a Leica DMRBE confocal scanning laser microscope.	Pernell et al. (2002)
Rheological Analysis (Viscosity)	● Foam samples were analyzed using a Haake Mars II rheometer with Rheoscope modulus, at 20°C, and controlled shear rate from 0.1 to 50/s, 180 s.	Miquelim and Da Silva Lannes (2009)
	● Viscosity of protein solutions was measured on a controlled stress rheometer over a range of shear rates (0.5 s^{-1}/225 s^{-1}).	Yang and Foegeding (2010)
	● Foam viscosity was measured by using a stress controlled rheometer AR1000-N Rheolyst^{TM}.	Nicorescu et al. (2011)
Density determination (interfacial tension)	● A Mettler-Toledo DE40 density meter was used equipped with a viscosity correction card at room temperature.	Yang and Foegeding (2010)
	● A density meter DMA 45 device at 5°C was used.	Kuropatwa et al. (2009)
Surface tension	● The surface tensions of the protein solutions was measured with a K12 tensiometer at 10°C for 3 h.	Nicorescu et al. (2011)
	● An automated contact angle goniometer was used to measure surface tension at room temperature (22 or 23°C).	Pernell et al. (2002)

2.10.4 Foam collapse

There are many factors influencing the foam stability. The occurrence of liquid drainage is associated with bubble surfaces moving closer together, leading to the bubble coalescence, foam collapse and loss of foam structure and texture (Figure 2.3) (Murray & Ettelaie, 2004). The mechanism behind the collapse of foam can be summarized as follows. After foam formation the drainage starts almost immediately. The final volume of liquid drainage approaches the initial volume of the liquid phase in the foam (Lau & Dickinson, 2005). Foam is also characterized by small diameter bubbles and the films between bubbles which are thick (Eisner et al., 2007). After some time, small air bubbles begin to expand and increase in their size, leading to foam collapse (Lau & Dickinson, 2005; Eisner et al., 2007).

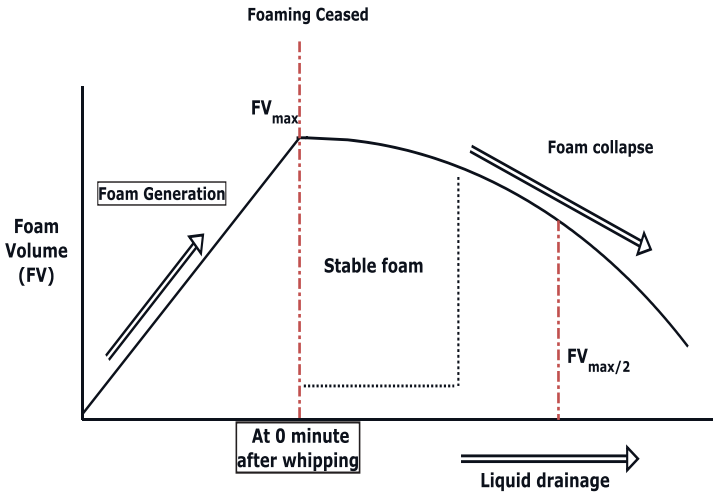

Figure 2.3 Typical foam collapses versus time during and after formation.
Source: Muthukumaran et al. (2008)

2.10.4.1 Gravity

One of the main destabilising mechanisms is drainage or leaking of lamellar liquid due to gravity and loss of surface tension (Muller-Fischer & Windhab, 2005). Loss of fluid due to gravity depends on the viscosity of the interfacial film and the surface that is exposed to the aqueous phase. High viscosity is required to avoid gravity deformation of the film and to hold the liquid within the lamella between air bubbles (Kinsella, 1981; Bovskova & Mikova, 2011).

2.10.4.2 Gas diffusion

A second foam destabilizing mechanism is gas diffusion and Ostwald ripening. The gas diffusion mechanism involves the passage of gas (air) from small to large bubbles as a result of the differences in the internal pressures between bubbles (Dutta et al., 2004; Damodaran, 2005). The net diffusion flux between bubbles is generally greatest from small to large bubbles, resulting in the foam containing a lot of large bubbles, which is called disproportionation (Muller-Fischer & Windhab, 2005). This causes shrinkage of small bubbles, which eventually disappear, and leads to the formation of large bubbles (Ostwald ripening) (Dutta et al., 2004). As the number of large bubbles increases, the film between bubbles becomes thinner, the liquid drains faster, leading to a decrease of foam volume and eventually foam collapse (Saint-James et al., 2012; Dutta et al., 2004; Murray & Ettelaie, 2004; Damodaran, 2005; Eisner et al., 2007).

2.10.4.3 Laplace capillary pressure

The third foam destabilising mechanism is rupture of the liquid lamellae separating gas bubbles. Bubbles are affected by several forces. Firstly, Laplace capillary pressure pulls the bubbles together, reflecting the tendency of the liquid surface to have minimum surface exposure between the bubbles. Secondly, Van der Waals attractive forces, also known as the London or dispersion forces, tend to thin films between bubbles (Damodaran, 2005; Lomakina & Mikova, 2006). Hence, drainage and rupture are related because when the space between films is decreased, film coalescence occurs, leading to lamellar liquid drainage and gradual collapse of the foam (Saint-James et al., 2012; Murray & Ettelaie, 2004).

2.10.5 Foam stabilisation

Stabilisation of foam depends on a wide range of different factors, primarily the structural, rheological and mechanical properties of the interfacial film. However, environmental factors, processing factors (temperature and pH), protein concentration, salt addition and the composition of continuous phase have to be considered when studying the stabilisation of foam (Kinsella, 1981; Rodríguez Patino et al., 1995; Yang & Foegeding, 2011). Recently, Bovskova and Mikova (2011) demonstrated other

factors that impact the formation and stability of egg white foam including hen age, egg age, storage conditions, speed and time of whipping, pasteurisation conditions, ionic strength and presence of egg yolk, stabilisers, surface active compounds and metal ions. There are some rheological effects that play important roles in maintaining egg white foam stability through different mechanisms. The disjoining force maintains the thickness of the aqueous film between air bubbles, aiming to prevent them from merging. An electrostatic double-layer interaction results from the mutual repulsion of charge at each side of the film, providing more stability to the foam (Saint-James et al., 2012).

The disproportionation process, which results from the formation of lots of large bubbles, can be countered by the strength of protein films. Film strength depends on the adsorbed amount of protein and the ability of the adsorbed molecules to combine. It also depends on limited protein denaturation, providing additional amino acid side chains which can enter into intermolecular interactions and form strong cross-linkages between proteins and promote film stability and strength (Belitz et al., 2004). The drainage rate may be decreased by increasing the bulk viscosity of the liquid (Lau & Dickinson, 2005). Raising the surface viscosity can be achieved by the addition of polysaccharides at high concentration, causing high adhesive or cohesive bonding. Bulk viscosity decreases the thinning of thick films and consequently slows the rate of draining (Lau & Dickinson, 2005).

2.11 Factors affecting the properties of egg white foam

2.11.1 Protein concentration

Protein concentration helps to maintain foam stability, depending on the influence of the rheological properties of the interfacial films (Patino et al., 1997). High protein concentration increases the viscosity of the protein solution. In a study reported by Sanchez and Patino (2005) a high concentration of caseinate (0.1–1% w/w) increased the viscosity of the solution, which lowered the liquid drainage and thus resulted in a more stable foam (Sanchez & Patino, 2005). Additionally, high protein concentration reduces the surface tension of aqueous protein solutions, producing smaller bubbles and a more stable foam (Rodríguez Patino et al., 1995). Kinsella (1981) and Rodríguez Pantino et al. (1995) both found that the volume and stability of egg white foams was

increased with protein concentration, and the foams prepared using higher concentrations of foaming proteins resulted in more stable, finer and denser foam because of the thicker interfacial films. When ovalbumin ranging between 0.01 and 1% (w/w) was used to generate foam by a sparging method (Rodríguez Patino et al., 1995), the foam stability was demonstrated to increase with protein concentration up to approximately 0.5% (w/w). At higher egg white protein concentrations, the number of small bubbles increased, which was manifested in the appearance of the foam and the volume of liquid held in the foam. They also found that when the concentration of egg white protein was less than 0.2% the foam collapsed due to the effect of gravity. However, high protein concentration (6%) can also have an adverse effect on foamability. This is due to the fact that high protein concentration enhances the viscosity of protein solutions which in turn does not allow a high volume of air to be incorporated at the interface, due to slowing the rate of diffusion and unfolding of protein at the interface (Lau & Dickinson, 2005).

2.11.2 Whipping time

Whipping time is defined as the time that is required to generate foam (Macherey et al., 2011). The whipping time has a significant effect on the foam stability and its mechanical strength. Generally, increasing whipping time leads to the production of smaller diameter bubbles while their stability is decreased as a result of thinner films. This effect is mainly caused by changes in the protein conformation rather than by the bubble size. This means that low whipping time results in insufficient changes in the conformational structure of protein molecules, thus decreasing the adsorption of proteins at the air-water interface and resulting in low foam volume (Vega & Sanghvi, 2012). In contrast, prolonged whipping causes excessive aggregation of egg white protein (e.g. ovalbumin) at the air-water interface, which induces it to form insoluble aggregates that limit water-holding capacity, thereby leading to increased drainage and subsequent foam collapse (Vega & Sanghvi, 2012).

Kampf et al. (2003) found that whipping time (6-8 min) decreased the mean size of bubbles and narrowed their size distribution, which eventually increased the foam stability (Hoppe, 2010). However, whipping egg white for a relatively long time (> 8 min) tends to cause excessive coagulation of ovalbumin which aggregates at the air–

water interface and becomes insoluble. As a result, proteins lose the ability of holding liquid, leading to foam collapses (Kinsella, 1981; Lau & Dickinson, 2005). Also, excessive whipping can cause more liquid film thinning, more mechanical deformation, and also more bubble-wall rupture, all of which contribute to a decline in the stability of foam (Lomakina & Mikova, 2006; Hoppe, 2010).

Girton et al. (1999) studied the foamability of egg white solution by whipping it for different times of 120-188 seconds at the lowest speed in a Kitchen Aid mixer. With increasing the whipping time between 148 and 188 seconds, the foamability increased and was about 35% higher than that produced by lower or higher times. Mott et al. (1999) studied the effect of whipping times (2.5, 5, 10, 15 and 20 min) on foamability using whey protein isolate at different concentrations (1.0, 2.5 and 5.0%). The results showed that the foamability increased with increasing whipping time of up to 10 min whipping and then decreased after 10 min.

It has been shown that when the beater speed increases, the volume of foam formed increases (Chavez-montes et al., 2007). However, whipping egg white at high speeds for a long time caused a decrease in foamability because of pressure fluctuations between bubbles, causing the formation of large bubbles that become large enough to cause sufficient stretching of films between bubbles to induce coalescence and foam collapse (Dutta et al., 2004). Coalescence is a process that occurs due to rupture of the film between bubbles, and during beating these films are frequently stretched. This also means that the film becomes thinner. Furthermore, films can be stretched (increasing bubbles diameter) because bubbles are pressed toward each other by pressure fluctuations (Dutta et al., 2004; Damodaran, 2005).

2.11.3 pH and isoelectric point

Usually, protein surface has a net charge because of their amino acid composition but the electrical net charge changes according to the pH of their environment. At low pH below the isoelectric point (pI), protein has a net positive charge because of the protonation of acidic amino acids (aspartic acid and glutamic acid). However, at higher pH values (above pI), protein is negatively charged because of the deprotonation of basic amino acids (lysine, arginine, histidine) (Figure 2.4) (Langel et al. 2011). If

protein has a net zero charge at the pI, then the protein molecules tend to lose water solubility due to their interaction resulting from loss of electrostatic repulsion (Ferreira Machado et al., 2007). At this point (pI), the intermolecular repulsion between proteins is minimal, leading to increase the protein adsorption at the air-water interface and consequently the high foamability and stability (Foeding et al., 2006; Macherey et al., 2011). Accordingly, protein adsorption to the interface generally occurs rapidly at the isoelectric pH as electrostatic repulsion is minimised (Foegding et al., 2006).

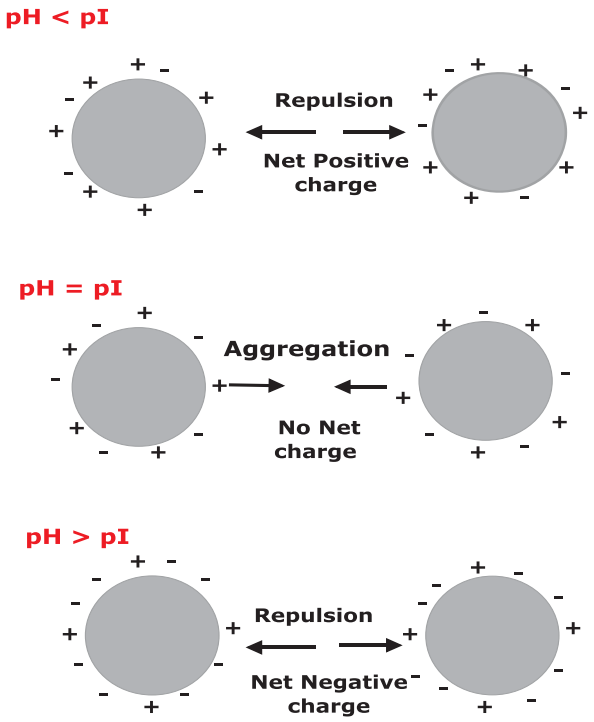

Figure 2.4 Effect of electrical net charge of protein molecules at pH below or above pI on protein interactions.
Source: Hui (2007)

The maximum stability of egg white foam was found to be at pH near or at the isoelectric pH, due to the increasing rate of protein adsorption as a consequence of decreased repulsive forces at the air-water interface and lower protein solubility (Clarkson et al., 2000). Egg white is known to produce the best foams at about pH 4-5, which is near to the pI of many egg white proteins. Another mechanism contributing to foam stability around the pI is the high density of adsorbed proteins at the interface.

This higher density of the adsorbed layer provides better steric stabilisation, resulting in more stable foam (Wierenga & Gruppen, 2010). On the other hand, at pH higher or lower than the pI of egg white proteins, proteins are more soluble. This is because of the presence of excess charges of identical polarity, producing repulsion among protein molecules (Ferreira Machado et al., 2007). This charge imbalance increases repulsion and works against foam stability causing foam collapse (Liang & Kristinsson, 2005).

Bovskova and Mikova (2011) observed a greater increase in the foaming ability of egg white at the neutral and acidic pH values but not at extremely acidic pH 1.0. At pH 8.6 (natural pH of egg white proteins), the foam stability was the highest but decreased with changing its pH (Chang et al., 1999; Girton et al., 1999; Hoppe, 2010; Bovskova & Mikova, 2011). With an aqueous egg white solution, foamability was the highest between pH 4.8 and 5 while it was the lowest between pH 9 and 10.7 (Liang & Kristinsson, 2005; Kuropatwa et al., 2009; Hoppe, 2010; Bovskova & Mikova, 2011). The highest long-term foam stability and the least amount of liquid drainage were found for egg white at pH 4.5 (Bovskova & Mikova, 2011). However, Rodríguez Patino et al. (1995) showed that the behaviour of ovalbumin in foam was different at different pH levels in regard to its pI. At pH lower than the pI of ovalbumin, the foam stability decreased whereas at pH levels higher than the pI of ovalbumin the foam was more stable (Rodríguez Patino et al., 1995). On the contrary, in another study reported by Girton et al. (1999), the maximum stability was observed at pH lower than the pI of egg white proteins. Rodríguez Patino et al. (1995) pointed out that at pH values higher than the pI of egg white proteins, the foam stability was not only affected by pH but there were other factors such as protein type and concentration. This was supported by the results reported by Ferreira Machado et al. (2007) that the protein solubility and foam stability were higher at pH above the pI of egg white proteins than at pH below the pI.

2.11.4 Addition of hydrocolloids

Generally, hydrocolloids used in food are polysaccharides of high molecular weight that are extracted from plants and seaweeds or produced by microbial synthesis. Hydrocolloids are used for a wide range of different functional applications in food as thickening, gelling, film-forming, foaming, stabilising and water-holding agents (Miquelim & Da Silva Lannes, 2009). The mechanism by which hydrocolloid gums

enhances the foam stability is mainly due to its influence on the strength of air cell walls. This is achieved by providing an additional polymeric network that increases stiffness in the foam structure. They also enhance the water binding capacity of the foam matrix, thus strengthening its mechanical integrity and stability (Kampf et al., 2003). The high viscosity of hydrocolloids also prevents bubble coalescence and retard liquid drainage from the film lamellae (Vardar-Sukan, 1998). The viscosity effects depends on the type of hydrocolloids used (Chavez-montes et al., 2007). For instance, xanthan solutions can be 100 times more viscous than guar gum solutions at equal concentrations (Chavez-montes et al., 2007).

The most common polysaccharides used in stabilising egg white foam are guar gum and xanthan gum. Guar gum is neutral, with a high molecular weight of about 103 kDa, and it is used as a bulking agent to increase viscosity (Miquelim et al., 2010). It has shear-thinning and non-Newtonian flow behavior (Srichamroen, 2007). It has the ability to form viscous solutions with a long texture for thickening and is also used in confectionery due to its gelling properties (Miquelim et al., 2010). The mechanism behind increasing the viscosity of aqueous solution when guar gum is dispersed in water is because the branches of a galactose unit across the main chain of guar gum molecules interact with water molecules, leading to inter-molecular chain entanglement of guar gum in the solution, thereby increasing the viscosity of the solution (Srichamroen, 2007).

Xanthan gum is an anionic polysaccharide with a molecular weight greater than $2x10^6$ kDa. It produces high viscosities at low concentrations. Xanthan gum forms pseudoplastic viscous solutions which are stable to pH and temperature compared to other hydrocolloids. This pseudo-plasticity makes xanthan gum also suitable as a stabiliser of suspensions, emulsions and foams (Carp et al., 2001). Xanthan gum is highly hydrophilic and has no significant hydrophobic groups, hence it is not adsorbed at the air–water interface, but it can retard foam destabilisation due to its thickening effect on the aqueous phase (Carp et al., 2001).

Gum arabic is a mixture of ionic polysaccharides which are highly branched and small amount of protein (2%, w/w) (Dickinson, 1993). Thus, it has a complex chemical structure consisting of six main carbohydrate components in its branches, such as galactopyranose, arabinopyranose, arabinofuranose, rhamnopyranose, glucuropyranosyl uronic acid and 4- *O*-methyl glucuro-pyranosyl uronic acid (Jayme et al., 1999). It has a

molecular weight between 260 and 1,160 kDa (Belitz et al., 2004). It has high solubility in water because of its low viscosity, thus a solution up to 50% gum arabic can be prepared. Gum arabic has a good emulsifying and film-forming properties although its surface activity is low compared with most food proteins (Dickinson, 1993; Belitz et al., 2004).

Protein-hydrocolloid interactions may enhance the stabilisation of interfacial layer thickness, leading to the stability of foam against bubble coalescence (Dickinson, 1993). Cox et al. (2009) studied the effect of hydrocolloids on the stabilisation of milk protein foam. It has been shown that the addition of xanthan gum has a significant effect on the apparent yield stress of the continuous phase. Initially, this effect prevents the buoyancy of bubbles since the yield stress opposes that force (Cox et al., 2009). Mott et al. (1999) showed that xanthan gum (0.05%) increased the stability of milk protein foam due to its increase in viscosity. However, in another study by Ercelebl and Ibanoglu (2009), the addition of guar gum (0.1% and 0.5%) significantly decreased the foamability of egg white protein compared to the control samples (without guar gum).

2.11.5 Addition of sucrose

The main effect of sucrose on foam stability is to increase the liquid viscosity around the bubbles, consequently lowering the drainage rate (Kinsella, 1981; Rodríguez Patino et al., 1995; Hoppe, 2010; Macherey et al., 2011; Vega & Sanghvi, 2012). However, the addition of sugar (e.g. sucrose, lactose, dextrose and maltose) inhibits the formation of foam during whipping because it hinders air bubbles being incorporated in the solution due to a rise in the viscosity of the continuous phase, resulting in slower diffusion and unfolding of the protein molecules in the vicinity of the air-water interface (Lau & Dickinson, 2005). Similarly, the addition of glycerol, sorbitol or several other chemicals which increase egg white viscosity improves the foam stability but it reduces the foaming ability (Bovskova & Mikova, 2011). Thus, after the addition of 50% sucrose, the time required for whipping to produce foam was more than 9 minutes compared to only 3-4 min without sugar addition (Stadelman & Cotterill, 1995; Yang & Foegeding, 2010). However, the effect of sucrose on foamability can differ, depending on its concentration. Raikos et al. (2007) showed a decrease in foamability when the concentration of sucrose added was high (12%) but at low concentration (6%) the

foamability increased slightly. Overall, the addition of sucrose has not been shown to improve the foamability (Rodríguez Patino et al., 1995; Macherey et al., 2011; Vega & Sanghvi, 2012).

The liquid drainage by gravity force can be significantly reduced by the addition of sugar, which enhances the stability of foam (Rodríguez Patino et al., 1995; Macherey et al., 2011; Vega & Sanghvi, 2012). This is achieved through the binding of sugar and excess water, resulting in less liquid drainage from the foam (Rodríguez Patino et al., 1995). The protein responsible in egg white for this binding is a glycoprotein of ovomucin, due to its long carbohydrate chains that can retain water (Hoppe, 2010). Lau & Dickinson (2005) showed the foam stability increased at sucrose concentrations higher than 15%. Raikos et al. (2007) found that higher concentrations of sucrose (12%) added to preheated egg white samples (64°C for 2 minutes) showed low liquid drainage and high foam stability.

2.11.6 Salt (ions) and ionic strength

Different ions can also influence the foaming properties of proteins. Generally, the addition of salt increases the protein coagulation and protein-protein interactions, and this reduces the adsorption of proteins at the interface, but the interaction of adsorbed proteins at the interface increases, hence increasing foamability (Kinsella, 1981; Hoppe, 2010). Ercelebl and Ibanoglu (2009) showed that at a low ionic strength (0-0.03 M NaCl), the solubility of protein increased, resulting in the enhanced foamability, but the foamability decreased at higher concentration above 0.03 M NaCl with the lowest volume at 0.2 M NaCl. High concentration of salt (high ionic strength) causes a reduction in protein–protein repulsion and leads to protein aggregation and decreased protein solubility and consequently inferior foaming ability and stability. Raikos et al. (2007) studied the effect of a combination of salt, heat treatment and whipping time. They found an increase in the volume of foam when the whipping time was 13 minutes for samples that had been preheated between 60°C to 64°C for 2 minutes, due to the high adsorption of proteins at the air-water interface. But they also reported a negative effect on foam stability from a combination of a longer whipping time and addition of NaCl.

2.11.7 Metallic cations

The addition of metallic cations may affect the functional performance of egg white as a foaming agent because of the ability of conalbumin (ovotransferrin) to react with polyvalent metal cations (e.g. aluminium, copper, iron and zinc) to form complexes (Cotterill et al., 1992). The amount of conalbumin in egg white is about 13% of the total egg white protein and it has a significant contribution in foam formation but its denaturation starts at 53°C (Hui, 2007). Even a mild thermal treatment, e.g. pasteurisation of egg white (60°C), may cause damage of its functional properties, which is important in view of conalbumin having the best foaming ability of all egg white proteins. However, the complex of conalbumin with a metal cation is resistant to thermal induced denaturation and proteolysis, and thus increasing the foaming properties of egg white (Lomakina & Mikova, 2006). The ability of conalbumin to bind to heavy metal ions is due to the presence of cysteine containing free sulphydryl groups (Stevens, 1991 & Yildiz, 2009).

Cotterill et al. (1992) examined the effect of different ion solutions with different concentrations (0.05-1 mM) on the foaming properties of both egg white liquid and spray-dried egg white before and after heat treatment at 54°C. The results showed significant differences in the foamabilities between the unheated and heated samples, especially for spray-dried egg white samples. For the heat treated samples, the foamability was high compared to the untreated samples. This was due to the high resistance of the complexes formed between conalbumin and metal ions against heat treatment. The most effective metallic cation for increasing the foamability in heat treated samples was copper ion followed by iron ion while aluminium showed a moderate foamability. The liquid and foam stability was also shown to increase with the addition of copper ions, followed by zinc and iron whereas aluminium had a negative effect. In summary, foams were more stable when copper ions were present and the foam stability improvement was increased with increasing copper concentration up to 1 mM.

2.11.8 Small molecule surfactants

Amphiphilic compounds containing both hydrophilic and lipophilic groups possess the surface active properties that can be used as emulsifying or foaming agents, including

some proteins and carbohydrates emulsifiers and small molecule surfactants (Dickinson, 1993; Wilde, 2000). Small molecule surfactants typically contain a polar group linked to one or more fatty acid chains and are classified according to the hydrophile-lipophile balance (HLB) (Dickinson, 1993; Wilde, 2000). A surfactant with a low HLB value is more lipophilic (oil soluble) while surfactant with a high value is more hydrophilic (water soluble) (Dickinson, 1993).

Some effects of surfactant that can have on protein structure and adsorption at the interface (Dickinson, 1993) can be described as follows: i) reduction in surface activity of proteins due to binding of the lipophilic tail of surfactant to the hydrophobic site on protein, ii) unfolding of tertiary structure of protein, improving molecular flexibility and hence the rate of rearrangement of proteins at the interface, iii) change in visco-elasticity of adsorbed layer due to interfacial protein-surfactant complex or protein-surfactant competitive adsorption, depending on the type of surfactant "ionic or non-ionic" and iv) incorporation of proteins into surfactant micelles.

Proteins form immobile viscoelastic films at the interface, whereas most small molecule surfactants form fluid, mobile interfacial layers. Surfactants induce a reduction in the interfacial viscoelasticity by a rapid diffusion at the interfacial layer; this process is called the Gibbs-Marangoni mechanism (Wilde, 2000; Rodríguez Patino et al., 2007; Maldonado-Valderrama & Patino, 2010). The Gibbs-Marangoni mechanism requires lateral mobility in the foam lamellae, and it plays a major role in stabilising foam. When a film is subjected to stretching due to some external disturbance such as whipping or shaking, the surface area decreases which results in the generation of a surface tension gradient (the Gibbs effect). In this situation the foam stabilisation depends on the change of the surface tension with time (the Marangoni effect) (Blomqvist et al., 2004; Maldonado-Valderrama & Patino, 2010). The surface tension can be returned to its normal level by either the adsorption of surfactant from the bulk or diffusion of adsorbed proteins at the surface. The diffusion mechanism causes stabilisation by facilitating the adsorption of proteins at the film, thus restoring the film thickness. This is associated with repulsion due to electrostatic double-layer forces and steric hindrance, which may also provide the film stabilisation (Blomqvist et al., 2004).

The effect of surfactants on the egg white protein foam properties has been widely studied. Generally, water-soluble surfactants (e.g. Tween) increase foam stability and

decrease slightly foamability whereas oil-soluble surfactants decrease both foam stability and foamability (Maldonado-Valderrama & Patino, 2010). This is due to the fact that small amounts of surfactant disrupt the surface layer of egg white protein and the adsorbed protein layer is no longer able to stabilise the foam via the viscoelastic mechanism. At this stage there is not enough surfactant present to stabilise the foam by the Gibbs–Marangoni mechanism. Therefore, as the concentration of surfactant increases, the surfactant displaces the egg white protein from the surface, and is able to stabilise the foam (Maldonado-Valderrama & Patino, 2010). Small molecule surfactants usually diffuse faster than proteins for their adsorption at the surface of air bubbles but surfactants can act differently depending on their concentrations, particularly at critical micelle concentration (CMC) at which surfactants can also undergo self-assembly to form micelles (Eisner et al., 2007). Patino et al. (1997) showed that a foam liquid drainage time increased with increasing surfactant concentration at below the level of CMC, which was due to a decrease in both the surface tension and the film thickness, while the drainage time decreased above the CMC resulting from to the reduction of elasticity. Eisner et al. (2007) studied the effect of small molecule surfactant (Tween 85) on the foamability of milk protein by using a sparging method. The addition of Tween 85 reduced foamability significantly due to a decline in elasticity of the interface between air bubbles, resulting in rapid coalescence. In contrast, the foamability of milk protein added with Tweens 20 and 80 was not affected significantly (Eisner et al., 2007; Maldonado-Valderrama & Patino, 2010).

2.11.9 Heat treatment

Heat treatment causes denaturation of egg white proteins by inducing changes in their chemical structure, which leads to aggregation of denature proteins (Hui, 2007). In other words, protein denaturation disrupts the secondary and tertiary structures of protein molecules which cause uncoiling their polypeptide chains and exposure of some reactive amino acid groups from the interior of their conformational structure. As a result, the denatured proteins interact with each other via chemical and physical bonds (e.g. hydrophobic, electrostatic interactions and disulphide linkages). This often leads to the protein coagulation and precipitation, depending on the degree of protein denaturation that can be affected by many variables (e.g. temperature and holding time

and type and concentration of protein) (Hui, 2007).

Heat treatment of egg white protein solution reduces foam stability and increases foamability via several mechanisms. An increase in temperature reduces the viscosity of the continuous phase and causes additional instability in the foam which leads to an increase in bubble size, leading to the reduced stability of foam (Kinsella, 1981). It also decreased foam stability through the enhancement of gas diffusion between bubbles and evaporation of liquid phase (Rodríguez Patino et al., 1995). However, Kinsella (1981) demonstrated that heat treatment of egg white at temperatures lower than its pasteurisation temperature (60°C) induces partial unfolding of globular proteins without causing thermal coagulation and this facilitates the formation of foam with smaller bubbles and increased rigidity. Rodríguez Patino et al. (1995) studied the effect of temperatures (5-40°C) of egg white liquid on the stability of egg white foams prepared using a sparging method. At lower temperatures of 5-20°C the foams produced were more stable. It was pointed out that the effect of temperature on the foam stability is also associated with the amount of protein used. As the concentration and temperature of egg white protein solutions were increased, the size of air bubbles was larger, leading to the foam instability (Girton et al., 1999; Lomakina & Mikova, 2006). According to Vega and Sanghvi (2012), whipping egg whites at temperatures (21-27°C) resulted in improved foam stability whereas at lower temperature (2°C) it did not show any significant difference in foamability. Ibanoglu and Ercelebi (2007) also investigated the effects of temperature and protein concentration on egg white foam. Egg white solutions (0.01-0.5% protein, w/v) were heat-treated at 65, 70, 75 and 80°C for 2 min and then used to produce foams using a sparging method. For the heat-treated samples at 80°C, the foamability was the lowest due to a rapid increase in the liquid drainage, but it was shown to be improved with increasing protein concentration to 0.5%. This was due to the presence of large molecules resulting from an increase in the denaturation and aggregation of proteins in the solution (Ibanglu & Ercelebi, 2007).

2.11.10 High pressure treatment

High hydrostatic pressure is being used more and more as an alternative to thermal processing. This technique is advantageous because it avoids the deterioration of food components and nutrients, and it enhances the safety of food. Other major advantages of

using the high pressure process are that it makes unnecessary the use of chemicals for preservation, and it produces foods with new functional properties. However, there is a negative effect of this process, that is, it also induces protein denaturation. This effect also depends on other factors including protein type and concentration, ionic strength, pressure range, temperature and pH (Van der Plancken et al., 2005).

The effect of high pressure treatments on the foam properties of egg white has been shown to be different according to the pH of egg white. Van der Plancken et al. (2007) examined the effect of different levels of pressures (0.1-700 MPa) at pH 7.6 and 8.8. Foaming ability increased when egg white solutions were treated with pressure above 500 MPa at pH 8.8. This effect was greater when the temperature was increased to 60°C. At pH 7.6, no significant effect of pressure was shown even with increasing temperature higher than 60°C but when the level of pressure applied was 400 MPa and at temperature below 60°C, the foamability was shown to be improved. A positive effect of pressure-treated samples above 500 MPa at 10-40°C on the foam stability was also reported. It was significantly more stable even though low foamability was produced from egg white solutions. Low foamability could be related to the formation of aggregates that did not allow the incorporation of large volumes of air (Van der Plancken et al., 2007). However, the formation of the intermolecular interactions between protein molecules enhances the stability of egg white foam (Van der Plancken et al., 2007).

In a study by Hoppe (2010), egg white solutions were treated at the pressure levels of 400–800 MPa for 5 min at 4°C. The foaming properties of egg white solutions were also shown to be highly dependent on their pH levels. Increasing the pressure applied resulted in an increase in foamability, especially at 800 MPa. The highest foaming ability was achieved at pH 4.5. This increase could be attributed to major egg white proteins, such as ovalbumin and ovomucin, which have the pI of 4.5 and 4.1, respectively. At pH 4.5, these two proteins have either very low or net zero charge, resulting in a reduction of electrostatic repulsion between the protein molecules that allows them more readily to absorb into the air-water interface due to protein unfolding (Hoppe, 2010). However, a study by Van der Plancken et al. (2007) showed that high pressure significantly reduced the foam stability of egg white solutions.

2.12 Preparation of aerated foods

There is a wide variety of aerated food products, such as mousses, meringue, ice cream, cakes, bread and confectionery products and whipped cream, all of which contain air bubbles. All these aerated products differ in their colors, flavors and textures. Gases commonly used for `aeration` include air, nitrogen and carbon dioxide (Arnaudov et al., 2012). The formation of foams in these products provides a range of unique sensorial properties, making their texture lighter and softer, increasing their volume, and improving the products' appearance, especially with added coloring (Foegeding et al., 2006).

There are two important factors to take into account when developing aerated foods: i) foamability during manufacturing processes and ii) foam stability during storage and cooking (Arnaudov et al., 2012). Specifically, the foam stability of some products, such as meringues, angel food cakes and nougat is hard to maintain during the baking process. For example, the foam of meringues produced must be resistant to expansion or collapse when heat is applied during the baking process. Baking them in an oven causes the evaporation of water and converts the foam from a liquid to a solid state (Foegeding et al., 2006). In making angel cake, the protein foam prepared is mixed with wheat flour (starch and protein) and sugar, and then the mixture is baked. Nougat presents the complication of maintaining a foam structure in a high sugar and low moisture environment (Foegeding et al., 2006).

The preparation method of making meringues involves the formation of egg white foam by whipping a mixture of egg white and sugar (Schonover, 1967; Foegeding et al., 2006). The proportion of egg white and sugar depends on the taste and type of meringues. Soft meringues contain a small portion of sugar, less than or equal to the amount of egg white. Hard meringues contain a relatively large amount of sugar and twice the amount of egg white, and are stiffer than soft meringues. Hard meringues are generally used as a dessert (Foegeding et al., 2006). The importance of temperature while baking soft meringues arises because of the accumulation of moisture on the surface of meringues, making it undesirably moist. The optimum proportion of sugar is 4-5 tablespoons per one egg white (Schonover, 1967). Meringue preparation may also include the addition of acids, such as cream of tartar, citric acid, lemon juice and vinegar. The recommended amount is 1.5% of the total weight of egg whites (Vega & Sanghvi, 2012). It should not exceed 2.4% based on the weight of egg white. Above this

concentration, the foam formation is compromised. The presence of acid also brings the pH closer to the pI of egg white proteins, hence decreasing the electrostatic repulsions. This leads to an increase in the diffusion and the adsorption of proteins at the air-water interface, enhancing the film between air bubbles and producing stable foam (Vega & Sanghvi, 2012).

Meringues can be produced by either cold or hot methods. A cold method includes mixing of egg whites and sugar at room temperature. On the other hand, hot methods are used when sugar is dissolved in a small quantity of water, and then the solution is boiled and beaten into egg whites. Meringues can be prepared with or without the addition of stabilisers. Stabilisers such as gum and starch act to absorb excess moisture, thereby helping to prevent the leakage of its aqueous components from the foam. Soft meringues are baked at 204 - 232°C for about 8-12 min, while the hard meringues are baked at 121- 148°C, for about 30-60 min (Schonover, 1967). Foegeding et al. (2006) demonstrated that the baking temperature of soft and hard meringues is important to avoid foam collapse. Both can be baked at 180°C for 15 min.

Another type of meringues is a dried confectionery meringue, which is characterised by its light, brittle texture, provided by sucrose and egg white protein (Perry et al., 2011). This method involves the mixing of 3-15% egg white powder, 45-65% sucrose, at least one monosaccharide between 1% and 20%, and optionally cocoa powder and salt, with sufficient water to form a mixture. The whipped mixture is then baked to remove the moisture (Perry et al., 2011).

2.13 Literature review conclusions

Egg white is one of the most important dietary sources of food proteins. It contains about 10% protein, 89% water, <1% carbohydrate and some minerals and vitamins. The fat content of egg white is almost negligible. Egg white proteins are known to be superior in quality and biological value to other food proteins (e.g. milk, meat and vegetables) because they contains all essential amino acids and are readily digested, absorbed and metabolised efficiently for utilisation in human body. In addition, egg white proteins have multiple protein functionalities such as foaming, emulsifying and gelling.

Egg white containing egg white proteins is widely used and applied in various food products as foaming agents. Its functional behaviour to produce and stabilise foam can be affected by many different factors, including protein concentration, pH, temperature, ionic strength, presence of other components (e.g. sucrose and hydrocolloids) and foaming methods. Therefore, it is important to understand how these factors affect the foaming ability and foam stability of foams produced from egg white. Although some studies have been conducted on egg white foams but there are still some knowledge gaps to be further investigated. The current research was to determine the properties (foamability and foam stability) of foams prepared using two different egg white raw materials (egg white liquid or egg white powder) by using two different methods (e.g. whipping and gas sparging methods). Various factors described above, including microwave cooking of foams, were investigated for their impact on egg white foams.

Chapter 3 Formation and properties of egg white foams prepared by using a standard mix beater

3.1 Introduction

Egg white has been known to have excellent foaming ability which can increase six to eight times its original volume when whipped. The simple way to create foam from egg white solution that is to apply mechanical forces to the egg white proteins. Whipping method (e.g. standard kitchen mix beater) (Breville wiz, EM3, New Zealand) is widely used to create egg white foam. Many researchers highlight the importance of using this method and its influence on egg white functional properties (Girton et al., 1999; Pernell et al., 2002; Raikos et al., 2007; Yang et al., 2009; Yang & Foegeding., 2010; Yang & Foegeding, 2011). Numerous variables, such as mixing speed and time, temperature, pressure, egg white protein concentration and presence of other components (oil, salt, hydrocolloids, stabiliser and emulsifiers including sucrose, sodium chloride, calcium chloride, xanthan gum, guar gum and gum arabic) have also been investigated to understand their effects on the functional properties of egg white as a foaming agent.

As a device of producing foams, standard mix beaters are widely used as a whipping method to create egg white foams. Some advantages of using this mechanical equipment are easy and simple operation and fast foam production. More importantly, the conditions for making foams (whipping speed and time) can be maintained constant and optimised readily according to the size and composition of materials to be whipped

One of the important properties of materials to be whipped to generate foam is their ability to generate and stabilise foams readily during and after production. In areas of studying foam, several parameters are usually measured to determine, including foamability and foam stability. Foamability refers to an increase in the volume of liquid after whipping and is expressed as percentage in response to an initial volume of liquid prior to whipping. Foam stability is normally defined as foam volume stability and foam liquid stability with time after foam preparation. Foam volume stability is related to the retention of foam volume against foam collapse for a specific time whereas foam liquid retention is defined by the volume of liquid drainage from foam over time.

The objective of the research presented in this chapter was to determine the effects of several variables on the formation and properties of egg white foams produced by using a standard kitchen standard mix beater. Some factors affecting the foamability and foam stability of egg whites investigated included processing conditions (whipping time and speed), environmental factors (pH and temperature) and ingredient and composition variables (type and concentration of egg white, sugar and hydrocolloids).

3.2 Materials and Methods

3.2.1 Materials

Frozen pasteurised egg white liquid (EWL) (10% w/v protein), and egg white powder (90.4% protein in dry basis) were purchased from Eggcel (Eggcel, New Zealand) (Figure 3.1). Hydrocolloids, including xanthan gum E415 (ADM, Novaxan, Food Grade, U.S.A), guar gum E412 (Roeper, Food Grade, Germany), gum arabic "*Acacia* gum" E414 (Nexira, France) and locust bean gum E410 (LBG Sicilia, Food Grade, Italy) were obtained from Hawkins Watts, New Zealand. Sucrose *(*Analytical Grade, *LabSe*rv, Australia) was purchased from Biolab (Australia) Ltd. Sodium chloride and calcium chloride dihydrate (Analytical Reagent Grade, LabServ, Australia) were purchased from Thermo Fisher Scientific Inc., New Zealand. Citric acid E330 (Jungbunzlauer, Food & Pharmaceutical Grade, U.S.A) as a flavour agent was obtained from Hawkins Watts, New Zealand.

Figure 3.1 Pictures of egg whites in two different forms. (A) Frozen pasteurized egg white liquid (10% w/v protein) after thawing at 4oC and (B) spray dried egg white powder (90.04% protein in dry basis)

3.2.2 Preparation of EWL and EWP solutions

Frozen pasteurised egg white liquid (EWL) was thawed and kept at 4°C until it was used. Before whipping, the EWL was placed in a water bath and warmed to 20°C. Egg white powder (EWP) was kept in a freezer at minus 16±1°C until use. It was used to make egg white foams in some experiments. EWP solutions containing different protein concentrations (5, 10, 15 and 20% w/v) were prepared by dissolving in distilled water at 20±1°C.

3.2.3 Preparation of foams

Egg white foam was prepared using a standard kitchen mix beater, which was an standard mixer with two stainless steel beaters (5 speed control) (Breville Wizz Mix EM3, New Zealand). A 50 g of egg white solutions at 20^{o}C unless otherwise stated was weighed into a glass bowl (6.35 cm diameter) and used to make foam by whipping at 20°C for 5 min. A whipping time of 5 min and the level of speed 5 were used after examining the optimal whipping time and speed to generate stable foams. Upon completion of whipping, the beaters were removed from the foam gently to minimize disruption of the foam. Stiffer foams had a tendency to remain attached to the beaters, in which case the foam was removed with a rubber spatula.

3.2.4 Analysis of foamability and foam stability

Immediately after whipping, the foam was transferred into a plastic measuring cylinder (500 g) by gently scooping it out using a rubber spatula. The measuring cylinder was gently shaken to remove any air gaps or spaces within the foam. The volume of foam in the measuring cylinder was then recorded to determine foamability. The stability of foam was also measured over time at a 10 min interval for 5 hr by monitoring the amount of foam volume remaining without collapse, defined as foam volume stability, and the volume of liquid remaining without being drained from the foam, defined as foam liquid stability.

Foamability represents the volume of air entrapped by a solution. This was measured in this study according to the method of Chang et al. (1999) and calculated using Equation (1). As indicated above, foam stability was measured in two different aspects: the ability

of foam to resist collapse and retain its liquid without drainage over time after foam preparation (Marinova et al., 2009). The foam volume and foam liquid stability was calculated using Equations (2) and (3), respectively.

$$\text{Foamability } \% = \frac{V}{V_I} \times 100 \qquad (1)$$

$$\text{Foam volume stability } \% = \frac{V_T}{V} \times 100 \qquad (2)$$

$$\text{Foam liquid stability } \% = \frac{V - V_{LD}}{V} \times 100 \qquad (3)$$

Where, V is the volume of foam after whipping,
V_I is the volume of initial liquid used for foam preparation,
V_T is the volume of foam measured over time after foam preparation, and
V_{LD} is the volume of liquid drained over time after foam preparation.

3.2.5 Effects of various factors on egg white foam

Various factors, including whipping time and speed, types of egg white (EWL and EWP), egg white protein concentration, pH, type and ionic strength of salts and addition of sugar and hydrocolloids, were investigated to determine their influence on foam properties. The form properties (foamability and foam stability) of all samples were analysed as described in Section 3.2.4.

In all experiments described in Chapter 3, unless otherwise stated, the conditions used for making foam using the standard mix beater were: i) whipping speed 5 and whipping time 5 min and ii) 50 g of EWL (10% protein) at 20^{o}C.

3.2.5.1 Whipping time

Whipping time was defined as the time that EWL needed to be whipped to achieve stiff foam. A 50 g of EWL (10% w/v proteins) at 20°C poured into a glass bowl was whipped for different times (1, 3, 5, 7 and 9 min) using the method described in Section 3.2.3 and the foams produced were analysed as described in Section 3.2.4.

3.2.5.2 Whipping speed

Effect of a rotating speed of the mix beater for whipping egg white was examined at two different speed levels: speeds 3 and 5. Egg white foams were produced from 50 g of EWL at 20±1^{o}C by whipping for 5 min as described in Section 3.2.3. The 5 min whipping time was chosen based on the results of the experiment from Section 3.2.5.1 to generate the most stable foam. The foams produced were analysed as described in Sections 3.2.4.

3.2.5.3 EWL temperature

To study the effect of temperature of egg white prior to whipping, EWL was warmed in a water bath to bring their temperature to 20±1°C, and other EWL sample was cooled to 4±1°C using an ice water bath. Egg white foams were produced by whipping as described in Section 3.2.3 and analysed as described in Section 3.2.4.

3.2.5.4 Type of egg white solution (EWL versus EWP)

In order to compare the impact of using two different forms of egg white (EWL and EWP) which are available commercially, both of them were used to make foams and compared. EWP solution containing 10% protein was prepared as described in Section 3.2.2. Foams were produced by whipping 50 g of EWL and EWP solution using the method as described in Section 3.2.3 and analysed as described in Section 3.2.4.

3.2.5.5 Protein concentrations of EWP solution

The effect of protein concentration of egg white on foamability and foam stability was determined using EWP. Aqueous solutions of EWP containing different protein concentrations (5, 10, 15 and 20% w/v) were prepared as described in Section 3.2.2. The pH of all EWP solutions was measured using a pH meter (Sartorius Basic pH meter, pB-20) which was around to be pH 7.7 ± 0.5. It should be noted that this was different to pH of EWL (~ pH 8.8). Foams were produced from the EWP solutions (50 ml) by whipping as described in Section 3.2.3 and analysed as described in Section 3.2.4.

3.2.5.6 pH of EWL

To examine the effect of pH, the pH of EWL was adjusted from its original pH 8.8 ± 0.09 to pH 3, 4, 5, 6, 7 and 8 using 2 M or 4.7 M HCl and to pH 10 using 2 M NaOH. After pH adjustment, foams were produced by whipping as described in Section 3.2.3 and analysed as described in Section 3.2.4.

3.2.6 Effects of some ingredients on egg white foam

3.2.6.1 Addition of salts to EWL

The impact of two types of salts on egg white foam properties was determined using sodium chloride (NaCl) and calcium chloride ($CaCl_2$) at different concentrations. EWL solutions containing different salt concentrations were prepared.

The pH of distilled water was first adjusted to be around the pH of EWL (pH 8.8) using 0.1 M NaOH. Salts were added to the pH-adjusted distilled water at different concentrations and stirred with a magnetic stirrer until the salt was fully dissolved. Then, the salt solutions containing different concentrations were mixed with EWL at the same ratio of 1:9, thereby maintaining the same protein concentration (i.e. 9%) between the samples (Table 3.1). The final NaCl concentrations in the EWL solutions to test their effect on egg white foam were 0, 10, 50, 100, 200 and 400 mM. The pH of the EWL solutions was also measured before and after the addition of salts as shown in Table 3.1. The EWL solutions containing $CaCl_2$ at different concentrations of 0, 50 and 100 mM were also prepared, similarly to the EWL solutions containing NaCl, as shown in Table 3.2. Foams were prepared from the salt-containing EWL solutions (50 g) at 20±1^{o}C by whipping described in Section 3.2.3 and analysed as described in Section 3.2.4.

The EWL solutions with added NaCl and $CaCl_2$ were also analysed to determine their optical properties (turbidity) and electrical charge of proteins (refer to Sections 3.2.7.2 and 3.2.7.3). These measurements were carried out one day after storage at 4±1°C.

Table 3.1 Preparation of EWL containing NaCl at different concentrations.

NaCl (mM)	NaCl (g)	Water (g)	EWL (g)	Total (g)	pH[a]	pH[b]	Protein (%)[c]
0	0	10	90	100	8.74	8.8	9
10	0.0584	9.9416	90	100	8.74	8.8	9
50	0.2922	9.7078	90	100	8.74	8.8	9
100	0.5844	9.4156	90	100	8.95	9.04	9
200	1.1688	8.8312	90	100	8.86	9.03	9
400	2.3376	7.6624	90	100	8.83	9.03	9

[a] Original pH of EWL batch
[b] Final pH of EWL solution with added salt
[c] Protein concentration after addition of salt

Table 3.2 Preparation of EWL containing $CaCl_2$ at different concentrations.

$CaCl_2$ (mM)	$CaCl_2$ (g)	Water (g)	EWL (g)	Total (g)	pH[a]	pH[b]	Protein (%)[c]
0	0	10	90	10	8.96	8.94	9
50	0.5549	9.45	90	9.45	8.96	8.00	9
100	1.1098	8.89	90	8.89	8.96	7.48	9

a Original pH of EWL batch
b Final pH of EWL solution with added salt
c Protein concentration after addition of salt

3.2.6.2 Addition of sucrose to EWL

EWL solutions containing sucrose at different concentrations (0, 5, 10 and 20%) were prepared (Table 3.3). It should be mentioned that two EWL solutions formulated with 10 and 20% sucrose contained low protein concentrations (8.2 and 7.5% protein) and the other two samples (9% protein). After mixing with sucrose, the EWL solutions were stored at 4±1°C overnight for full dissolution. Foams were produced from the EWL solutions (50 g) by whipping as described in Section 3.2.3 and analysed as described in Section 3.2.4.

Table 3.3 Formulation for EWL with sucrose at different concentrations.

Sucrose (%w/w)	Sucrose (g)	Water (g)	EWL (g)	Total (g)	pH[a]	pH[b]	Protein (%)[c]
0	0	10	90	100	8.9	8.9	9
5	5	5	90	100	8.87	8.95	9
10	10	0	90	110	8.84	8.95	8.2
20	20	0	90	120	8.9	8.78	7.5

[a] Original pH of EWL batch
[b] Final pH of EWL solution with added sucrose
[c] Protein concentration after the addition of sucrose

3.2.6.3 Addition of hydrocolloids to EWL

Three different types of hydrocolloids, such as xanthan gum (XG), guar gum (GG) and gum arabic (GA), were used at different concentrations to determine their influence on egg white foam. Hydrocolloid solutions were prepared by dissolving them in distilled water (pH 7) at 20^{o}C using a magnetic stirrer and then storing overnight at 20^{o}C for full dissolution and hydration. A 10 g of hydrocolloid solution with different concentrations was then mixed with 90 g of EWL using a magnetic stirrer (Table 3.4). Egg white foams were produced from 50 g of EWL solutions (20^{o}C) containing hydrocolloids by whipping as described in Section 3.2.3 and analysed as described in Section 3.2.4.

Some samples of the EWL solutions containing hydrocolloids were analysed for their viscosities and compared with the viscosity of the control (i.e. EWL without containing hydrocolloids) (refer to Section 3.2.7.1).

3.2.7 Analyses of viscosity, turbidity and zeta potential of EWL

3.2.7.1 Viscosity of EWL solutions containing hydrocolloids

The viscosity of three samples for the EWL solutions added with each of three types of hydrocolloids (0.04% GA, 0.04% GG or 2% GA) and the control sample, which was described in Section 3.2.6.3, was measured with a rheometer (TA AR550 Rheometer, TA Instruments, New Castle, Delaware, USA) equipped with a cone and plate geometry (cone angle 2^{o} and cone diameter 60 mm diameter). To determine the viscosity of the

EWL solutions, a small amount of sample taken by dropper was placed on the Peltier plate. The geometry was then lowered to the gap distance once the sample was loaded correctly and the gap was properly filled. The flow curve was monitored over a controlled shear rate range between 100 s^{-1} and 1000 s^{-1}. The data were analysed using the Rheology Advantage Software (version v5.6.0).

Table 3.4 Formulations for EWL solutions with hydrocolloid (HC), such as xanthan gum (XG), guar gum (GG) and gum arabic (GA), at different concentrations.

	HC (w/w%)	HC (g)	Water (g)	EWL (g)	Total (g)	pH[a]	pH[b]	Protein (%)[c]
XG	0.01	0.01	9.99	90	100	6.69	8.90	9
	0.02	0.02	9.98	90	100			9
	0.04	0.04	9.96	90	100			9
GG	0.01	0.01	9.99	90	100	6.55	8.88	9
	0.02	0.02	9.98	90	100			9
	0.04	0.04	9.96	90	100			9
GA	1	1	9	90	100	4.71	8.72	9
	2	2	8	90	100			9

[a] pH of hydrocolloid solution
[b] pH of the mixture of EWL and hydrocolloid solution
[c] Protein concentration after the addition of hydrocolloid

3.2.7.2 Turbidity of EWL solutions

Two different sets of samples (EWL solutions with different levels of pH described in Section 3.2.5.6 and EWL solutions containing salts at different concentrations described in Section 3.2.6.1) were analysed to determine their optical properties (turbidity) by measuring absorbance at 600 nm using a UV/Vis spectrophotometer (UV-1800, Shimadzu, Japan).

3.2.7.3 Zeta potential of proteins in EWL solutions

Those samples analysed for the measurement of turbidity described above were also analysed for the electrical charge (zeta potential) of proteins in the EWL solutions using a Malvern Zetasizer (Nano ZS90, Malvern Instruments Ltd., Malvern, Worcestershire, UK). Prior to the measurements, all samples were diluted to 100 fold (v/v) using distilled water of appropriate pH and/or salt concentration the same as the samples in order to prevent multiple light scattering effects. All sample measurements were performed at 20±1°C.

3.2.8 Statistical data analysis

All experiments for the sample preparation and analysis were carried out at least in duplicate. The results were reported as average and standard deviation. The data were statistically analysed using a Minitab statistical software version 16 (Minitab Inc., USA). A one-way ANOVA using a Turkey method was used to determine the significance of means at a 95% confidence level ($p < 0.05$).

3.3 Results and Discussion

3.3.1 Whipping time

The effect of whipping time on the foamability and stability of egg white foam was initially studied to determine an optimum time required for producing egg white foam at 20°C. Five different whipping times of 1, 3, 5, 7, and 9 min were used to test their effect. A total of 50 g of EWL at 20^{o}C was whipped in a glass bowl using a standard mix beater at speed 3.

3.3.1.1 Foamability

Whipping EWL for 5 min produced foam that had the highest volume compared to the other whipping times (Figure 3.2). The foamability of EWL increased with increasing whipping times from 1 to 5 min but it decreased when whipping time was extended to 7

and 9 min (Figure 3.2). This means that at a given condition of whipping used in this experiment (e.g. 50 g of EWL at 20^{o}C when whipping in a glass bowl using a standard mixer beater at a speed 3 setting), the highest foam volume was observed after whipping for 5 min with an increase in volume to 724 ml from the initial volume of 50 ml of EWL solution prior to whipping. This reflects there was an increase in its original volume by up to about 15 times. On the other hand, at 1 min the foam volume increased to 510 ml (i.e. 10 times volume expansion) and at 7 and 9 min it was 630 and 665 ml, respectively, both about 13 times increase in volume. Overall, the results of the foaming ability of EWL observed in this study were larger than that widely reported in the literature (Girton et al., 1999; Lomakina & Mikove, 2006). This may be due to differences in the methods and conditions used to create foams.

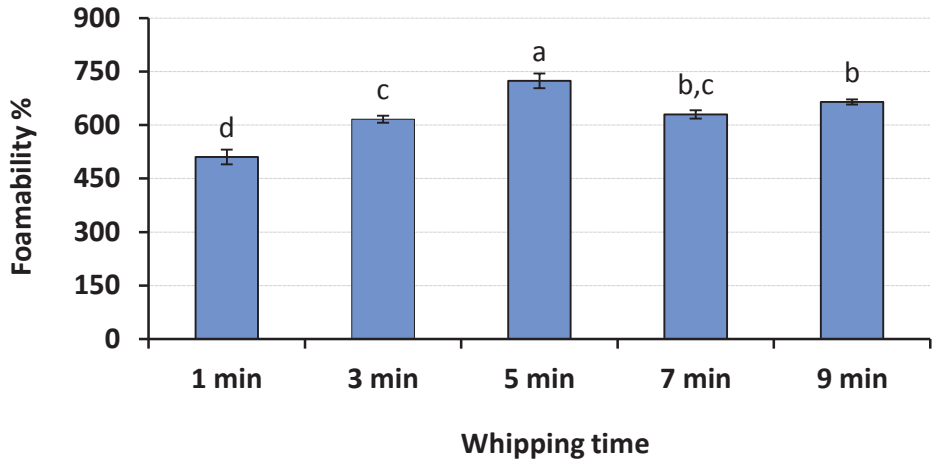

Figure 3.2 Effect of whipping time on the foamability of egg white liquid prepared using a standard mixer.
Statistical data analysis using one way ANOVA with Fisher method. a,b,c,d means with the different letters are significantly different ($p < 0.05$). Each data point: Mean ± SD for n=2.

The possible explanation for the observed lower foamability resulting from the shorter whipping time (e.g. <5 min) could be due to the introduction of less air bubbles into egg white solution due to insufficient whipping time. In other words, egg white proteins present in the egg white solution might have not been used fully to create foams and some proteins still remained in the egg white solution without being adsorbed at the air-water interface, thus reducing the formation of new interfaces and more air bubbles (Vega & Sanghvi, 2012).

Excessive whipping time (e.g. ≥7 min) was also shown to cause a reduction in foamability which was in agreement with the results reported by Mott et al. (1999). This can be due to two main reasons as follows: i) a long whipping time leads to an induction of more bubbles with a small diameter (Mott et al., 1999). These small air bubbles can undergo a process called coalescence which results from film rupture of the interfacial layer between bubbles as prolonged whipping leads to excessive stretching of these films (Walstra, 2003; Lomakina & Mikova, 2006; Hoppe, 2010), and ii) excessive denaturation and aggregation of proteins at the air–water interface, limiting the function of protein as surface active agents and/or causing the disruption of interfacial layers surrounding air bubbles (Kinsella, 1981; Lau & Dickinson, 2005).

3.3.1.2 Foam stability

The measurement of foam stability is referred to as 'liquid drainage stability' and 'foam volume stability'. These two parameters were determined by measuring the amount of liquid drained from the foam and the volume of foam remaining without collapse with time after foaming. The stability of foam volume and foam liquid has been shown to improve through the induction of bubbles with small diameters and more film thinning (Walstra, 2003; Lomakina & Mikova, 2006; Hoppe, 2010).

The changes of foam volume stability pattern between samples produced at different whipping times are shown in Figure 3.3A. Overall, the results indicated that the stability of foam volume was higher at 7 and 9 min of whipping than the short time of whipping, 1, 3 and 5 min. Whipping egg white for 1 min resulted in low foam volume stability, with a rapid reduction of the foam volume to 80% and 65% after 30 min and 3 hr, respectively. On the other hand, 9 min whipping time resulted in the highest foam volume stability with 95% and 80% of the initial foam volume remaining stable after 30 min and 3 hr, respectively. The foam volume stability observed between 1 and 9 min of whipping time was the most significant over the whole period among all the whipping times tested, while the other whipping times (3, 5 and 7 min) resulted in a relatively similar pattern with only slight differences in the foam volume stability during 3 hr after the foam preparation before the patterns changed after 3 hr (Figure 3.3A).

Regarding the foam liquid stability, one minute of whipping also exhibited remarkably a lower liquid stability (i.e. high liquid drainage), compared to the other samples prepared with longer whipping times, as more than 50% of the liquid was drained from the foam rapidly within 10 min (Figure 3.3B). Among all whipping times, the egg white foam produced by whipping for 5 min showed the highest stability to liquid drainage during 5 hr after it was whipped.

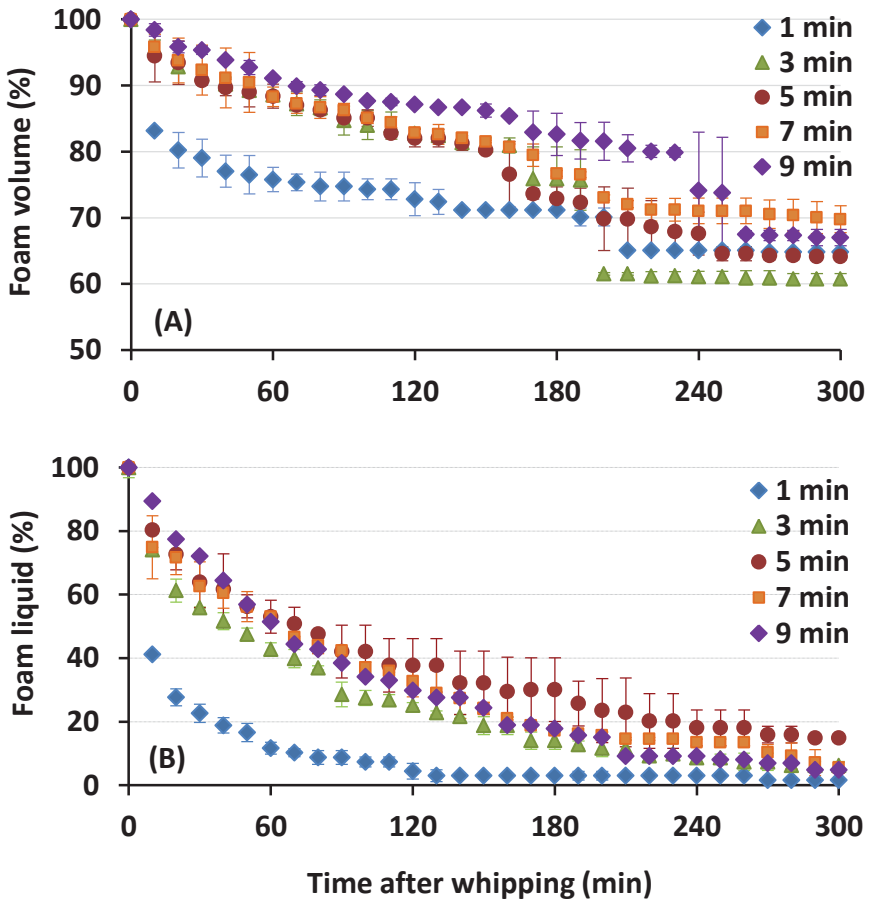

Figure 3.3 Stability of (A) foam volume and (B) foam liquid from foams produced by whipping liquid egg white for different times.
Each data point is mean ± SD for n=2.

The low foam liquid stability observed from a short whipping time (e.g. 1 min) can be partly attributed to the entrapment of a relatively larger amount of liquid within a given volume of the foam than the other samples as the initial foam volume produced was much lower (Figure 3.3B). This may lead to a higher pressure built up on the foam,

causing a faster foam rupture, collapse and liquid drainage. A short whipping time can result in air bubbles with large diameter and low protein adsorption at the air-water interface (Vega & Sanghvi, 2012); thus decreasing the stability of foam against coalescence and collapse.

Relatively long whipping times, such as ≥ 3 min used in this study, enhanced the stability of foam and its liquid drainage through the induction of bubbles with small diameters, and more film thinning (Walstra, 2003; Lomakina & Mikova, 2005; Hoppe, 2010; Vega & Sanghvi, 2012). Some changes in conformational structures of protein molecules due to moderate protein denaturation and aggregation by adequate whipping times can induce the formation of soluble aggregates at the air–water interface that have a high water-holding capacity, thereby leading to a decreased liquid drainage and subsequent high foam stability for a longer period (Vega & Sanghvi, 2012).

The final conclusion that can be made from this experiment is that although high foam volume stability was able to be obtained by whipping EWL for 9 min, the whipping time of 5 min was preferred to produce foam with high liquid stability. However, 3 and 7 min also showed moderate stability with respect to both volume and liquid drainage.

3.3.2 Whipping speed

The effect of whipping speed on foamability and foam stability was studied by whipping 50 g of EWL solution at 20^{o}C using a standard mix beater at two different speed levels (3 and 5). The whipping time used in this study was 5 min which was identified to be optimal to use as described above.

3.3.2.1 Foamability

Applying two different whipping speeds used did not show a significant influence on the foamability of EWL ($p < 0.05$) (Appendix 1). The volume of EWL after whipping was increased 724 ± 21% and 723 ± 7% at speed 3 and 5, respectively (Figure 3.4). However, when EWL was whipped at a lower speed (i.e. speed level 1 or 2), the foamability was much lower and a longer whipping time was required at low speed to produce foams (data not shown) (Walstra, 2003; Chavez-montes et al., 2007). This

indicates that there is a relationship between beater speed and whipping time. If egg white is whipped at low speed but for a long time, its foaming ability can be enhanced whereas whipping egg white solution at high speed for a long time may reduce the foamability because of the pressure fluctuations between air bubbles (Walstra, 2003).

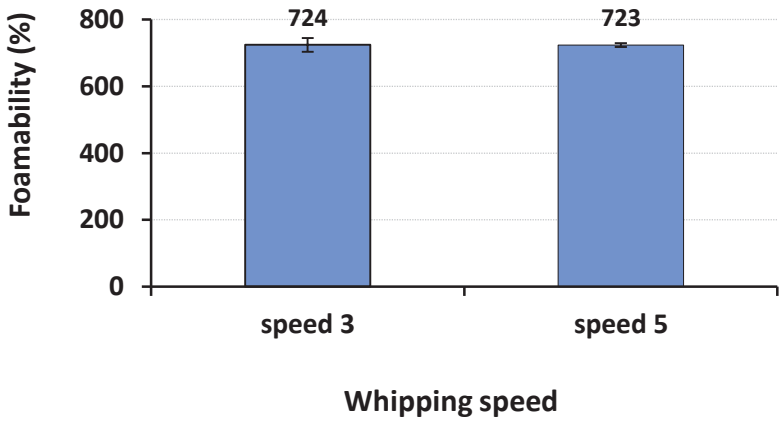

Figure 3.4 Foamability of liquid egg white produced by whipping at two different speeds using a standard mixer.
Each data point is mean ± SD for n=2.

3.3.2.2 Foam stability

Figure 3.5B shows that the stability of foam volumes produced at two different speeds decreased gradually over time after preparation and that the foam was slightly more stable when prepared at speed 3 than at speed 5. Foam volume stability of EWL when it was whipped at speed 5 was 83% and 73% at speed 3 (Figure 3.5A). However, the stability of foam volume dropped quickly after 4 hr with no significant difference between different speeds.

For the stability of foam against liquid drainage, a small difference was also observed between the two samples (Figure 3.5B). The foam produced at speed 5 was slightly more stable to liquid drainage during the first 1 hr after preparation but after 1 hr the rate of liquid drainage occurred faster in this sample than the foam prepared at speed 3.

Although it is not clearly understood, the differences between the two samples for their foam stability and foam liquid stability which had no correlation may be due to some

differences in air bubble size and film thinning between them caused by two different whipping speeds (Walstra, 2003).

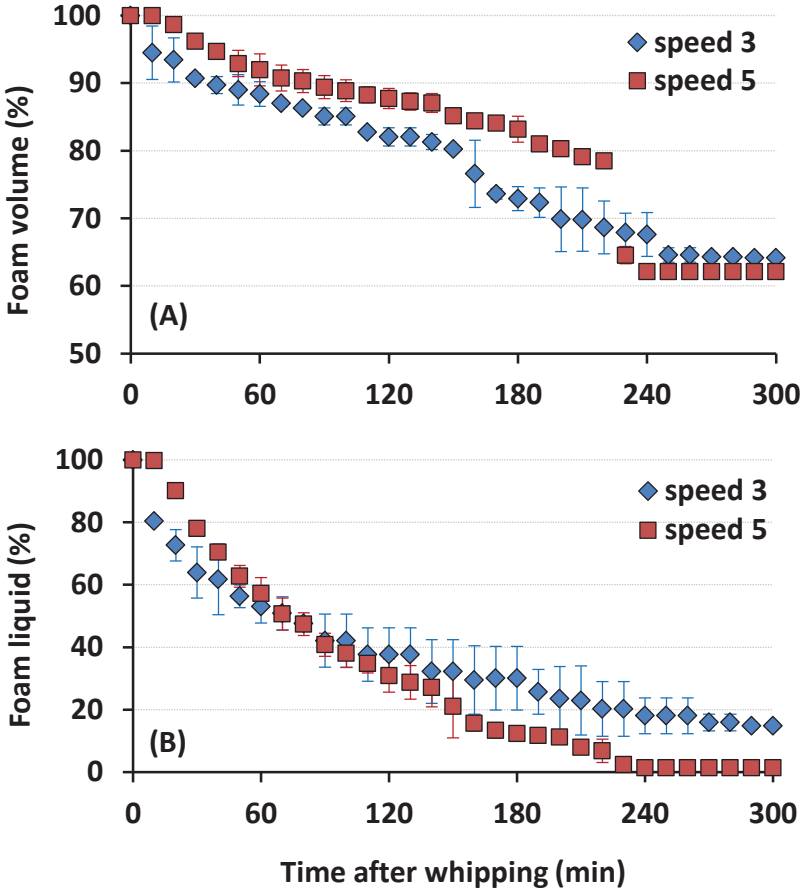

Figure 3.5 Stability of egg white foams with time after whipping at two different speed levels (speed 3 and 5); (A) foam volume stability and (B) foam liquid stability. Each data point is mean ± SD for n=2.

3.3.3 Temperature studies using egg white liquid

EWL solutions with two different temperatures (4°C and 20°C) were used to determine the effect of temperature on egg white foam. In this experiment, EWL was whipped for 5 min at speed 5.

3.3.3.1 Foamability

The foamability of EWL solution prepared at two different temperatures was

significantly different with 682 ± 6% and 724 ± 7.18% at 4°C and 20°C, respectively (Figure 3.6), indicating egg white foam was affected by the temperature of egg white solution ($p < 0.05$) (Appendix 2).

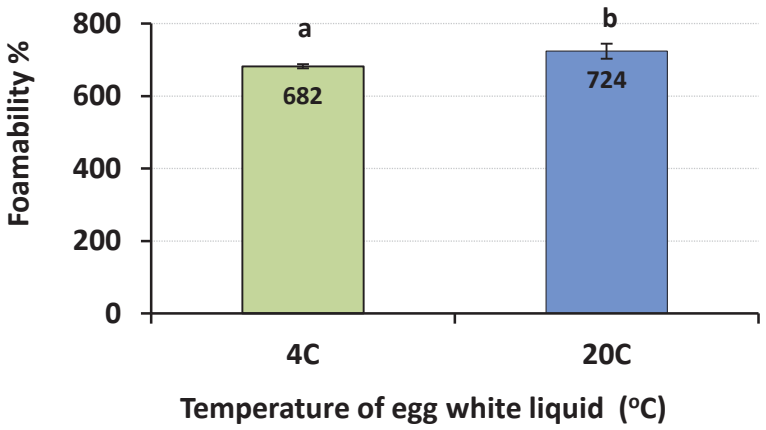

Figure 3.6 Foamability of egg white at two different temperatures.
[a,b] Means with the different letters are significantly different ($p < 0.05$). Each data point is mean ± SD for n=2.

3.3.3.2 Foam stability

Overall, the stability of foam against both foam volume and foam liquid drainage was higher when the EWL temperature was 20°C than 4°C prior to whipping (Figure 3.7A). The results show that the foam volume stability for the sample prepared at 4°C was slightly higher during the first 2 hr after the foam formation (Figure 3.7A). However, after 3 hr, the trend changed with the foam volume stability prepared at 4°C dropped significantly. Whereas the foam prepared form EWL at 20°C had a slow gradual decline in its foam volume. After 5 hr, the foam volume was 56 ± 2.3% and 65 ± 0.01% for their initial foam volumes at 4°C and 20°C, respectively. The results suggest that the foam prepared from EWL at a higher temperature of 20°C was more stable for a longer time than the foam produced from EWL at 4°C.

Regarding the foam liquid stability, there was no significant difference between the foams prepared from EWL solutions at 4°C or 20°C, with the 20°C foam showing slightly higher liquid drainage stability until 3 hr after the foam formation (Figure 3.7B). This was in accordance with the data that were recorded for the time required for 50% of liquid to drain. From the data, the liquid drainage of the foam produced from a EWL

solution at 20°C took around 70 min, while it took 40 min for the foam prepared at 4°C. There was a slight difference as shown in Figure 3.7B.

The temperatures of 4°C and 20°C used in the experiments did not exceed the denaturation temperature of egg white proteins, however, it might affect the viscosity of egg white solution, thus influencing the formation and properties of egg white foams, in terms of air bubble size and thinning of the liquid film between air bubbles, especially at relatively low protein concentration (10% protein) (Girton et al., 1999; Lomakina & Mikova, 2006). The foaming ability and foam stability which were observed to be higher in the foam produced from the EWL solution at 20°C might thus be due to the viscosity of egg white solution being lower at 20°C than at 4°C.

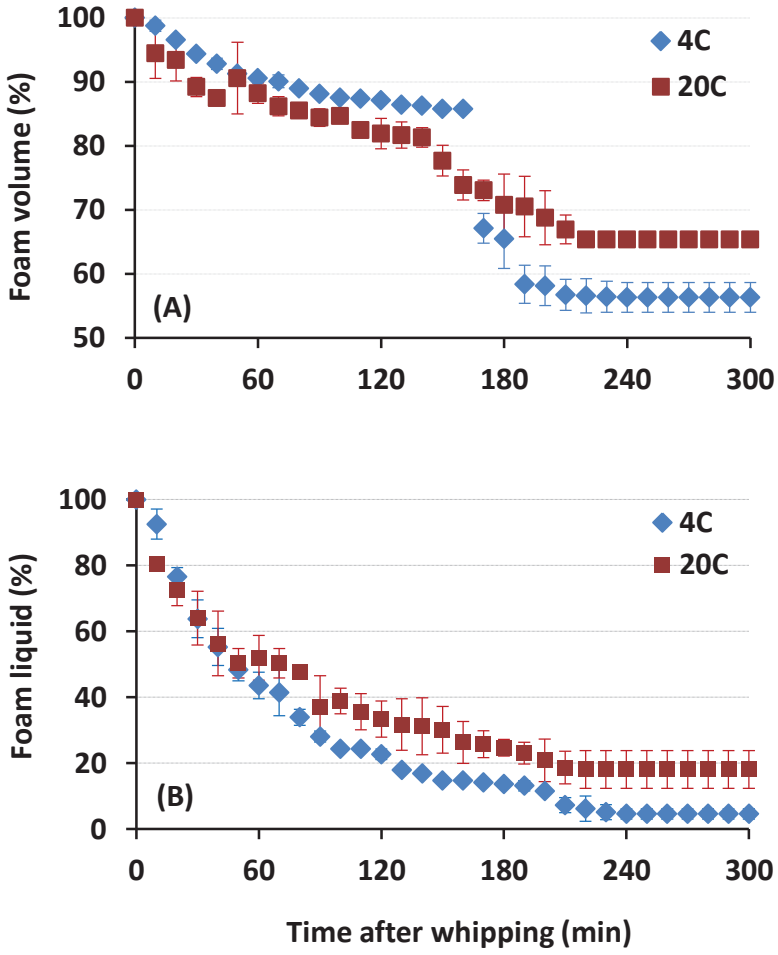

Figure 3.7 Stability of foams produced from egg white solutions at two different solution temperatures (4 and 20°C); foam volume stability (A) and foam liquid stability (B).
Each data point is mean ± SD for n=2.

3.3.4 Spray-dried egg white powder

Egg white is commercially available in three different forms, such as frozen fresh egg white, fresh egg white liquid and spray-dried egg white powder. In the previous experiments described above, frozen fresh egg white was used to prepare foam after thawing. In this experiment, spray-dried egg white powder was also used to prepare foam by dissolving in distilled water at 20^{o}C and then whipping for 5 min using a standard mix beater at speed 5. Hereafter, frozen fresh egg white is denoted as egg white liquid (EWL) and spray-dried egg white powder is egg white powder (EWP). The foamability and foam stability of EWP were compared to the results of EWL shown in Figures 3.6 and 3.7.

3.3.4.1 Foamability

When two different solutions of egg white containing 10% egg white protein derived from EWL or EWP were whipped using the standard mix beater under the whipping condition described above, the foamability of egg white from EWP was found to be significantly higher with a foaming capacity of 851 ± 2% compared to EWL with 724 ± 21% (Figure 3.8).

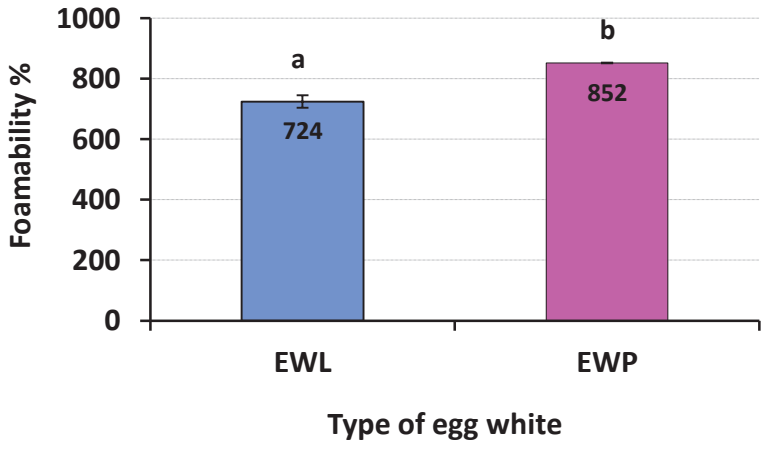

Figure 3.8 Foamability of egg white derived from egg white liquid (EWL) and egg white powder (EWP).

[a,b] Means with the different letters are significantly different ($p < 0.05$). Each data point is mean ± SD for n=2.

3.3.4.2 Foam stability

Figure 3.9A shows that there were also some differences in the foam stability between the two types of egg whites. The foam volume produced from EWP was more stable than that of EWL, in particular, much more pronouncedly after 2 hr. The foam volume for both EWL and EWP decreased gradually with time during the first 2 hr after the foam preparation. However, after 2 hr, the EWP foam remained stable and had no further significant decrease in its foam volume, resulting in 81% of the initial volume that remained after 5 hr. In contrast, the foam volume made from EWL dropped more markedly to around 62% after 5 hr (Figure 3.9A).

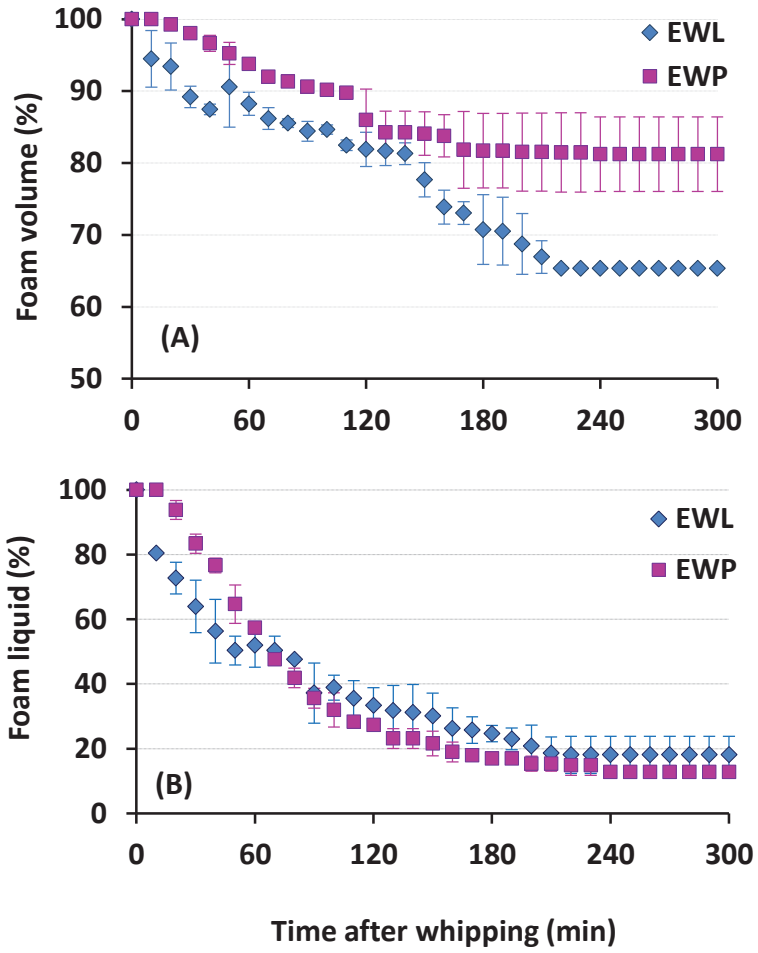

Figure 3.9 Comparison of foaming properties between fresh egg white liquid (EWL) and spray-dried egg white powder (EWP); (A) foam volume stability and (B) foam liquid stability.
Each data point is mean ± SD for n=2.

Regarding the foam liquid stability, the liquid drainage of both samples occurred gradually and steadily over the whole period of 5 hr (Figure 3.9B). The liquid stability of EWP foam was observed to be high during the first 1 hr after the foam preparation compared to that of the EWL foam. Overall, the results indicate that the functionality of egg white as a foaming agent was more effective with EWP than EWL. This may be due to changes in the physical and chemical properties of egg white proteins caused by spray drying, which may induce partial denaturation of egg white proteins. However, it should also be mentioned that freezing and thawing processes may however cause some changes in the functional properties of egg white proteins desirably or adversely, depending on the functional role of egg white proteins to be utilised and applied.

3.3.5 Egg white protein concentration using egg white powder

The effect of egg white protein concentration on the foaming ability and stability of egg white was investigated using spray-dried EWP. Aqueous solutions of egg white containing different protein concentrations (5, 10, 15 and 20%) were prepared by dissolving EWP in distilled water. They were whipped using the standard mix beater under the same whipping conditions.

3.3.5.1 Foam ability

Figure 3.10 shows that egg white protein concentrations had a significant influence resulting in an inverse relationship between protein concentration and foamability. The results showed a gradual decrease in the foamability as the protein concentration increased from 5% to 20%. Notably, the EWP solution containing 5% protein showed the highest foamability, up to nearly 936%, while the EWP solution containing 20% protein showed the lowest foamability with 696%. At 10% and 15% protein concentrations, their volumes were increased to 852% and 838%, respectively, after whipping (Figure 3.10).

The overall decline in foamability with increasing protein concentration of a solution can be due to the fact that high protein concentration enhances the viscosity of protein solutions which in turn does not allow a high volume of air to be incorporated at the

interface (Lau & Dickinson, 2005). Therefore, only a limited number of protein molecules can diffuse and unfold at the interface (Lau & Dickinson, 2005).

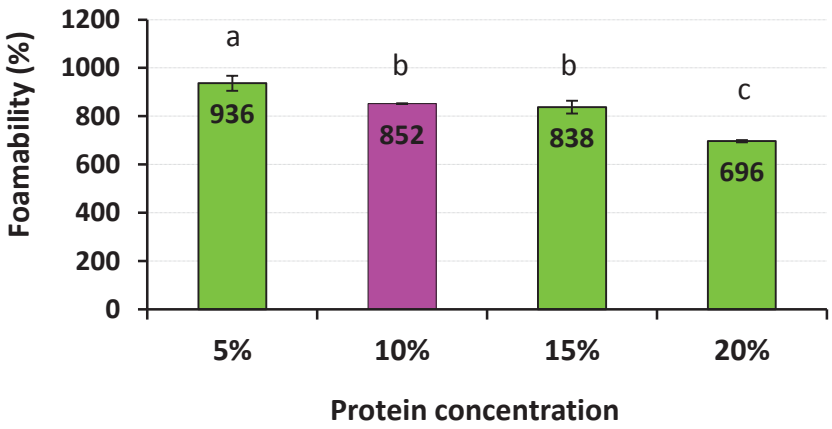

Figure 3.10 Foamability of spray-dried egg white powder (EWP) at different protein concentrations (5, 10, 15 and 20%).

Each data point is mean ± SD for n=2. [a,b] Means with the different letters are significantly different ($p < 0.05$).

3.3.5.2 Foam stability

Although the stability of foam in volume seemed to be higher at higher protein concentrations, it was not significantly different between the samples with different protein concentrations (Figure 3.11A). Notably, the foam liquid stability at 15 and 20% protein was substantially higher over the whole period than that at 5 and 10% protein (Figure 3.11A) (Appendix 3 & 4).

In contrast, the foam liquid stability trend at different protein concentrations showed significant variances (Figure 3.11B). The foams made from the egg white solutions containing 20% or 15% protein displayed a higher liquid stability over the whole period than those of 5% or 10% protein which exhibited a relatively low foam liquid stability and quick foam collapse (Figure 3.11B).

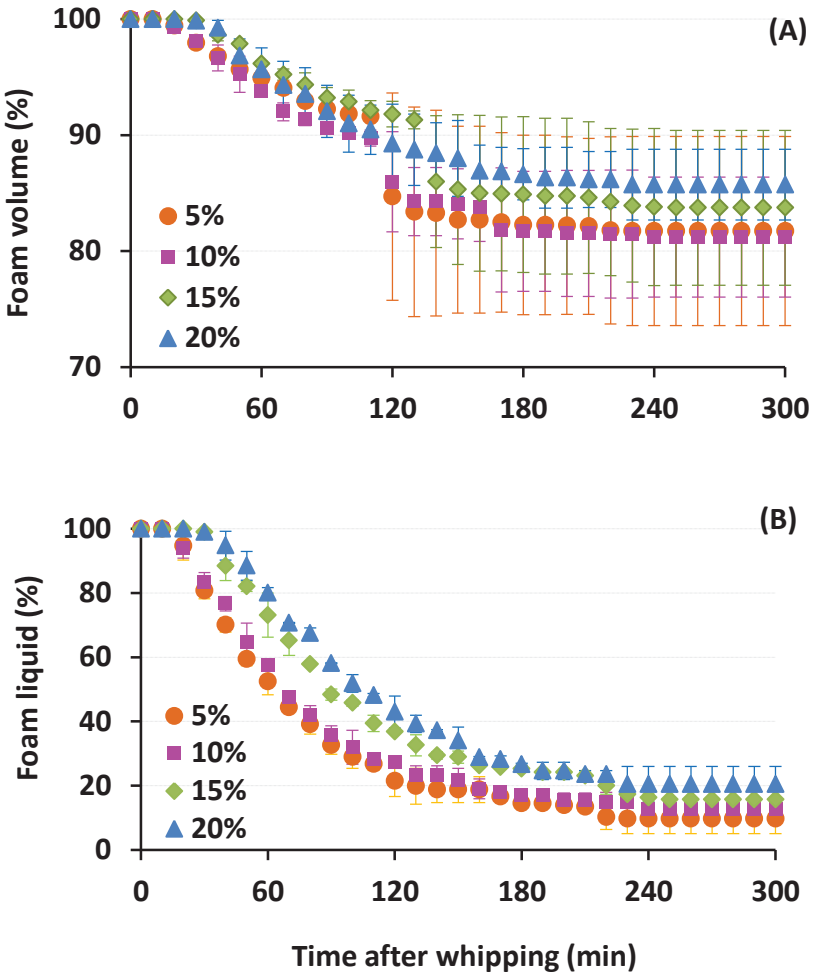

Figure 3.11 Foam stability of egg white at different protein concentrations (5, 10, 15 and 20%) made from EWP. (A) Foam volume stability and (B) foam liquid stability.
Each data point is mean ± SD for n=2.

The mechanisms behind the enhancement of foam stability regarding liquid drainage stability are considered to be rheological factors and conformal changes of protein structure at the air-water interface (Sanchez & Patino, 2005). First, when the protein concentration is high, the interfacial tension is reduced. This results in an improvement in bubble formation with smaller diameters. These bubbles contribute to film thickening which increases rupture time and thus delays foam collapse through conservation of the film liquid (Rodríguez Patino et al., 1995; Sanchez & Patino, 2005). Second, a high protein concentration (e.g. 20% and 15%) increases the viscosity of the protein solution and enhances the ability of foam to retain its liquid, resulting in an increased time for the whole liquid to be drained (Rodríguez Patino et al., 1995). The results shown in

Figure 3.8C indicate that the time taken to drain to half the volume that it started with was significantly longer for the foams with increasing protein concentration (e.g. about 1 hr at 5% protein while about 2 hr at 20% protein).

3.3.6 Effect of pH of egg white liquid

Proteins are susceptible to changes in their physicochemical properties due to denaturation when they are exposed to various environmental factors, such as pH, ionic strength and salt. Consequently, they undergo changes in their functional properties adversely or favourably, depending on the extent of protein denaturation.

The original pH of EWL used in this study was around pH 8.82. A range of different pH levels was examined to determine its influence on the foamability and foam stability of egg white solution at 20°C after whipping for 5 min at speed 5.

3.3.6.1 Foamability

Overall, the foamability of EWL at different pH values did not show significant differences between samples, except for pH 3. At pH 3, EWL had the highest foamability with an increase in its volume to 9.6 times (i.e. 960% foamability). At the original pH 8.82 of EWL, the foam volume was increased by 7.3 times (i.e. 735% foamability) which were similar to those shown in the previous sections (Figures 3.12).

It has been reported that pH values higher than the pI of egg white proteins have an adverse effect on foamability (Liang & Kristinsson, 2005; Kuropatwa et al., 2009; Hoppe, 2010; Bovskova & Mikova, 2011). The main reason for this reduction was explained to be due to the high level of solubility resulting from a high electrostatic repulsion between the protein molecules (Ferreira Machado et al., 2007). Therefore, the ability of proteins being adsorbed and interacted with each other at the air-water interface less favourable, resulting in less air bubble formation and consequently lowerfoamability (Ferreira Machado et al., 2007).

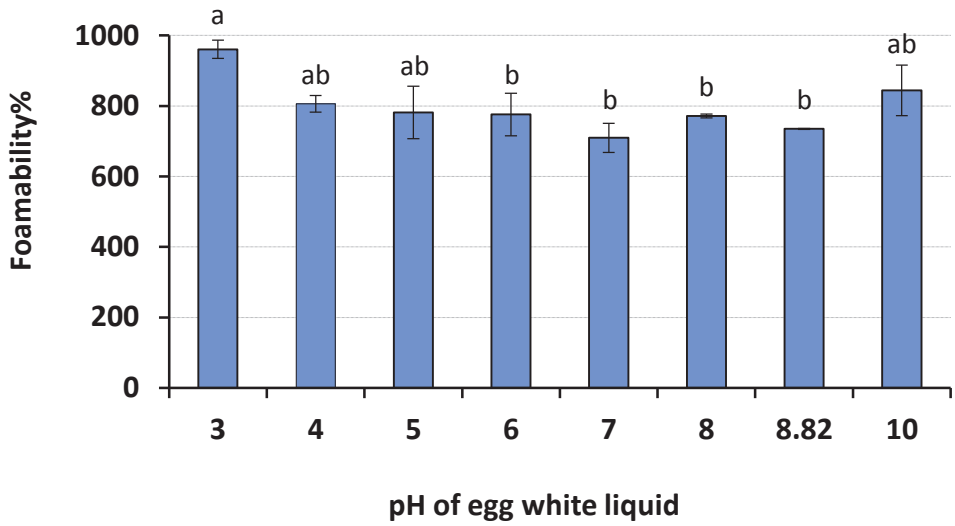

Figure 3.12 Foamability of EWL solutions at different pH 3-10. Original pH of EWL=pH 8.82.
Results are expressed as the means ± SD for two replications. [a,b] Means followed by the same letter are not significantly different ($p < 0.05$).

3.3.6.2 Foam stability

Foam volume stability was found to be significantly affected by pH. The differences among the EWL solutions with different pH values were significant at some pHs (Figure 3.13A). The EWL solutions at acidic pH 3, 4 and 5 or extreme alkaline pH 10 showed a significant ($p < 0.05$) enhancement of foam volume stability with higher than 85% after 5 hr (Appendix 5). On the other hand, the trend for EWL at pH 6, 7, 8 and 8.82 exhibited a relatively low level of foam volume stability over the same period with lower than 80%. In particular, at pH 6, the foam volume stability was observed to be very low compared to the other samples (Figure 3.13A).

The stability of foam against liquid drainage between samples with different pH values also exhibited some differences (Figure 3.13B & Appendix 6). Overall, the high stability of foam liquid drainage among EWL samples was observed from the foams made at low acidic pH 3, 4 and 5. In contrast, the foams prepared from EWL at alkaline pH 8, 8.82 and 10 exhibited relatively low foam stability against liquid drainage compared to the other samples.

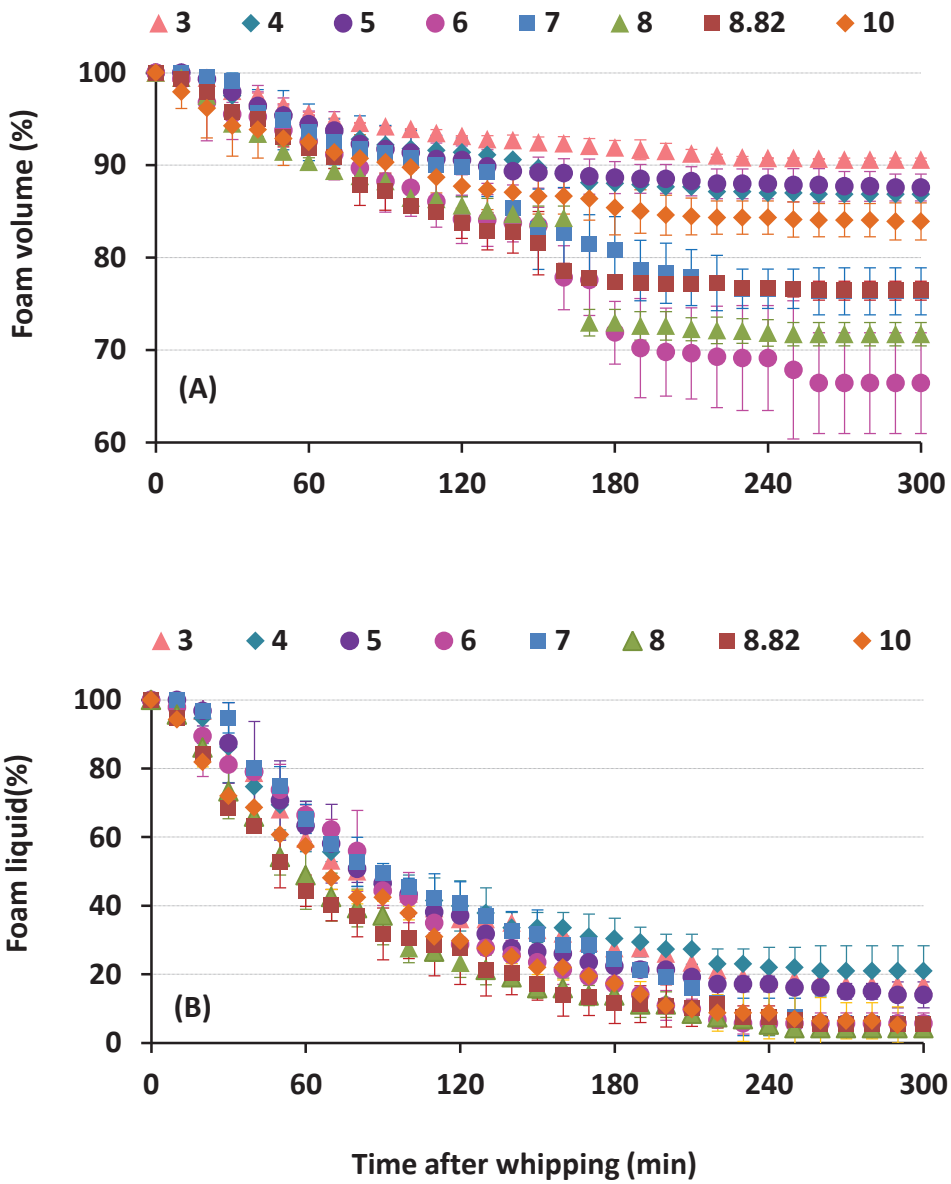

Figure 3.13 Stability of foams produced from EWL solutions at different pH 3-10. Original pH of EWL = pH 8.82. (A) Foam volume stability and (B) foam liquid stability.
Each data point is mean ± SD for n=2.

Some studies have shown that the maximum stability of egg white foam and the lowest amount of liquid drainage were observed at pH values near to the pI of egg white proteins (Clarkson et al., 2000). The pI of each egg white protein varies, as shown in Table 2.9 in Chapter 2, and differs from each other but many of them have their pI at around pH 4-5 and some proteins at around pH 6 (e.g. conalbumin) and at pH 10 (e.g.

lysozyme and avidin). The enhancement of foam liquid stability at acidic pH values was due to an increased rate of protein adsorption. This increase in protein adsorption at the interface resulted from low levels of aggregate formation through the hydrophobic interactions at the air-water interface (Clarkson et al., 2000).

Zeta potential (ζ) measures an electrical charge of particles (or molecules) dispersed in a solution. It indicates a degree of repulsion or attraction forces between adjacent particles dispersed in suspensions. The higher the zeta potential, the more stable the dispersed particles against their interaction. In general, dispersion systems with zeta potential values of greater than -30 mV or +30 mV confer system stability against aggregation, whereas the zeta potential values less than ±30 mV indicate less stable system (Silva, 2012). This means that the stability of a system can be reduced or lost when the zeta potential gets close to a net zero charge (i.e. 0 mV). In this study, the zeta potentials of egg white solutions with different pH values were measured, and the results are shown in Figure 3.14.

The zeta potentials of EWL solutions at pH 3, 4 and 5 were +32.65, +25.05 and +1.06 mV, respectively, whereas the zeta potentials of EWL solutions at pH 6, 7, 8, 8.82 and 10 were -14.38, -17.70. -18.63, -21.45 and -23.48 mV, respectively (Appendix 7). The zeta potential of +1.06 mV at pH 5 was very close to a zero charge because of the pH being around the pI of egg white proteins. As expected, at pH below 5, the zeta potential became more positively charged as the pH decreased whereas it became more negatively charged as the pH increased above the pH 5.

The original EWL solution at pH 8.82 was transparent while at pH 3, 4 and 5 it was observed to become turbid in appearance, indicating the formation of protein aggregates (Figure 3.15). This was in spite of the fact that the EWL solution at pH 3 had the zeta potential of +32.65 mV which was not very low and was believed to be large enough to be able to prevent protein aggregation via electrostatic repulsion. Also, the EWL at pH 4 was opaque and more turbid than the EWL at pH 3 and 5. This could be due to the fact that the pI of some egg white proteins is around pH 4-4.5 as shown in Table 2.9 in Chapter 2. This visual observation corresponded to the spectral absorbance of EWL which was measured at 600 nm as shown in Figure 3.16. The highest turbidity was observed from the EWL with pH 4 followed by pH 5 and then pH 3**.** This increase in turbidity of the EWL solutions at acidic pH 3, 4 and 5 was also in agreement with the work by Girton et al. (1999) and Bovskova and Mikova (2011).

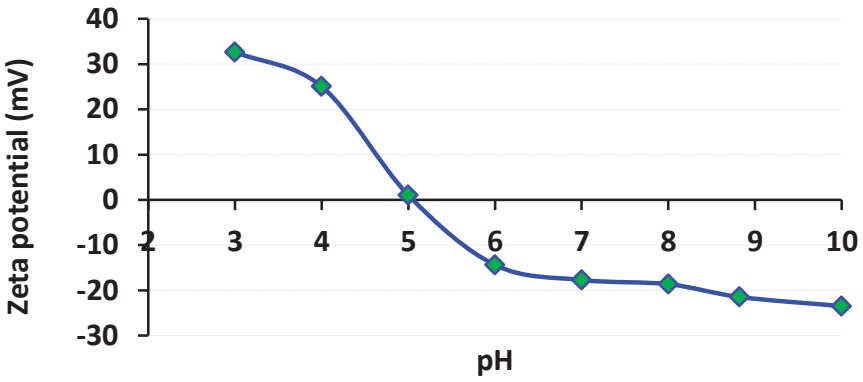

Figure 3.14 Zeta potentials (ζ) of EWL solutions containing 10% egg white protein at different pH values.

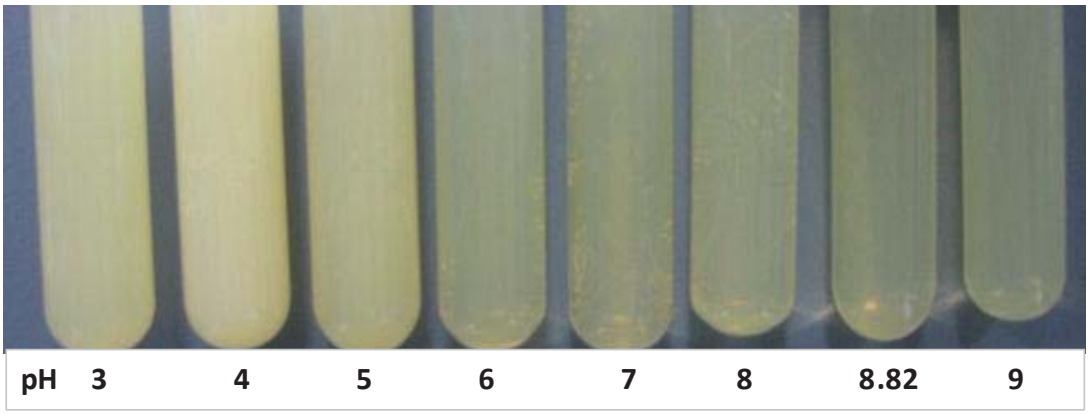

Figure 3.15 Pictures of EWL solutions (10% protein) with different pH values.

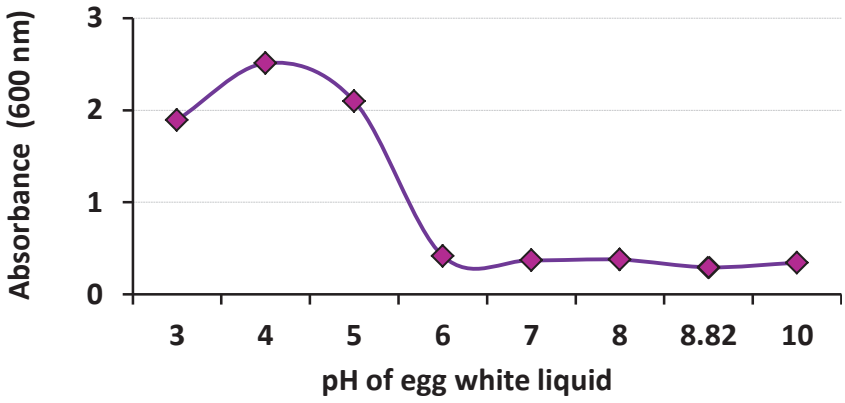

Figure 3.16 Turbidity of EWL solutions containing 10% protein at different pHs ranging from pH 3 to 10.
Each data point is mean ± SD for n=3.

At pH >6, the egg white proteins had a net negative charge. The appreciable aggregation of proteins was not observed at pH 6 or alkaline pH values, with the original EWL pH 8.82 giving the lowest turbidity result (Figure 3.16). This low aggregation rate can diminish protein adsorption at the air–water interface, which leads to low foam stability eventually (Liang & Kristinsson, 2005; Langel et al., 2011). This might have been one of the reasons for the low foam stability and high liquid drainage observed from the egg white foams at pH 6, 7, 8 and 8.82.

3.3.7 Addition of salt to egg white liquid solutions

The effect of two different types of salts (NaCl and $CaCl_2$) on egg white foam was analysed. The EWL solution with the original pH 8.82 at 20°C was whipped for 5 min at speed 5.

3.3.7.1 Foamability

The addition of sodium chloride (NaCl) at 10-400 mM or calcium chloride ($CaCl_2$) at 50-100 mM did not show significant differences in the foamability of EWL compared to the control (0 mM) (Figure 3.17). In the case of NaCl, although there were some differences but a consistent pattern that was attributable to different salt concentrations (e.g. low or high) was not observed (Figure 3.17A). Ercelebl and Ibanoglu (2009) demonstrated that the addition of NaCl at a range of concentrations from 0 to 30 mM increased the foamability of egg whites while a higher concentration above 30 mM NaCl had the opposite effect. The mechanism behind the adverse effect of salt addition on foamability may be attributed to the reduction in protein solubility at high salt concentrations. This decrease was due to a high level of protein aggregation which diminished protein adsorption at the interface and decreased foamability (Ercelebl & Ibanoglu, 2009).

In the case of $CaCl_2$, .the foamability for the control EWL sample with no added $CaCl_2$ was 774 ± 6% while it dropped to 709 ± 66% in the presence of 50 mM $CaCl_2$ and a further decrease to 666 ± 24% was obtained at 100 mM $CaCl_2$ (Figure 3.17B). However, the statistical data analysis revealed no difference between samples ($p > 0.05$) due to high variability in the data between replicate samples (Appendix 8). It has been reported

that an adverse effect of $CaCl_2$ on the foamability of egg white in comparison to NaCl can be attributed to the formation of salt bridges between proteins via $CaCl_2$ (Damodaran, 2005). As a result, it causes a slow rate of protein adsorption at the air-water interface during whipping, thus preventing the formation of a thin film (i.e. lamella) and impairing the foaming ability of egg white proteins (Damodaran, 2005).

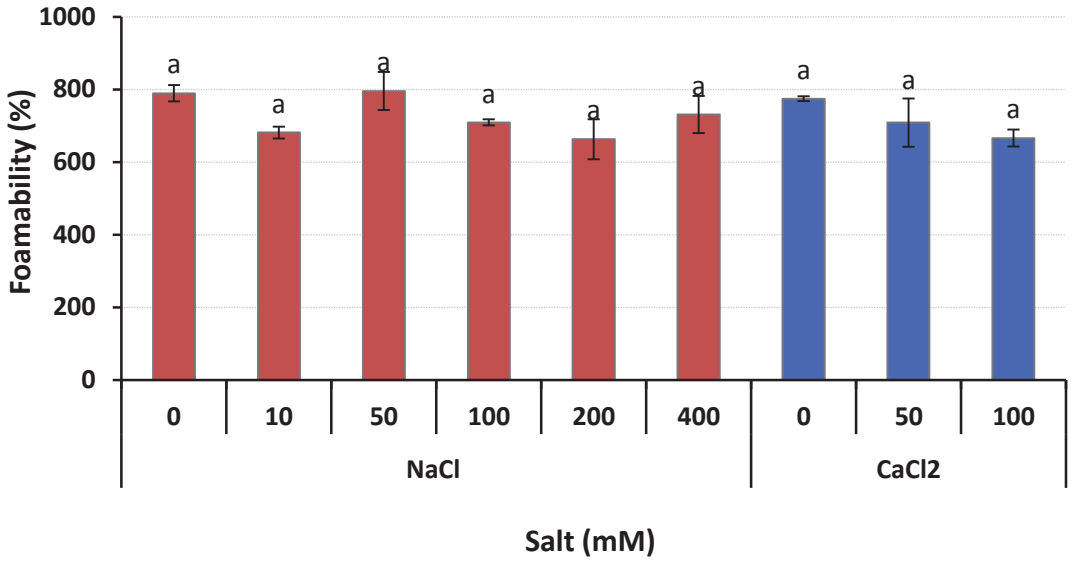

Figure 3.17 Foamability of EWL solutions added with different concentrations of NaCl at 0-400 mM or $CaCl_2$ at 0-100 mM.
Results are expressed as means ± SD for duplicate samples. [a] Means followed by the same letter within the same type of salt are not significantly different ($p < 0.05$).

3.3.7.2 Foam stability

The addition of NaCl or $CaCl_2$ also didn't cause a remarkable change in the foam stability of EWL foams compared to the control sample, regardless the concentrations used in this study. A minor difference was observed in foams with high salt concentrations (100, 200 and 400 mM), the stability of foam volume was less during the first 2 hr compared to the foams prepared with no added NaCl or low concentrations of NaCl (10 and 50 mM) (Figure 3.18A). After 2 hr the difference between EWL samples was significant ($p < 0.05$) (Appendix 9). Overall, the results show that all samples, regardless of the presence and absence of NaCl, had a similar pattern of a gradual decrease in foam volume stability with more than 85% of the total foam volume remaining after 3 hr (Figure 3.18A).

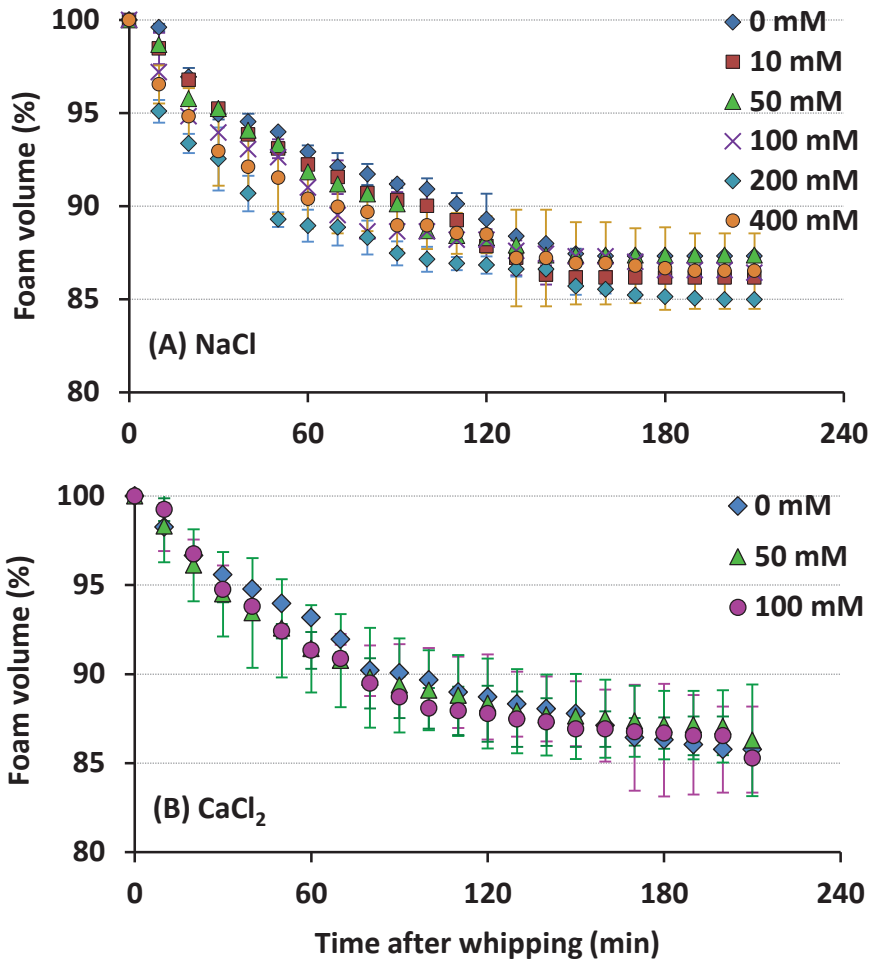

Figure 3.18 Foam volume stability of egg white foam in the presence of (A) NaCl and (B) $CaCl_2$ at different concentrations.
Each data point is mean ± SD for n=2.

A similar effect was also observed between samples with and without the addition of $CaCl_2$ in which the foam volume decreased gradually in all samples but there was no significant difference between them (Figure 3.18B). When two different types of salts (NaCl and $CaCl_2$) were compared for their effects on foam volume stability at the same concentrations of 50 mM or 100 mM, no differences were also observed between them.

The results of foam liquid stability against drainage are shown in Figure 3.19. Similar to the results of foam volume stability, the foam liquid stability was lower in the samples containing high concentrations of NaCl (100-400 mM) than the control sample or the samples containing low concentrations of NaCl (10 and 50 mM) during the first 2 hr

(Figure 3.19A). For the foams prepared with the addition of $CaCl_2$, the foam liquid stability did not differ from the control sample (Figure 3.19B).

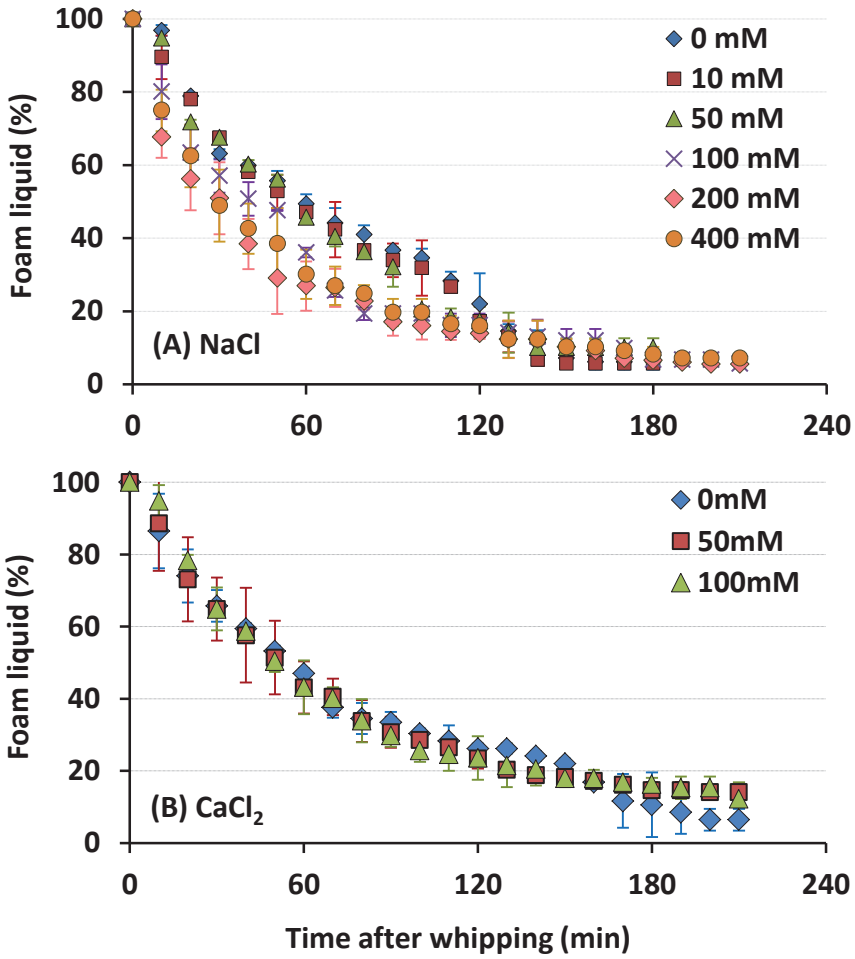

Figure 3.19 Foam liquid stability for egg white foam in the presence of (A) NaCl and (B) $CaCl_2$ at different concentrations.
Each data point is mean ± SD for n=2.

It was interesting to note that $CaCl_2$ had no significant influence on changes in the foam stability of EWL (i.e. foam volume and foam liquid) despite the fact that EWL had a considerable amount of protein aggregation prior to whipping, due to the addition of $CaCl_2$, as shown in Figure 3.20B. The extent of protein aggregation induced by $CaCl_2$ was higher at 100 mM than 50 mM which was visually noticeable and was also confirmed by the measurement of their turbidity. However, protein aggregation did not occur after the addition of NaCl as shown in Figures 3.20A and 3.21. The results of turbidity measurements indicated that the optical density decreased slightly with

increasing concentration of NaCl rather than increase, which may suggest disruption of some protein aggregates. It was also noticed that the addition of salts caused changes in the pH of EWL, especially with the addition of $CaCl_2$ rather than NaCl, as shown in Figure 3.22, despite of the fact that the protein concentration of all samples was maintained consistent at 9% including the control sample. Although the reason for the decrease in pH observed by the addition of $CaCl_2$ is not clearly understood, it may result from the release of hydrogen ions caused by the formation of salt bridges between acidic amino acids of egg white proteins and calcium ions.

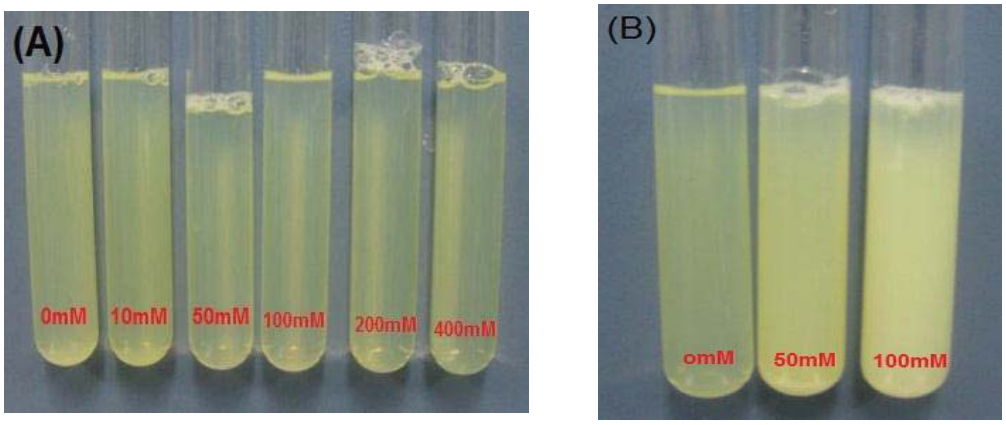

Figure 3.20 Pictures of liquid egg white solutions containing (A) NaCl and (B) $CaCl_2$ at different concentrations.

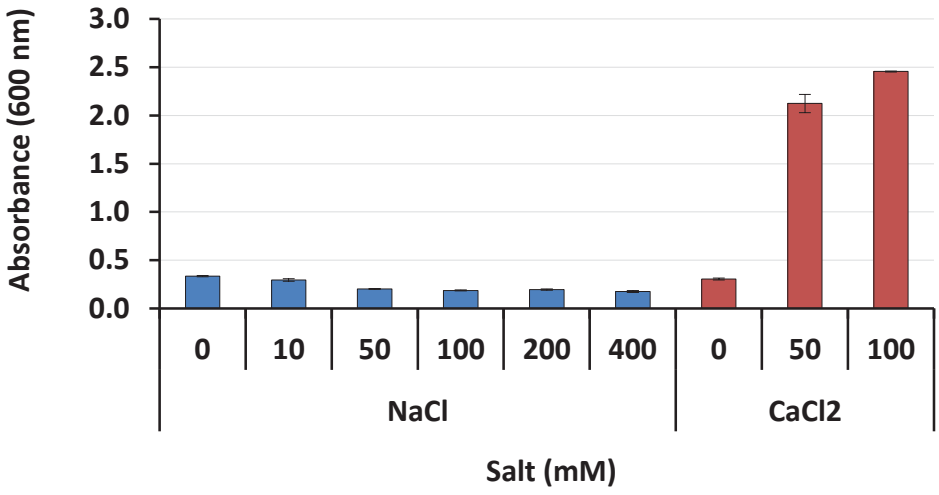

Figure 3.21 Turbidity of EWL solutions containing NaCl and $CaCl_2$ at different concentrations which was determined by measuring absorbance at 600 nm.
Each data point is mean ± SD for n=3.

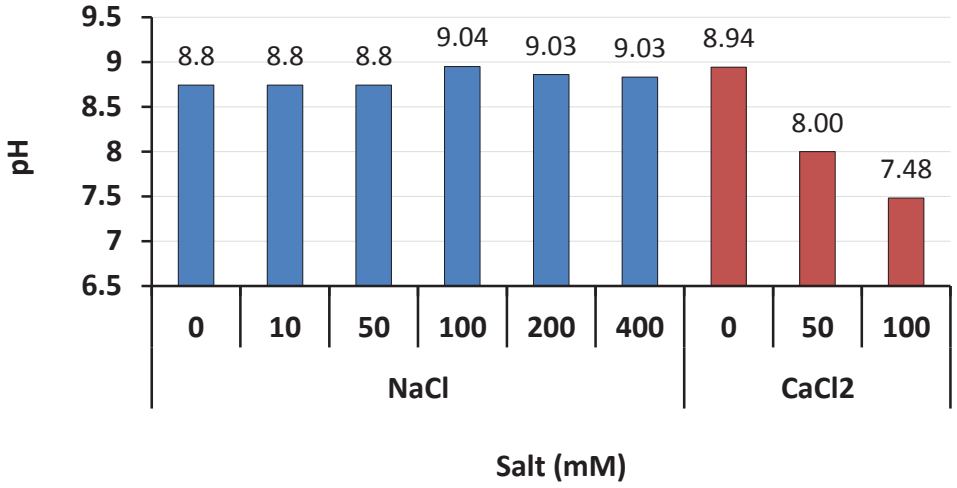

Figure 3.22 Changes in the pH of EWL due to the addition of NaCl and $CaCl_2$ at different concentrations.

It has been reported that protein aggregation causes an increase in liquid drainage rate due to a reduction in protein solubility and lower foam capability to retain the liquid (Stadelman & Cotterill, 1995). However, this effect was not observed in this study. On the other hand, as a result of the addition of salts, a significant change in the electrical charge of protein molecules was determined which decreased with increasing concentration of salt and its effect was more significant with $CaCl_2$ than NaCl (Figure 3.23). The electrical charge (zeta potential) of proteins in EWL prior to the addition of salts was between -15.63 mV and -16.73. This value decreased to -13.53 mV and -13.25 mV at 50 mM and 100 mM of NaCl, respectively, whereas it decreased further to -6.22 mV and -5.21 mV when $CaCl_2$ was added at 50 mM and 100 mM, respectively. The zeta potential of EWL containing NaCl at 400 mM was -8.77 mV (Figure 3.23).

In summary, although the chemical and/or physical properties of egg white proteins were significantly altered due to the addition of salts (NaCl or $CaCl_2$) into EWL, it did not lead to a significant change in the foamability and form stability of EWL compared to the control sample although some very minor differences in the foam stability occurred between the samples prepared with NaCl at different concentrations during the first 2 hr after whipping.

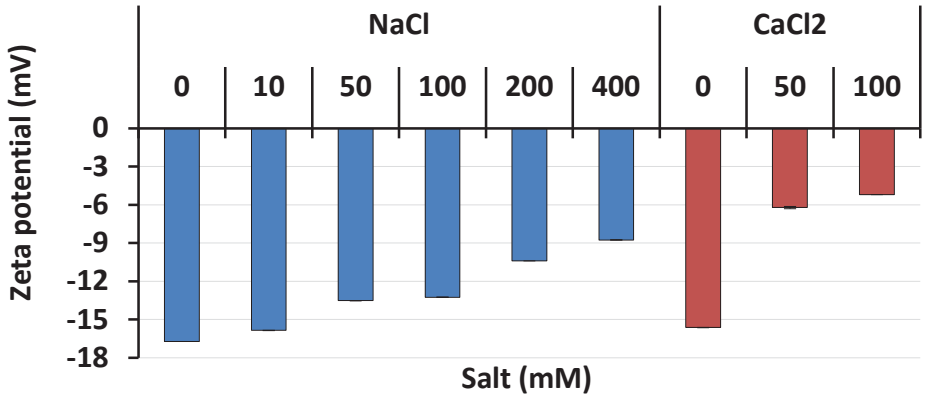

Figure 3.23 Zeta potential of EWL solutions containing NaCl and $CaCl_2$ at different concentrations.
Each data point is mean ± SD for n=2.

3.3.8 Addition of sucrose to egg white liquid solutions

The effect of sucrose on egg white foam was analysed by using the EWL solutions with the original pH 8.82 at 20°C and whipping for 5 min at speed 5.

3.3.8.1 Foamability

The addition of sucrose had a significant influence on the foamability of EWL (Figure 3.24A). It reduced the foamability of EWL from 769 ± 12.9% for the control sample with no added sucrose to 665 ± 19%, 680 ± 4% and 558 ± 60% for the EWL samples containing 5, 10 and 20% sucrose, respectively (Figure 3.24A).

The mechanism behind the effect of sucrose on the foamability of EWL is related to its effect on the viscosity of EWL (Kinsella, 1981; Rodríguez Patino et al., 1995; Hoppe, 2010; Macherey et al., 2011; Vega & Sanghvi, 2012). As a consequence of an increase in viscosity with added sucrose, the incorporation of air bubbles into the EWL can decrease. The increase in viscosity lowers reduction in the rate of protein-protein interactions and aggregations, and limits diffusion and unfolding of protein molecules at the air-water interface, thereby resulting in a reduction in foamability (Lau & Dickinson 2005; Raikos et al., 2007). Yang and Foegeding (2010) demonstrated that the addition of sucrose in a range between 4.27% and 63.6% decreased the foamability of egg white (10% protein), due to the increase in the viscosity of EWL solution mixed with sucrose. They revealed that the highest foamability was 800% at low value of apparent viscosity

of 1.5-2 mPa.s, whereas when the viscosity increased to 10-15 mPa.s, the foamability of EWL and sucrose solution dropped to around 300% (Yang & Foegeding, 2010). This means that the foam volume of EWL was reduced to more than a half when the viscosity increased.

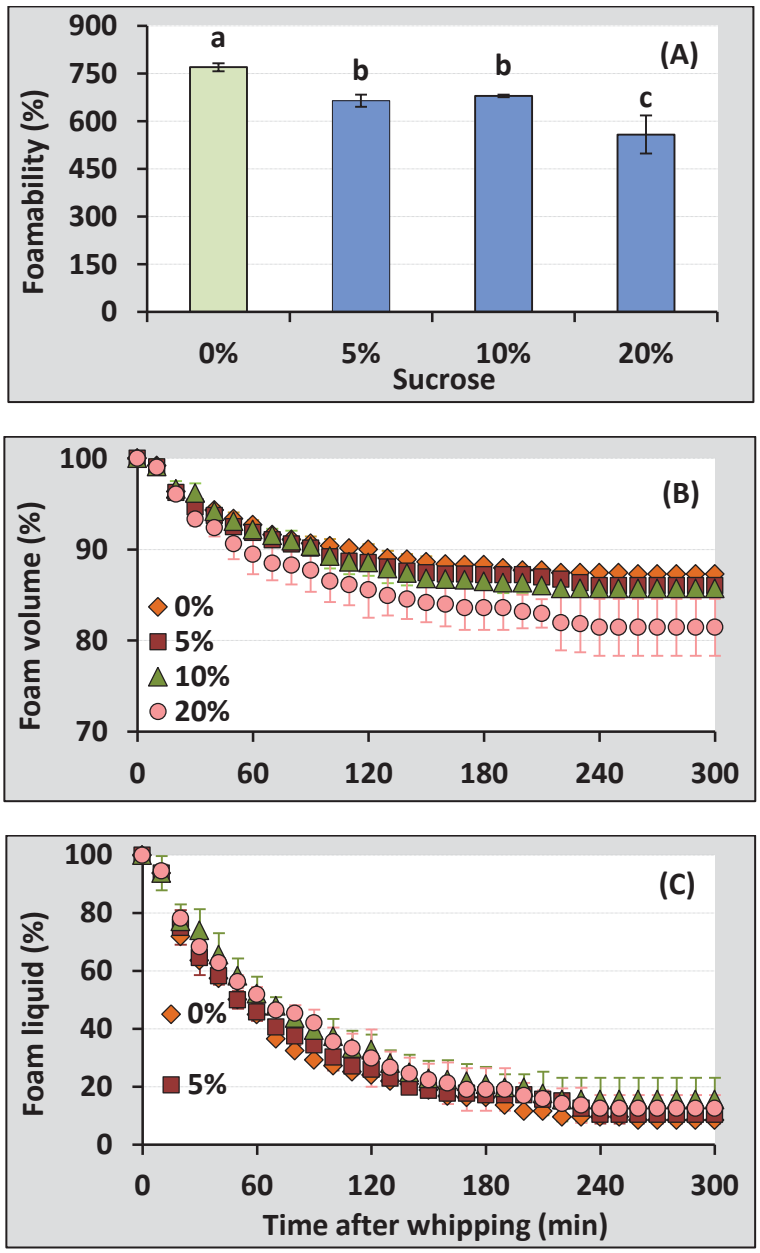

Figure 3.24 Effect of sucrose on the foamability and form stability of EWL; (A) foamability, (B) foam volume stability and (C) foam liquid stability against drainage.

Results are expressed as means ± SD for duplicate samples. Means followed by the same letter within the same type of salt are not significantly different ($p < 0.05$).

3.3.8.2 Foam stability

The stability of egg white foam in terms of foam volume was also found to be reduced by the addition of sucrose, especially at a high concentration of 20% sucrose, compared to the control and the other two samples containing 5% and 10% sucrose (Figure 3.24B). However, the stability of foam to liquid drainage seemed to be enhanced slightly by the addition of sucrose, particularly at 10% and 20% concentrations (Figure 3.24C) although the observed differences were not large. Studies have shown that sucrose enhanced the stability of egg white foam (Lau & Dickinson, 2005). The enhancement may be attributed to an increase in the viscosity of liquid in egg white solution (Lau & Dickinson, 2007) and an increase in protein molecules adsorbed at the interface (Lau & Dickinson, 2005) due possibly to the a decreased water activity (Rodríguez Patino et al., 1995).

3.3.9 Addition of hydrocolloids to egg white liquid solutions

Most hydrocolloids except some polysaccharides (e.g. gum arabic and modified starch) do not possess surface activity due to their chemical structure and properties, including no amphiphilic moieties, high viscosity, low flexibility and monotonic repetition of monomer units in the backbone of their chemical structures (Garti et al., 1999). In general, hydrocolloids are widely used in food as stabilisers and/or thickeners to confer viscosity to aqueous solutions, thus altering the rheological properties of fluids. Several different types of hydrocolloids (xanthan gum, guar gum and gum arabic at different concentrations).were added to EWL solution prior to whipping and determined for their effects on egg white foam properties, The results obtained were compared to the control sample (i.e. EWL with no added hydrocolloids). The EWL solutions mixed with hydrocolloids at 20°C were whipped for 5 min at speed 5.

3.3.9.1 Foamability

The addition of xanthan gum (XG) or guar gum (GG) to EWL did not significantly influence its foamability ($p < 0.05$), regardless of their concentrations (0.01, 0.02 and 0.04%), compared to the control sample with 783% foamability. However, gum arabic (GA) was found to adversely affect the foamability of EWL, resulting in a decrease to

672% and 599% at 1% GA and 2% GA, respectively (Figure 3.25). An adverse effect of hydrocolloids on foamability is associated with an increase in the viscosity of EWL. Increasing the viscosity of the EWL minimises the air incorporated into the solution, hence decreasing the number of air bubbles and reducing the foamability (Lau & Dichinson, 2005).

3.3.9.2 Foam stability

Unlike the foamability of EWL was not improved by the addition of XG or GG, the stability of foams produced in the presence of them seemed to be increased, particularly at the concentration of 0.04% XG or GG (Figure 3.25). The enhancement of foam volume stability was not observed at the lower concentrations of 0.01 and 0.02%. In contrast, GA was found to diminish the foam volume stability of EWL at both concentrations of 1 and 2% used, compared to the control sample (Figure 3.25). Nevertheless, the foam volume stability of all samples remained stable for a reasonably long time even after 5 hr of the foam preparation as more than 80% of the initial foam volume remained, regardless of the presence and absence of hydrocolloids.

Regarding the foam stability against liquid drainage, XG enhanced the stability of foam liquid drainage, particularly at 0.02% and 0.04% concentrations. However, the foam liquid stability improved by GG was not as significant as observed for XG (Figure 3.25). Interestingly, despite the fact that the addition of GA at 1 and 2% raised the viscosity of EWL, it did not result in an improvement in the foam stability unlike the other samples that were added with XG or GG (Figure 3.25).

The comparison between XG and GG for their influence on the foam stability (foam volume and foam liquid) was plotted at each level of three different concentrations in Figures 3.26. The stability of foam volume was slightly higher with XG than GG at 0.01% but it was not possible to differentiate between them at higher concentrations of 0.02% and 0.04%. For the stability of foam liquid drainage, XG showed the higher foam liquid stability against liquid drainage at all concentrations, particularly at 0.02% and 0.04% (Figures 3.26). The enhancement of foam liquid stability and delaying of the rate of liquid drainage may be related to the higher viscosity provided by XG compared to GG (Figure 3.27).

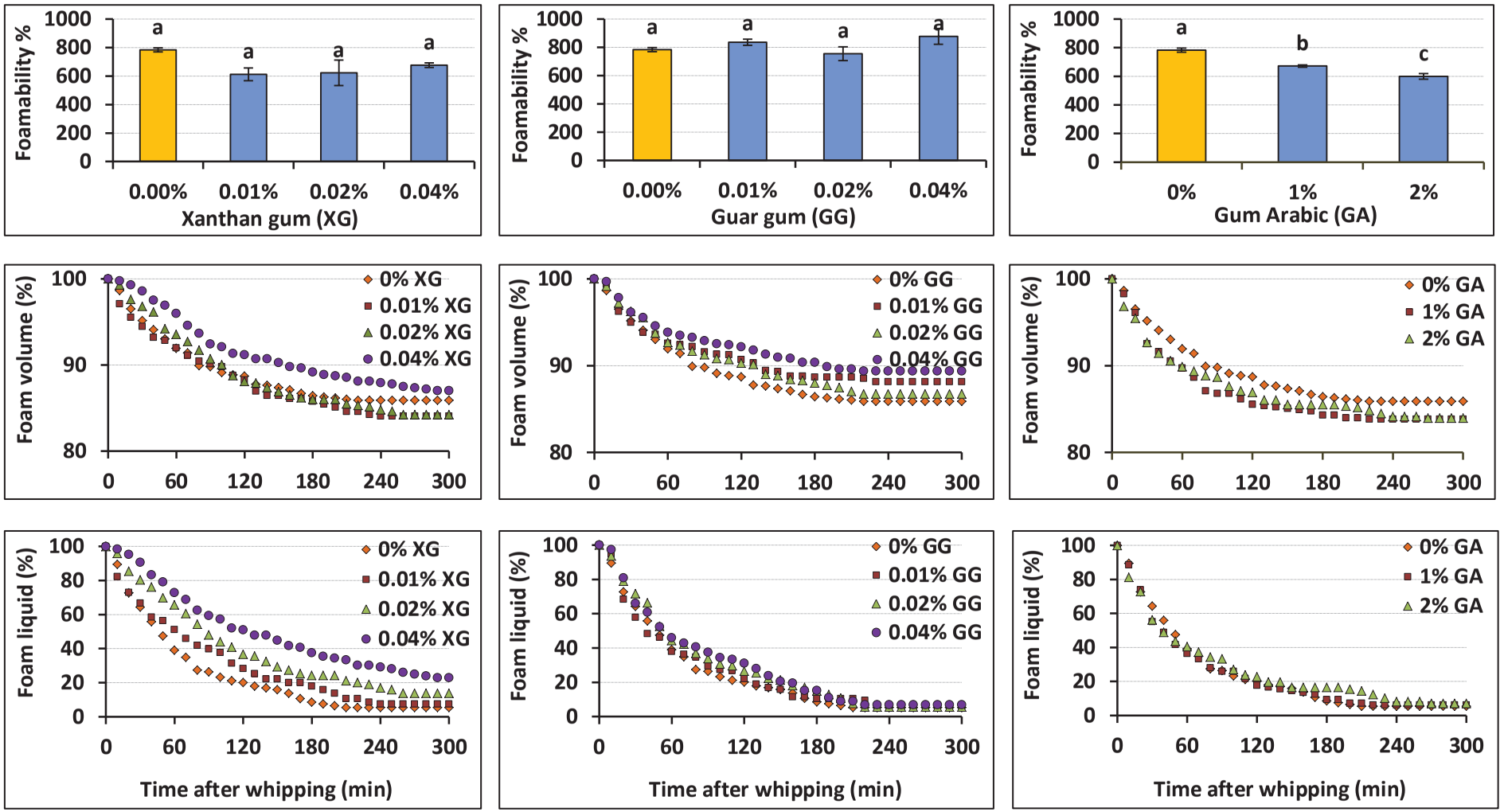

Figure 3.25 Effects of hydrocolloids on the foamability and foam stability of EWL

Each data point is mean ± SD for n=2. [a,b,c] Means with the different letters are significantly different ($p < 0.05$).

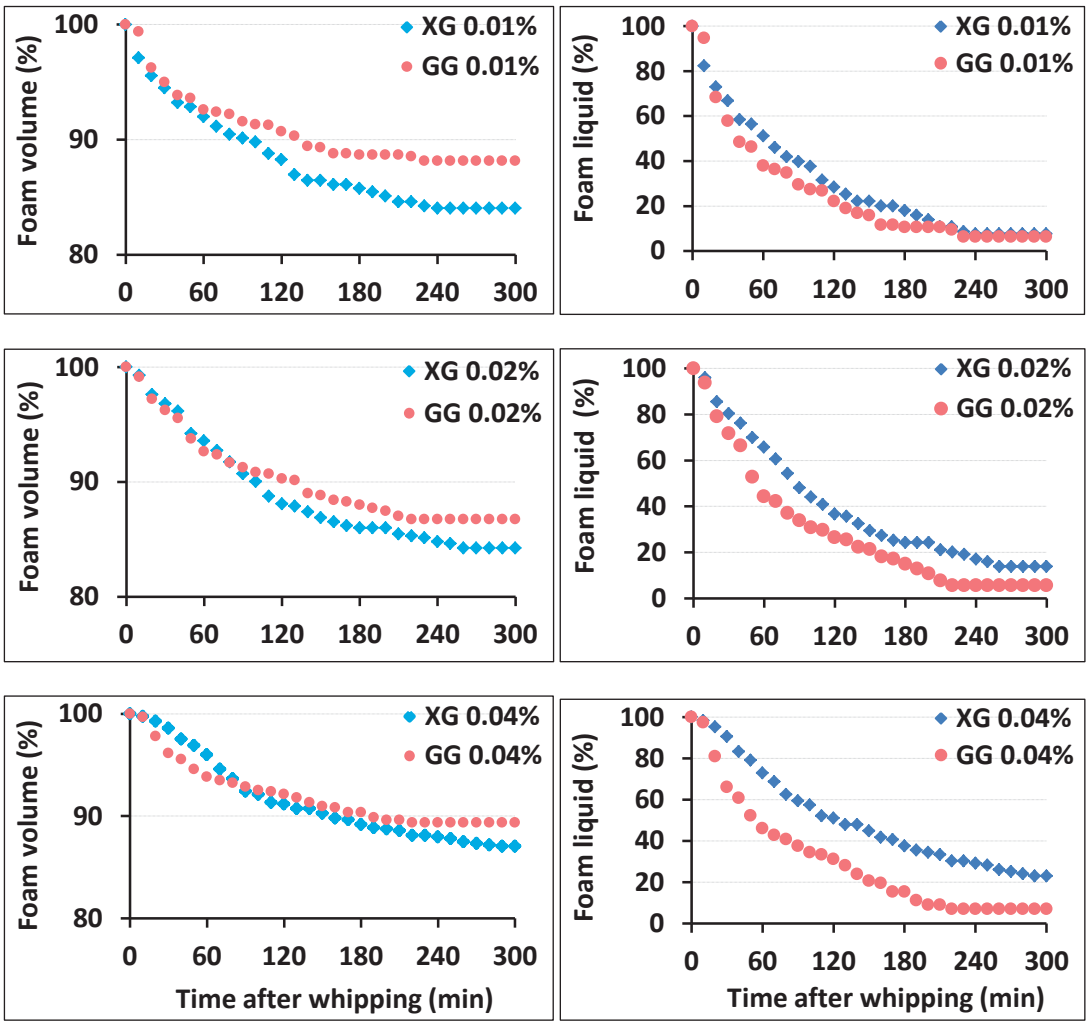

Figure 3.26 Comparison between xanthan gum (XG) and guar gum (GG) for their effect on the stability of foam volume and foam liquid drainage of egg white foam produced at different concentrations (0.01, 0.02 and 0.04%).
Each data point is mean ± SD for n=2.

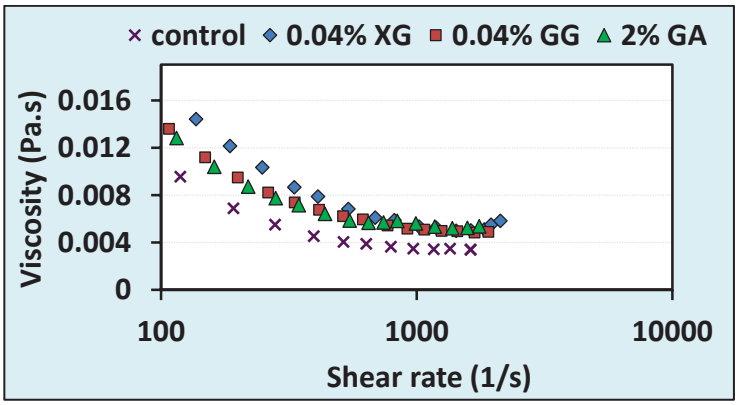

Figure 3.27 Viscosities of EWL solutions with and without added hydrocolloids.
Each data point is mean ± SD for n=2.

It can be concluded from this experiment that the addition of different types of hydrocolloids to EWL influenced the foamability and foam stability differently depending on their type and concentration. XG and GG enhanced the foam stability and foam liquid drainage at a high concentration of 0.4% whereas GA reduced the foam stability.

A slight enhancement of foam stability especially by XG and GG at high concentrations may be due to the interactions between XG and GG through hydrophilic interaction and the galactose side chain, respectively (Carp et al., 2001; Srichamroen, 2007). Both these interactions lead to a reduction in proteins' surface activity and an increase in film thickness (Dickinson, 1993). Increasing the film thickness eliminates rupturing of the liquid lamellae and inhibits the coalescence process (Damodaran, 2005; Lomakina & Mikova, 2006). Therefore, hydrocolloids increased foam volume stability and delayed foam collapse.

3.3 Conclusions

The volume of EWL solutions was increased to about seven to eight times its initial volume after whipping using the standard mix beater. The foamability of egg white was found to be affected by various factors, including whipping time, temperature and pH of EWL solution, type of initial egg white form used (i.e. EWL or EWP), egg white protein concentration and addition of sucrose. However, the addition of salts (NaCl and $CaCl_2$) at concentrations used in this study had no significant influence on foamability and foam stability compared to the control sample.

The foamability of EWL increased with increasing whipping times from 1 to 5 min but decreased when it was whipped for 7 min or longer. But the foam stability was observed to be higher when EWL was whipped for 9 min. Overall, whipping 50 g of EWL for 5 min using the standard mix beater was found to be optimal with regard to both foamability and foam stability. Temperatures of EWL between 4 and 20^{o}C had a significant influence on both foamability and foam stability which were higher at 20^{o}C than 4^{o}C. The concentration of egg white protein was another factor that had a significant impact but its effect differed between foamability and foam stability. Foamability decreased with increasing protein concentration from 5 to 20% protein but

foam stability (both foam volume and liquid drainage) was higher at higher protein concentration. In terms of the effect of type of egg white between EWL and EWP, EWP produced a significantly higher foam volume with higher stability, especially foam volume against foam collapse than EWL. This suggests that some physicochemical changes that egg white proteins encountered during spray drying process may have resulted in changes in their functionalities more suitable as foaming agents.

With regard to the effect of pH, a notable effect on foamability was observed when the pH value of EWL solution was adjusted to very low acidic pH 3 from its original pH 8.82 but no significant differences were observed in samples with other pH levels. On the other hand, it was found that egg white foams prepared at low acidic pH 3-5 or alkaline pH 10 were significantly more stable over time after foam preparation, especially against foam collapses. The high foam stability observed in EWL solutions at low pH could be due partly to protein aggregation that occurred at those low levels of pH but the reason for the high foam stability observed at pH 10 is not clearly understood.

Some ingredients such as sucrose and hydrocolloids affected the foam properties of egg white. The addition of sucrose at 10-20% concentrations decreased the foaming ability of egg white compared to the control sample with no added sucrose. However, the foam stability was not affected by the addition of sucrose. Regarding the influence of hydrocolloids (XG, GG and GA), their effects on foamability were not significant compared to the control sample, except for GA added at 1 and 2% concentrations which caused a significant decrease in foamability. On contrary, the foam volume stability was increased by the addition of XG and GG but decreased by GA. The results suggest that different types of hydrocolloids affect foamability and foam stability differently depending on their type and concentration.

Overall, the results showed that egg white produced a large volume of foam, indicating egg white proteins have excellent foaming properties but the formation of foam and the stability of foam after preparation are affected by many different variables as tested in this study. Their functional properties as foaming agents can be manipulated by altering processing conditions and compositional variables.

Chapter 4 Formation and characterisation of egg white foams produced by sparging method using a whipped cream dispenser

4.1 Introduction

Different methods have been used to produce foam, including whipping, sparging and shaking methods. Whipping method uses mechanical equipment such as Kitchen Aid blender and standard mix beater. Sparging method involves an injection of gas (e.g. nitrogen and carbon dioxide) into solution using a specific apparatus or a pressure device. Shaking method relies on shaking a container (e.g. can and bottle) containing a solution to be whipped until foam is produced.

In the experiments for Chapter 3, whipping method was used to make egg white foams from EWL or EWP solutions using the standard mix beater. In Chapter 4, sparging method was explored to produce egg white foams using a whipped cream dispenser with a nitrous oxide (NO_2) gas charger which is commonly used in food restaurants. With this device, the injected NO_2 gas is absorbed by the egg white solution loaded into the dispenser, producing high gas pressure inside. When the pressurised egg white solution is released through a nozzle from the dispenser, the absorbed gas expands rapidly to form bubbles stabilised by egg white proteins. The type of foam produced by using this device is similar in appearance to whipped cream as air bubbles are very small in size and highly condensed ("Stack exchange", n.d.). Some advantages of this method are a rapid foam formation and easy and simple operation On the contrary, disadvantages can include relatively low foam volume and foam stability and a high expense of foam production due to the cost of NO_2 gas chargers.

No studies have been shown in regard to the formation of egg white foam using a sparging method with a whipped cream dispenser. Therefore, the objective of this study was to investigate the use of this foaming device to produce and characterise egg white foam. Several variables associated with foaming conditions (e.g. shaking time, temperature and volume), thermal treatment and ingredient formulation (concentration, pH, salt and other ingredients) were investigated to understand their impact on the foamability and foam stability of foams produced by using the whipped cream dispenser.

4.2 Materials and Methods

4.2.1 Preparation of egg white foams

In this study, egg white foams were prepared from EWL or EWP solutions using a whipped cream dispenser (0.5 litres size) with a nitrous oxide (NO_2) gas charger (8 g pure NO_2 per charger) (Mosa cream whipper, Mosa Industrial Corp., Yunlin, Taiwan) (Figure 4.1). According to the manufacturer's guidelines, one charger can whips up to 0.5 litre of solution (e.g. whipping cream, desserts, mousses, sauces, etc). Briefly, an aliquot amount of EWL or EWP solutions (50 g unless otherwise stated) was poured into the whipped cream canister. The canister was tightly closed with a top head which had a metal nozzle part (attachable with a decorator tip), a lever arm and a metal holder (to be attached with a gas charger cylinder holder).

After inserting the NO_2 gas charger into its cylinder holder, the cylinder holder was attached to the metal holder on the canister head and twisted clockwise until it was locked into position. Upon placed into a lock position, the NO_2 gas was released into the canister containing the egg white solution. The canister was then shaken up for 20 times (unless otherwise stated) to enhance the sparged gas to be uniformly transferred into and absorbed by the egg white solution, thus generating gas pressure inside the canister. The dispenser was hold upside down pointing the nozzle tip down and triggered to release the foam from the canister into a glass beaker (250 ml) by pressing the lever (Figure 4.1).

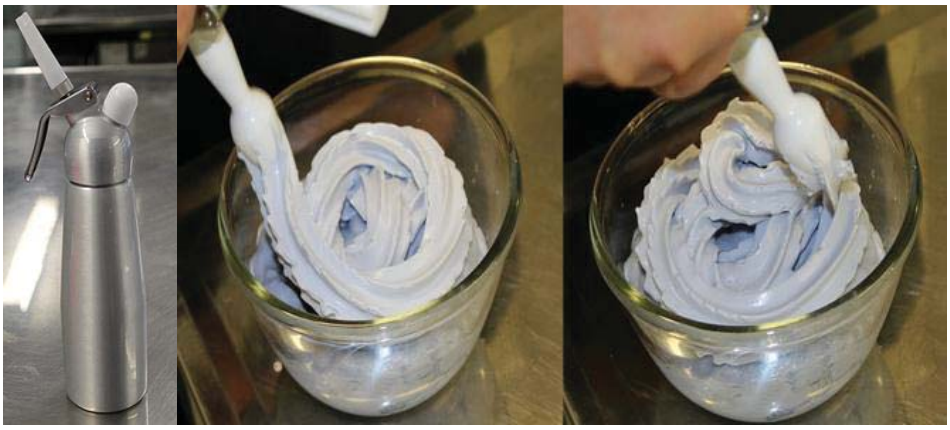

Figure 4.1 Pictures of egg white foams produced from EWL solution using a whipped cream dispenser.

4.2.2 Analysis of foamability and foam stability

All foams produced were analysed immediately after dispensing into a beaker (250 ml) for the measurements of foamability and foam stability using the methods described in Section 3.2.4 in Chapter 3. In this study, the stability of foams produced from a cream whipper was monitored and recorded at 5 min intervals over time for 15, 30 or 45 min, depending on types of samples prepared.

4.2.3 Effects of several factors on foamability and foam stability

Various factors (shaking time, volume, type of egg white, temperature, sucrose, hydrocolloids and protein concentration) were investigated to determine their effects on foamability and foam stability. All foams produced were analysed immediately for foamability and foam stability.

4.2.3.1 Number of Shakes for egg white powder solution

The effect of number of shakes prior to dispensing egg white solution from the whipped cream dispenser was studied. The dispenser containing a 50 g of EWP solution was shaken for 10, 20, 30, 40 and 50 times. Foams produced were analysed for foamability and foam stability.

4.2.3.2 Volume of egg white powder (EWP) solution

The effect of different volumes of egg white on foamability and foam stability was studied. Different volumes of EWP solution (10 w/v% protein) (50, 100, 200 and 400 g) were measured out and used for foam formation by shaking for 10, 20, 30, 40 and 50 times at 20±1°C. Foams produced were analysed for foamability and foam stability.

4.2.3.3 Type of egg white (EWL & EWP solution)

EWL and EWP solutions were compared for their ability to produce and stabilise foams at the same level of protein concentration (10%). A total of 50 g of sample at 20^{o}C was

used to produce foam after shaking 20 times. Foams were analysed for foamability and foam stability.

4.2.3.4 Egg white solution temperature (4 and 20^{o}C)

Effect of temperature of egg white solution at 4 and 20^{o}C on foamability and foam stability was determined. Foams were produced from a 50 g of EWP solution (10 wt. % protein) using the whipped cream dispenser after shaking for 20 times.

4.2.4 Effect of sucrose and hydrocolloids

4.2.4.1 Addition of sucrose at different concentrations

EWP solutions containing 10% and 20% w/v protein were prepared and mixed with sucrose at different concentrations to have five different formulations with different protein and sucrose concentrations (Table 4.1). The control sample with no added sugar was denoted as p/10/s9. A 100 ml of EWP solution containing different concentrations of sugar was taken and used to produce foam by using the cream whipper after shaking for 20 times. After preparation, foams were analysed immediately for foamability and foam stability as described in Section 3.2.4 in Chapter 3.

Table 4.1 EWP solutions containing different concentrations of sucrose and protein formulated to make foams using a cream whipper.

Sample code	Protein (w/v%)	Sucrose (w/v%)	Total solid (%)	Sucrose (g)	EWP solution (g)	Total (g)
p10/s0	10	0	10	0	100[a]	100
p9/s10	9	10	19	10	90[a]	100
p8/18	8	18	26	20	90[a]	110
p18/s10	18	10	28	10	90[b]	100
p16/s18	16	18	34	20	90[b]	110

[a] EWP solution containing 10% (w/v) protein
[b] EWP solution containing 20% (w/v) protein

4.2.4.2 Addition of hydrocolloids

All three types of hydrocolloids (XG, GG and GA) were dissolved into EWP solution (10 w/v% protein) at different concentrations and ratios by using a magnetic stirrer (Table 4.2). Each of all five formulations including the control sample was divided into two portions. One portion was stored at 4±1^{o}C and the other was at 20±1^{o}C. Then, foams were produced from each sample after shaking for 20 times by using the cream whipper and analysed for foamability and foam stability as described in Section 3.2.4 in Chapter 3.

Table 4.2 Formulations of EWP solutions containing a mixture of three types of hydrocolloids at different concentrations.

Sample code	XG (w/w%)	GG (w/w%)	GA (w/w%)	EWP solution[a] (g)	Total volume (ml)	Protein (%)
Control	0	0	0	100	100	10
X2/G2/GA	0.02	0.02	2	99	100	9.9
X2/G4/GA	0.02	0.04	2	99	100	9.9
X4/G2/GA	0.04	0.02	2	99	100	9.9
X4/G4/GA	0.04	0.04	2	99	100	9.9

[a]EWP solution containing 10 w/v% protein
Abbreviations, XG, GG and GA, represent xanthan gum, guar gum and gum arabic, respectively

4.2.5 Heat treatment of egg white liquid (EWL)

The effect of thermal treatment of EWL (10 w/v% protein) on denaturation and aggregation of egg white proteins was studied. First, EWL was equilibrated for 30 min to 20 ± 1°C in a water bath. Then, it was heat-treated to 30, 40, 50, 53, 55, 57, 59 and 60^{o}C by placing glass test tubes containing 8 ml of EWL in a 60 ± 0.5°C water bath. To monitor the temperature of samples, a glass test tube containing 8 ml EWL solution with a thermometer inserted was also placed in the water bath. When the EWL solution in the tubes reached the required respective temperature, the tubes were taken out from

the water bath and placed immediately in an ice water bath to cool down. The time taken for the EWL solution to reach each of the required temperatures was recorded (Table 4.3). After cooling, the samples was analysed for their turbidity using the method described in Section 3.2.7.2 to indirectly estimate the degree of protein denaturation and aggregation.

Table 4.3 Time taken for EWL solutions (8 ml) to reach from 20^{o}C to a desired temperature (30-60^{o}C) using a 60°C water bath

Temperature (^{o}C)	Time (s)
20	0
30	17
40	36
50	68
53	76
55	102
57	123
59	169
60	194

Another set of EWL samples were heat-treated at five different temperatures (55, 58, 60, 63 and 75°C). A 400 g of EWL was poured into a glass bottle and heated in a water bath. Once the temperature of EWL reached the required temperature, timing was started and kept for the required time (min) as shown in Table 4.4. Then, the bottle was placed in an ice bath for nearly an hour before foam production. A 100 ml of EWL was poured into the whipped cream dispenser. After shaking 20 times foams were produced and analysed.

Table 4.4 Heat treatment of EWL at different temperatures for different times.

Temperature (°C)	Time (min)
58	3.5 (pasteurization temp)
60	2
63	2

4.2.6 Heat treatment of EWL containing hydrocolloids and sucrose

The experiment in this section was performed same as Section 4.2.5, with minor differences. Before the heat treatment, EWL was mixed with sucrose, citric acid, xanthan gum, guar gum, locust bean gum and gum arabic (Table 4.5). Then, the EWL solution containing these ingredients were heat treated at 20, 58, 60 and 63^{o}C. After that, EWL foams were produced as described in Section 4.2.1 and analysed as described in Section 3.2.4.

Table 4.5 Formulation of EWL with ingredients

Ingredients	Percentage (w/v%)
Sugar	20
Citric acid	0.05
Xanthan gum	0.04
Guar gum	0.04
Locust bean gum	0.04
Gum arabic	2

4.2.7 Statistical analysis of data

All experiments for the sample preparation and analysis were carried out at least in duplicate. The results were reported as average and standard deviation. The data were statistically analysed using a Minitab statistical software version 16 (Minitab Inc., USA). A one-way ANOVA using a Turkey method was used to determine the significance of means at a 95% confidence level ($p < 0.05$).

4.3 Results and Discussion

4.3.1 Shaking time for egg white powder solution

The whipped cream dispenser containing a solution to be foamed was manually shaken, after charging with nitrous oxide (NO_2) gas, to enhance the absorption of the injected gas effectively into the solution by increasing their contact through shaking. Shaking should be done appropriately, neither too short nor too long shakes, as it can affect the foaming properties of egg white. In this study, the whipped cream dispenser containing a 50 g sample of EWP solution at 20°C was shaken for different times (10, 20, 30, 40 and 50 times) to determine the effect of shaking time on the formability and foam stability.

4.3.1.1 Foamability

It was found that the number of times the dispenser, containing EWP solution, was shaken had a significant effect on foam appearance as shown in Figure 4.2. Shaking more than 20 times produced a foam that was not stiff and had some large air bubbles. In contrast, shaking less than 20 times formed thick, dense, creamy foam with a bright whitish colour and glossy appearance due to more compact uniform gas bubbles in the foam.

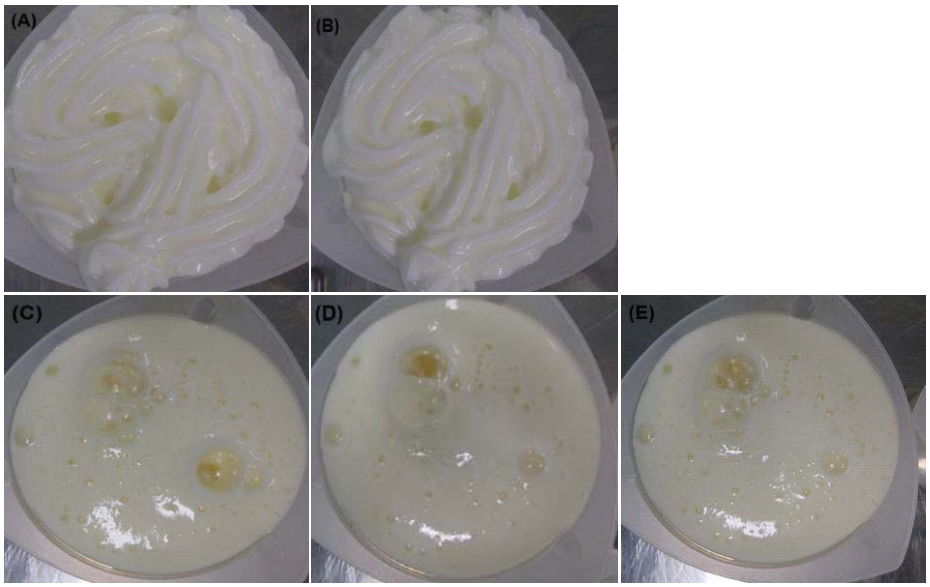

Figure 4.2 Egg white foam appearance after shaking different numbers of times made from EWP at 10% (w/v, protein); (A) 10, (B) 20, (C) 30, (D) 40, and (E) 50 times.

Although there was a remarkable difference in the foam appearance between foam produced after shaking for less than 20 times or more, there was no significant difference between the foam volumes produced ($p = 0.067$), ranging from 301 to 347% (Figure 4.3). The results indicate that the EWP expanded to about 6-7 times in volume from its initial volume of 50 ml. This was considerably lower than the foamability of EWP obtained by a whipping method using the standard mix beater, which was shown in Figure 3.8 for Chapter 3.

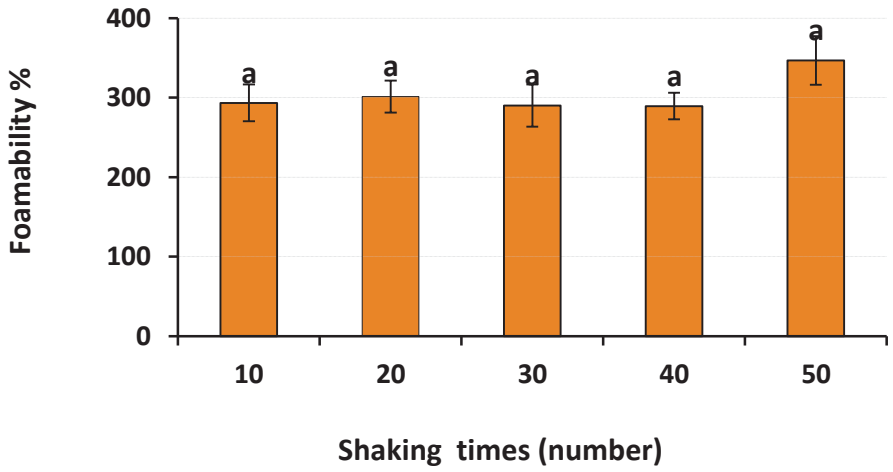

Figure 4.3 The volume of foams produced by a gas sparging method (whipped cream dispenser) after shaking EWP solution for different times (10– 50 times).
[a] Means followed by the same letter within a column are not significantly different ($p < 0.05$). Each data point is mean ± SD for n=3

4.3.1.2 Foam stability

The results for foam stability shown in Figure 4.4A show no pronounced differences between all samples in their foam volume stability and foam liquid drainage after 5, 10 and 15 minutes. This indicated that the structural and physical differences observed in Figure 4.3 did not cause a significantly noticeable difference in their foam stability. Overall the foam stability was also much lower than that observed in the foams produced by a whipping method described in Chapter 3. Foam liquid drainage occurred rapidly after preparation after 10-15 min by which time all liquid had drained from foams. Foam volume was very unstable and collapsed rather quickly from all samples with about 20-40% of foam volume remained stable after 15 min (Figure 4.4B).

In summary, the number of shakes had no significant influence on the foamability and foam stability of EWP but it affected the appearance of foam.

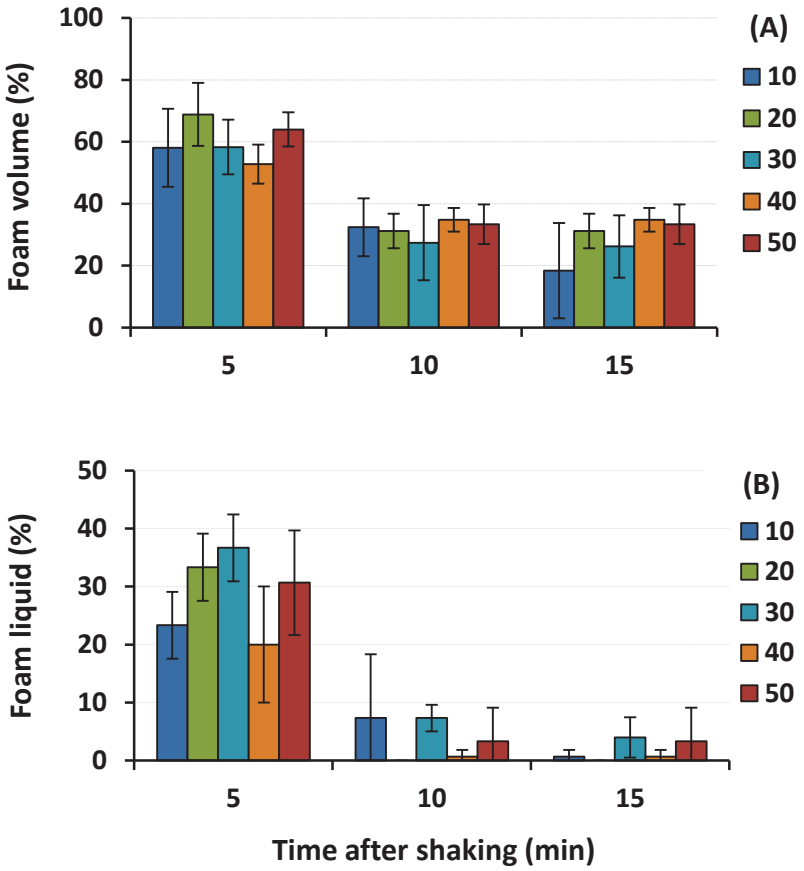

Figure 4.4 Stability of EWP foams produced by a gas sparging method after shaking for different times (0-50 times); (A) foam volume stability and (B) foam liquid stability.

4.3.2 Effect of volume of egg white powder (EWP) solution

The size of a whipped cream dispenser (canister) used to generate foam by the sparging method with NO_2 gas charger was 0.5 litre. According to the manufacturer's guidelines, the maximum volume of solution that can be placed in the dispenser was 500 ml. To determine how different volumes of EWP solution loaded into the dispenser would affect the foamability and foam stability, four different amounts of EWP solution (50, 100, 200 and 400 ml) were examined as described in Section 4.2.3.2. The amount of NO_2 gas to be charged into the a solution loaded in canister was constant in all experiments, regardless of the size of sample, using one NO_2 gas charger per each time

(8 g of NO_2). The canister containing EWP was then shaken various times (10, 20, 30, 40 and 50 times) after charging with NO_2 gas.

4.3.2.1 Foamability

It was anticipated that foamability would increase as the initial volume of EWP solution used increased. The results for foamability are shown in Table 4.6, and foamability was influenced by the amount of EWP solution and not by the shaking time as previously found.

Table 4.6 Foamability (%) of different volumes of EWL produced through gas sparging after shaking different times.

EWP volume (ml)	Number of Shakes				
	10	**20**	**30**	**40**	**50**
50	293 ± 23^{c}	301 ± 20^{b}	290 ± 27^{b}	289 ± 17^{c}	347 ± 31^{a}
100	337 ± 6^{b}	327 ± 6^{b}	322 ± 13^{b}	323 ± 13^{b}	325 ± 22^{a}
200	355 ± 9^{ab}	326 ± 25^{b}	278 ± 26^{b}	252 ± 3^{d}	307 ± 8^{a}
400	371 ± 4^{a}	399 ± 13^{a}	413 ± 10^{a}	420 ± 13^{a}	368 ± 46^{a}
p	<0.05	0.001	<0.05	<0.05	0.148

Results are expressed as the mean ± SD for three replications. $^{a–d}$ Means followed by the same letter within a column are not significantly different ($p < 0.05$). p values indicate the significant variance between samples with no significant difference at $p < 0.05$.

4.3.2.2 Foam stability

The stability of foam volume after preparation was also observed to be slightly higher when it was prepared with a greater volume of EWP solution (200 and 400 ml) than at 50 and 100 ml as shown in Figure 4.5A, B, C & D. Regarding the effect of numbers of shakes on the foam volume stability, overall its effect was not significant as already mentioned in Section 4.3.1.2 (Appendix 10).

The liquid stability of foams between samples also showed that two samples prepared at a higher volume of EWP solution (200 and 400 ml) had the higher stability compared to the other two samples made from a low volume of EWP solution (50 and 100 ml) (Figures 4.5E, F, G & H). However, using 200 ml of EWP solution showed the most

stable foam against liquid drainage up to 30 min after preparation (Appendix 11). This was in spite of the fact that its foamability was not greater than that of samples prepared with 50 and 100 ml after shaking for different times, except 10 times. The overall results indicate that the volume of EWP solution for creating foams with relatively high stability may be between 200 and 400 ml using the sparging method.

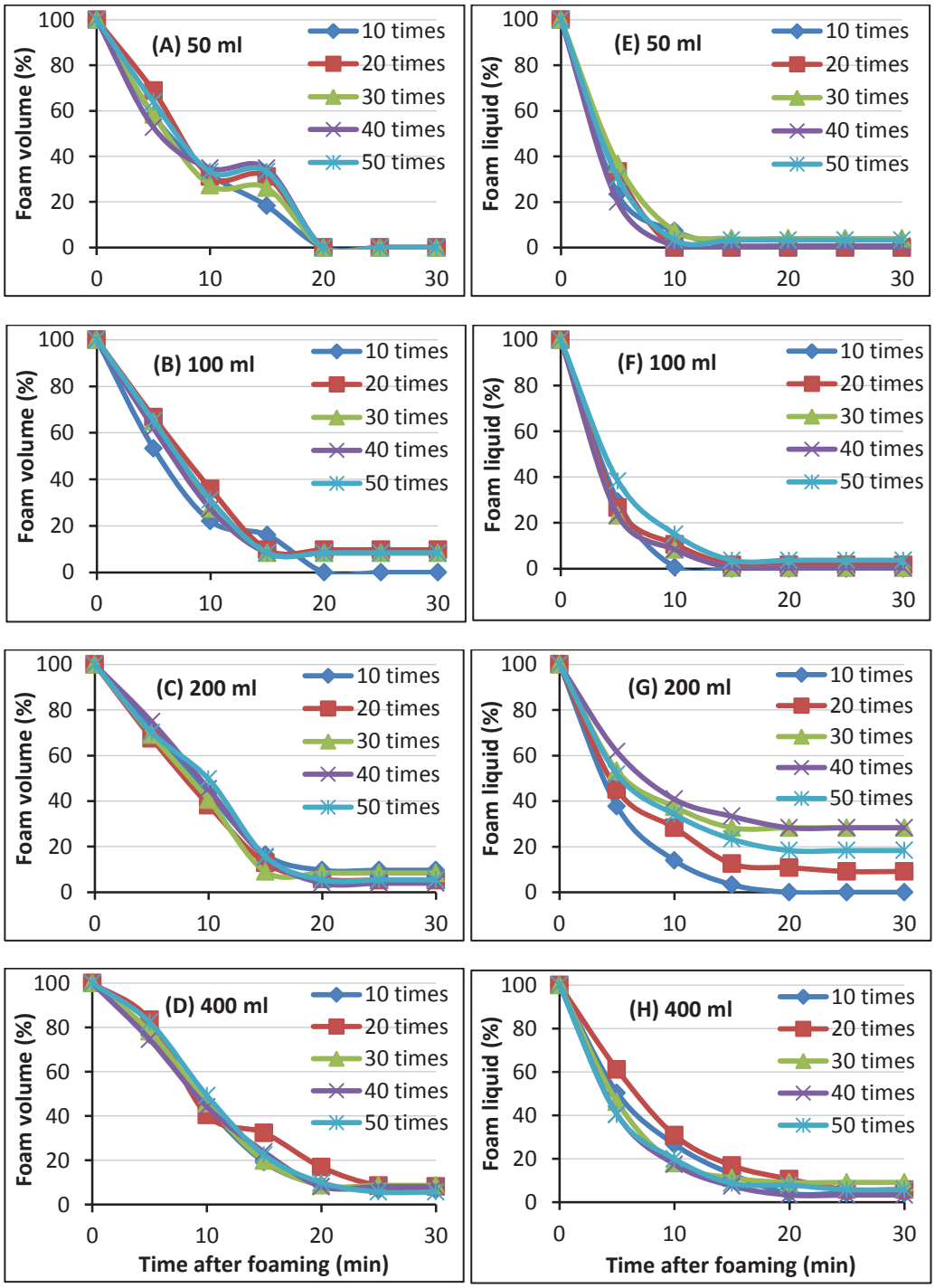

Figure 4.5 Changes to stability of foam volume (A, B, C and D) and foam liquid (E, F, G and H) over time after foam preparation. Foams were prepared by gas sparging in a whipped cream dispenser after shaking different volumes of EWP solution, for different times (10-50 times).

Each data point is the mean ± SD for n=3

4.3.3 Types of egg white raw materials (EWL & EWP)

As shown in Chapter 3 using the whipping method, two different types of egg white raw materials, such as fresh egg white liquid (denoted as EWL) and spray-dried egg white powder (EWP), were used to compare differences in their foamability and foam properties by using the sparging method. In this experiment, a 50 ml of EWL and EWP solutions containing 10 wt% proteins at 20^{o}C was used and it was shaken 20 times after gas sparging.

4.3.3.1 Foamability

Interestingly, the foam appearance between EWL and EWP was observed to be very different (Figure 4.6). The EWL solution produced a thick and creamy foam, whereas, EWP solution formed a more liquid-like foam as shown in Figures 4.6A & B. From these results, it can be inferred that EWL and EWP differ from each other in their properties, not only physical properties but also chemical nature. This is because EWP is produced by spray-drying involving a high temperature process, which can cause denaturation of egg white proteins. As a consequence, the chemical and functional properties of proteins in EWP can differ from those of EWL.

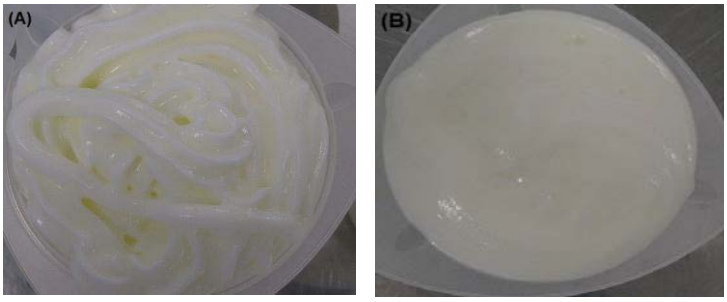

Figure 4.6 Appearance of foams prepared from (A) 50 ml of egg white liquid (EWL) and (B) 50 ml of egg white powder (EWP) solution by gas sparging using a whipped cream dispenser after shaking for 20 times.

Nevertheless, these two types of EWL and EWP did not show any significant difference in foamability with 280 ± 20% and 301 ± 20%, respectively (Figure 4.7). The observed no difference in their foamability between them was similar to the results illustrated in

Figure 3.8 for Chapter 3 with regards to the foamability of EWL and EWP solutions conducted by using the whipping method with a standard mix beater.

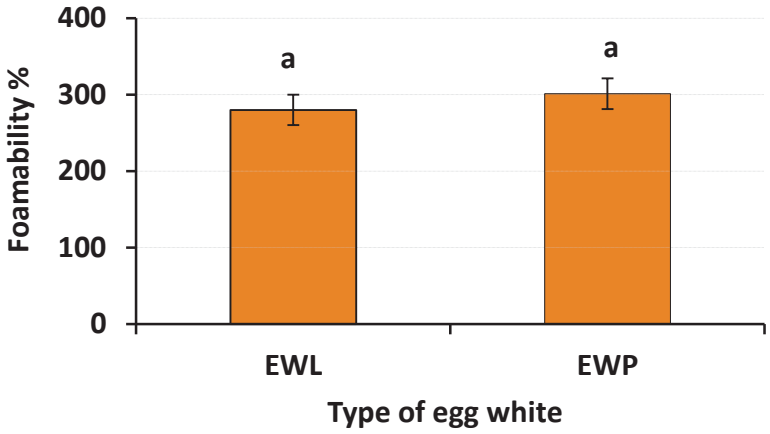

Figure 4.7 Foamability of EWL and EWP solutions produced by gas sparging using whipped cream dispenser.
[a] Means followed by the same letter within a column are not significantly different ($p < 0.05$). Mean values ± SD for n=3

4.3.3.2 Foam stability

The results of foam stability are shown in Figure 4.8. Overall, the foams prepared from both EWL and EWP solutions were unstable and collapsed rapidly, resulting in more than 60% foam being collapsed after 10 min, whereas, the EWP foam showed a higher stability to collapse after 10 min (Figure 4.8A). In the case of foam liquid stability, the difference was minor between EWL and EWP which was observed after 10 min with a slight higher liquid stability for the EWL foam (Figure 4.8B). Similar results were obtained using the whipping method. Foam volume of EWP was more stable than EWL foam but there were no differences in foam liquid stability between them (Figures 3.9 in Chapter 3). Between the two different methods of foam formation, the main difference was that the foam produced by the whipping method remained stable for about 2 hr while the stability was only for 10 min for the foams prepared by the sparging method.

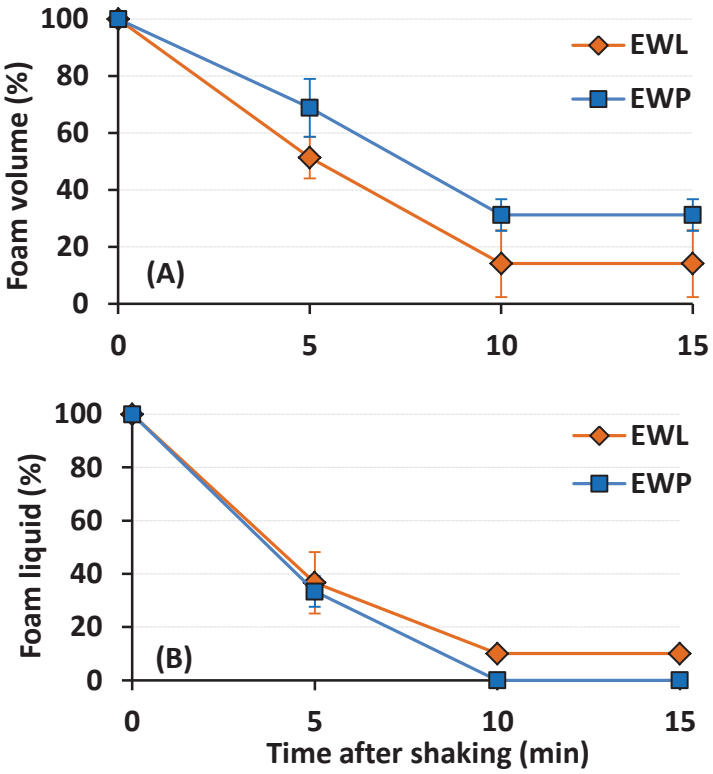

Figure 4.8. Stability of foams prepared with EWL and EWP solutions after shaking for 20 times with 50 ml solution; (A) foam volume stability and (B) foam liquid stability.
Each data point is mean ± SD for n=3

4.3.4 Effect of temperatures of egg white powder solutions (4 and 20°C)

The effect of temperature on foamability and foam stability was also determined for EWP solutions with two different temperatures, 4°C and 20°C, by using the sparging method with a 50 g of EWP solution (10 wt% protein) after shaking for 20 times.

4.3.4.1 Foamability

The foamability of EWP solution seemed to be slightly higher at 4°C than 20°C with 340 ± 20% and 301 ± 20%, respectively (Figure 4.9). However, the difference was not significant (p= 0.078). In the previous experiments as shown in Figure 3.6 for Chapter 3, the opposite results were however observed with a significantly higher foamability at 20^{o}C than 4°C when EWL was whipped using the standard mix beater.

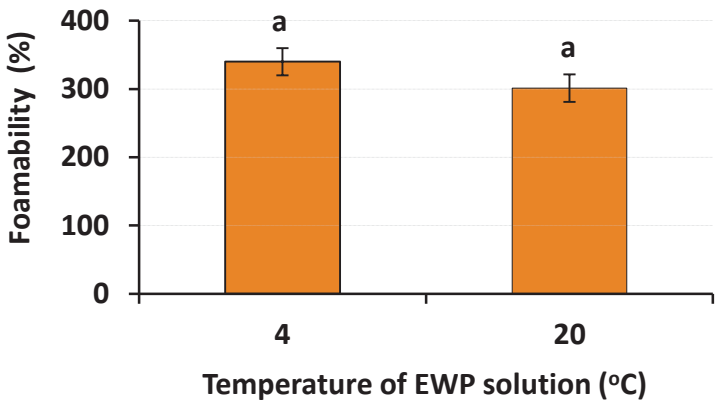

Figure 4.9. Foamability of EWP solution at two different temperatures of 4 and 20°C.
[a] Means followed by the same letter within a column are not significantly different ($p < 0.05$). Each data point is mean ± SD for n=3.

4.3.4.2 Foam stability

The behaviour of foam volume stability after preparation was not affected by the temperature of EWP solution as no significant difference was seen between 4°C and 20°C (Figure 4.10A). On the other hand, the stability of foam liquid prepared from the EWP solution at 4°C was slightly more stable (Figure 4.10B). However, both samples exhibited a rapid reduction in the liquid stability during the first 10 min after the foam preparation, more notably for the 20°C sample. In the case of egg white foam prepared from EWL using the whipping method as shown in Figure 3.7 for Chapter 3, the overall stability of foam volume and foam liquid was higher for the foam prepared at 20°C than 4°C. This may imply that the stability of foam prepared from two different egg white solutions (EWL and EWP) at two different temperatures (4 and 20°C) varies and they behaviour differently, depending on a method used between the whipping and gas sparging methods.

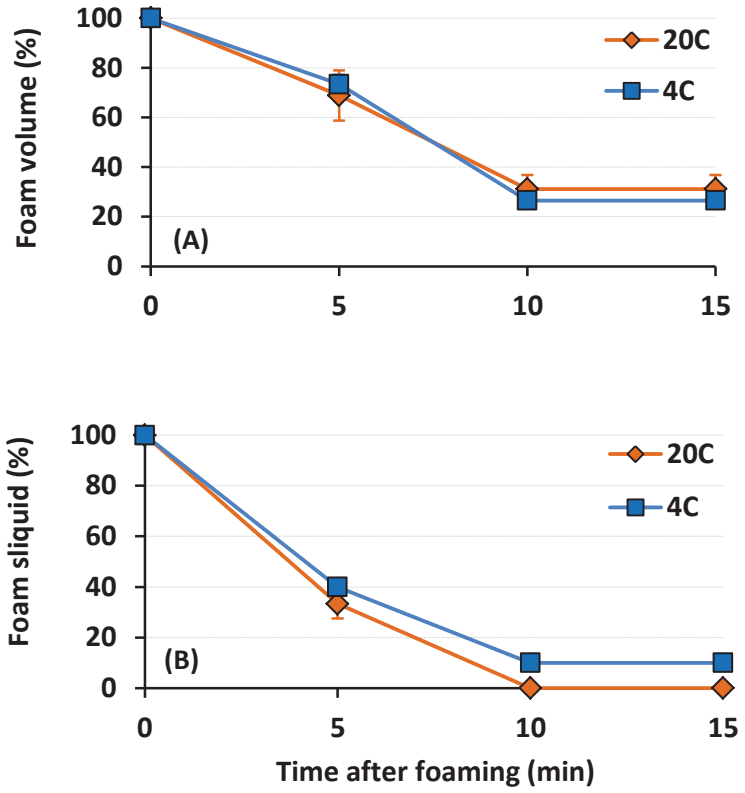

Figure 4.10 Stability of foams prepared from EWP solution at two different temperatures 4 and 20°C: (A) foam volume stability and (B) foam liquid stability.
Each data point is mean ± SD for n=3.

4.3.5 Addition of sucrose at different concentrations

The addition of sucrose into EWP solution at different sucrose concentrations was studied for its effect on egg white foam. The temperature of EWP solution of 20ºC and the number of shaking for 20 times were used to produce foams. The appearance of foams produced with the addition of sucrose to the EWP solutions was fluid at all concentrations (Figure 4.11) different from the dense, creamy foams formed from EWL solutions. In the previous experiment, shown in Figure 3.24A for Chapter 3, involving the foam preparation from EWL solutions using the whipping method, the foamability was shown to decrease when sucrose (5-20% w/v) was added to EWL solutions. The extent of reduction was more severe at higher sucrose concentrations (e.g. 20%). In contrast, in this experiment with EWP and using the gas sparging method, a decreased foamability was only seen at 10% sucrose (i.e. p9/s10 and p18/s10) whereas no significant difference was observed at 18% sucrose (p8/s18 and p16/s18) compared to the control sample (p10/s0) (Figure 4.12A). These results indicate that the presence of

sucrose coupled with increasing protein concentration had no significant impact on foamability. This may be a reason for no agreement observed between this study and the previous results shown in Figure 3.10 for Chapter 3 showing that the foamability decreased with increasing protein concentration. It should be mentioned that different forms of initial egg white (e.g. egg white liquid and spray-dried egg white powder) and different foaming methods (whipping and gas sparging) used in both experiments have shown contrasting results.

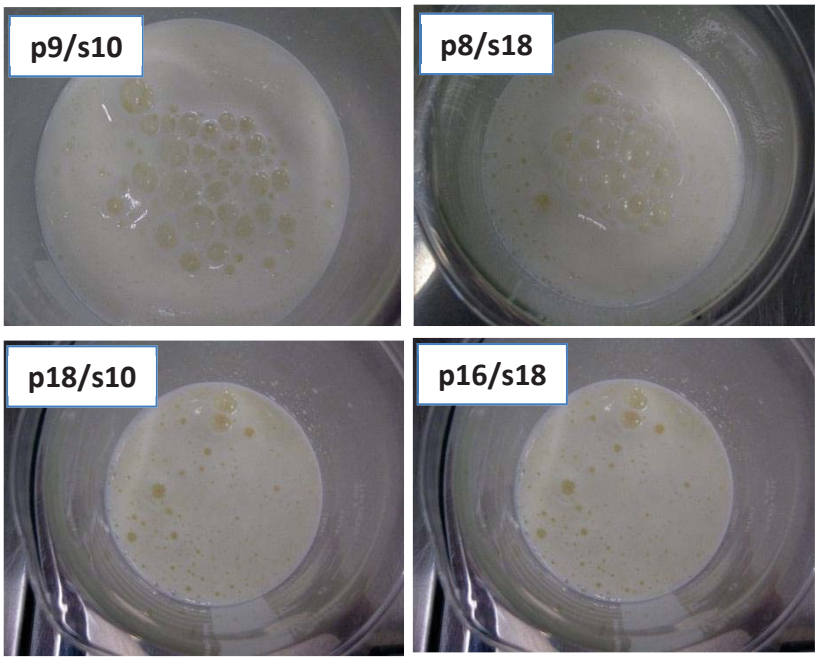

Figure 4.11 Pictures of foams from egg white powder solutions containing different concentrations of protein and sucrose.
Abbreviations p8, p9, p16 and p18 represent protein concentration (8, 9, 16 and 18 wt%) and s10 and s18 represent sucrose concentration (10 and 18 wt%).

Two samples (p9/s10 and p18/s10) containing 10% sucrose showed a greater reduction in their foaming ability compared to the control sample. On the other hand, the effect was not significant when 18% sucrose was added into the EWP solution containing 8 or 16% protein (p8/s18 and p16/s18). The foamability for the control samples was 325±7% while it was 273±4% for the sample containing 18% protein and 10% sucrose. The data showed that the protein concentrations at the levels between 8 and 18% used in this experiment did not significantly affect the foamability of EWP solution.

It is generally known that a diminished foamability with increasing protein concentration is associated with increasing viscosity of the protein solution, which limits diffusion and unfolding of protein molecules at the interface and thus makes it difficult for a large volume of air to be incorporated into the solution (Sanchez & Patino, 2005). However, this was not observed in this study for some samples, such as p8/s18 and p16/s18, which are believed to have a high viscosity due to a higher solid content of 28 and 35%, respectively, compared to the control sample (p10/s0) with 10% solid content. Another effect that can cause a reduction in foamability due to the addition of sucrose is the interaction between protein and sucrose at the interface.

Although the addition of sucrose and the increase in the protein concentration did not enhance the foamability of EWP solutions, the foam stability and foam liquid stability of two samples (p18/s10 and p16/s18), notably containing a high protein concentration (16% and 18%), were found to be significantly improved by an increase in protein concentration, compared to the control sample (p10/s0) containing 10% protein (Figures 4.12B & C). The results indicate that increasing the protein concentration raises the foam stability while the addition of sucrose has no positive effect on foam stability. In contrast, the control sample and the samples containing 8-9% protein and sucrose (p9/s10 and p8/s18) showed considerably lower foam volume and foam liquid stability**.**

The reason for the observed high foam stability for the two samples (p16/s18 and 18/s10) containing a high protein concentration (16 and 18% protein) compared to the control sample could be due to an increase in the viscosity of the egg white solution. The higher protein concentration helps the foam to retain its liquid, resulting in an increase in the time for the whole liquid to drain (Rodriguez Patino et al., 1995). Moreover, the higher protein concentration delays foam collapses because the interfacial film formed between water and air in the case of high protein concentration is thicker (more viscous), so the foam conserves the liquid for a longer time (Rodriguez Patino et al., 1995).

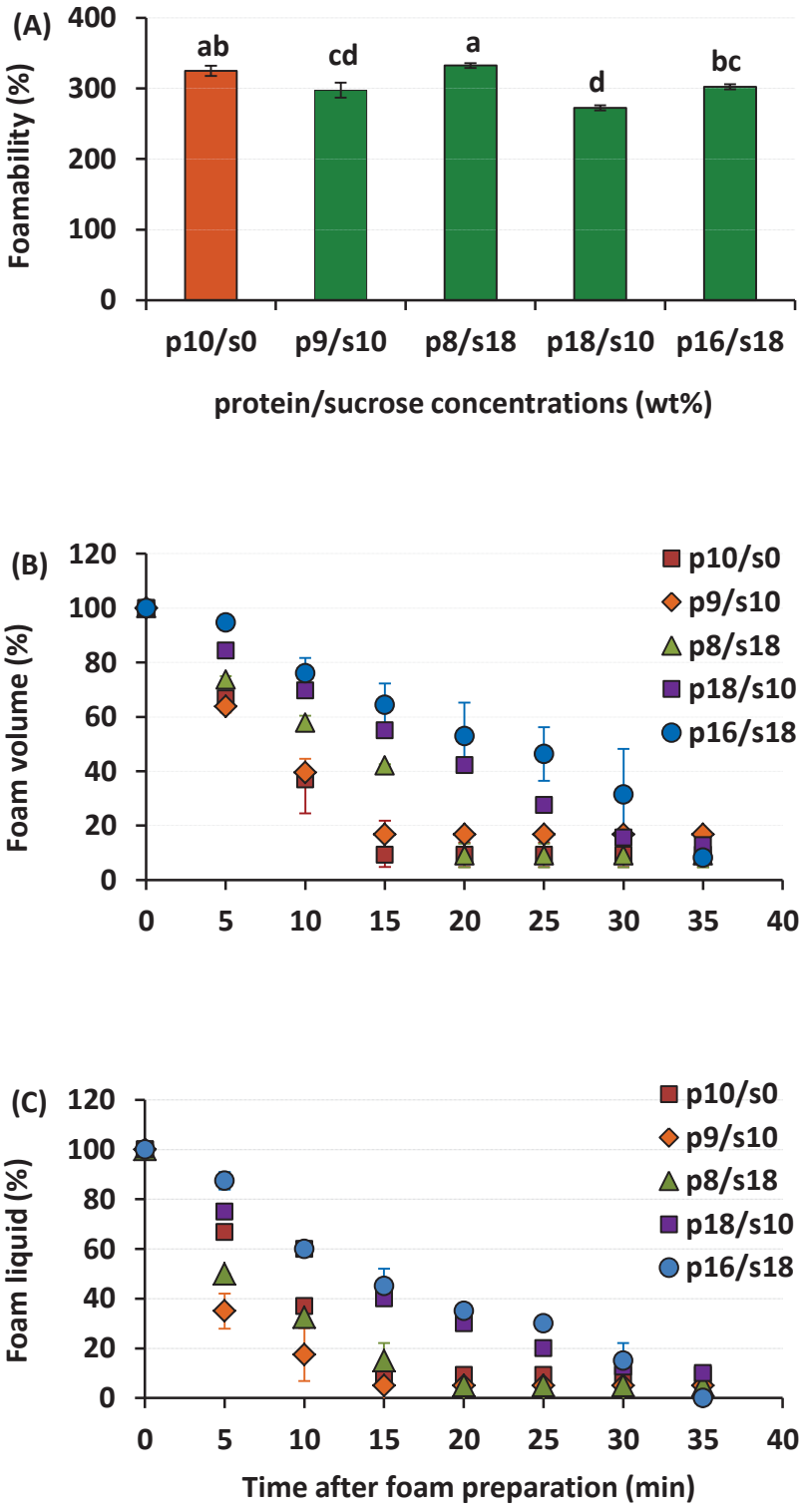

Figure 4.12 Effects of concentrations of sucrose and protein on (A) foamability, (B) foam volume stability and (C) foam liquid stability of foams produced from 100 ml of egg white powder (EWP) solutions after shaking 20 times.

[a-d] Means followed by the same letter within a column are not significantly different ($p < 0.05$). Each data point is mean ± SD for n=2. Abbreviations p8, p9, p10, p16 and p18 represent protein concentration (8, 9, 10, 16 and 18 wt%) and s0, s10 and s18 represent sucrose concentration (0, 10 and 18 wt%).

4.3.6. Addition of hydrocolloids

Based on the previous experiments examining the effect of hydrocolloids on foam properties using a standard mix beater, the concentration of individual hydrocolloids; xanthan gum (XG), guar gum (GG) and gum arabic (GA) which provided the optimal foam stability was 0.04% XG, 0.04% GG and 2% GA.

In this experiment, four different combinations of these hydrocolloids at different concentrations were formulated to determine their effects on the foamability and foam stability of EWP and compare with the control sample with no added hydrocolloids. Two different levels of concentrations (0.02 and 0.04%) were used for both XG and GG while the concentration of GA used was 2%. Four different formulations of hydrocolloids used are shown in Table 4.2. EWP solutions (10 wt. % protein) containing added hydrocolloids were shaken 20 times after charging with gas. The temperature of EWP solutions was also varied between 4 and 20°C.

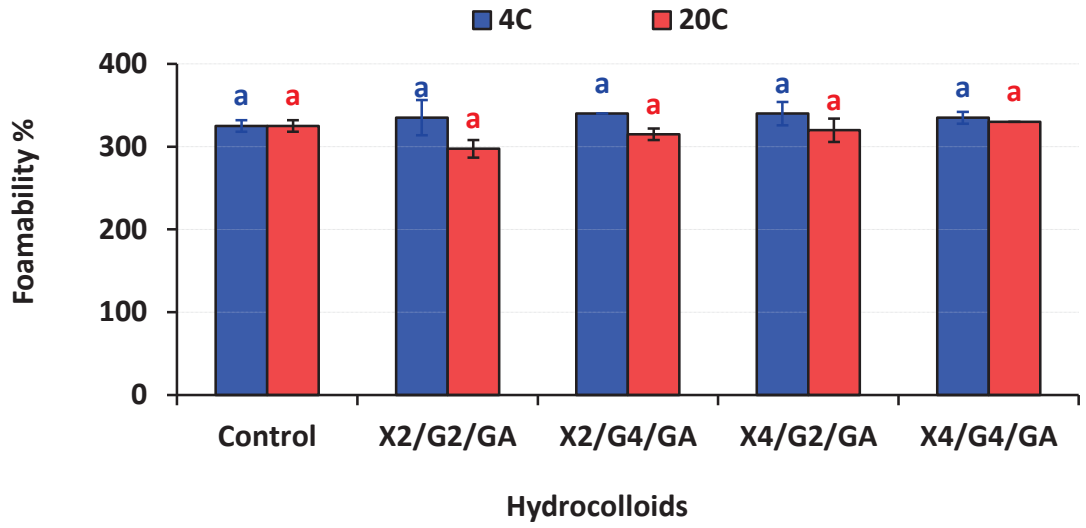

Figure 4.13 Foamability of EWP solutions (10% protein; 4 and 20°C) prepared from EWP with three different types of hydrocolloids at different concentrations.
Abbreviations X2 and X4 represent xanthan gum at 0.02 and 0.04%; G2 and G4 for guar gum at 0.02 and 0.04% and GA represent gum arabic at 2%. [a] Means followed by the same letter within a column are not significantly different ($p < 0.05$). Each data point is mean ± SD for n=2.

The results of foamability are shown in Figure 4.13. The addition of hydrocolloids did not show a significant increase in the foamability of EWP at both temperatures as there was no noticeable difference compared to the control sample. The appearance of egg

white foams produced in the presence of hydrocolloids was creamy in all cases at both temperatures as shown in Figure 4.14.

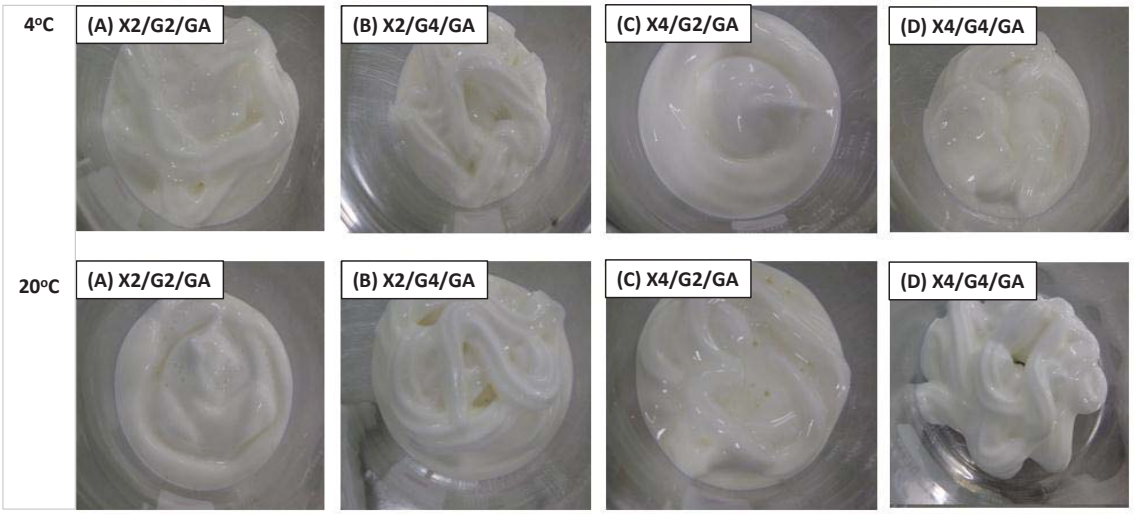

Figure 4.14 Pictures of foams prepared from EWP solutions mixed with three different types of hydrocolloids at different combinations and concentrations.
Abbreviations X2 and X4 represent xanthan gum at 0.02 and 0.04%; G2 and G4 for guar gum at 0.02 and 0.04% and GA represent gum arabic at 2%.

Although the addition of hydrocolloids had no positive effect on the foamability of EWP, it was shown that they conferred a significant enhancement in foam stability as illustrated in Figures 4.15A & B. The rate of foam collapse and liquid drainage was observed to be considerably slower for the samples containing hydrocolloids compared to the control sample during the first 20-25 min after the foam preparation, however, after 30-35 min the difference became less pronounced. In the absence of hydrocolloids, foam collapse occurred rapidly within the first 10 min and had around 10% foam remained after 15 min. Also, liquid drainage occurred quickly and almost all liquid drained after 15 min. Regarding the effect of temperature of EWP solution, the results showed that the foam stability prepared at two different temperatures (4 and 20^{o}C) did not differ significantly and exhibited a similar pattern and trend (Figures 4.15C & D).

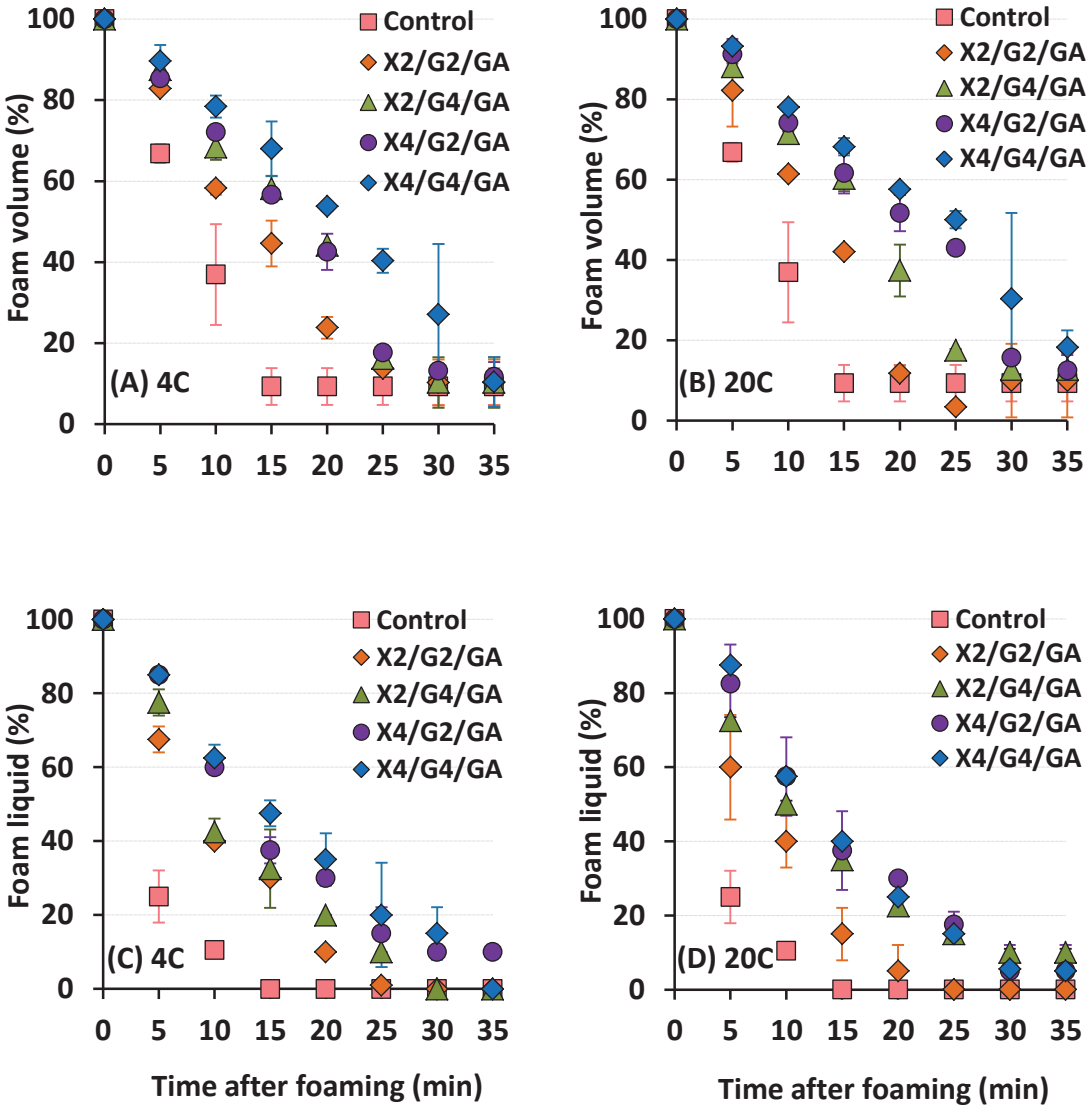

Figure 4.15 Stability of foam volume and foam liquid of egg white foams prepared, at two different temperatures, from solutions of egg white powder mixed with hydrocolloids at different concentrations.
Each data point is mean ± SD for n=2. Foam volume stability at 4°C (A) and 20°C (B) and foam liquid stability at 4°C (C) and 20°C (D).

The enhanced foam stability in both volume and liquid driven by hydrocolloids was particularly remarkable for those two samples (X4/G2/GA and X4/G4/GA) containing 0.04% XG while it was less pronounced for the other two samples (X2/G2/GA and X2/G4/GA) containing a low concentration of 0.02% XG. When comparing between the latter two samples, the one (X2/G2/GA) containing a lower concentration of 0.02% GG exhibited the lower foam stability. This implies that both type and concentration

have a significant impact on the foam stability of EWP. For instance, the sample (X4/G4/GA) containing the highest concentration of hydrocolloids (0.04% XG, 0.04% GG and 2% GA) showed the most stable foam properties, which is believed to due to a delay in foam collapse through an increase in the viscosity of egg white liquid.

Also, as mentioned earlier, the viscosity induced by hydrocolloids is another factor responsible for the stabilisation of air bubbles by decreasing the rate of liquid flow in the interior of lamellae and assisting the foam to retain its liquid, thus preventing drainage from the film lamellae. A synergistic effect for greater viscosity is known to take place when XG and GG are used together, resulting from their interaction and formation of junction zones (networks) in the system (Lachke, 2004).

4.3.7 Heat treatment of egg white liquid (EWL)

Initially, heat treatment of EWL was carried out to determine its impact on changes to physical appearance (e.g. aggregation, precipitation or gelation) through visual observation and turbidity measurement using a spectrophotometer. For this study, frozen fresh EWL was used after thawing at 4^{o}C and warmed up to 20^{o}C. Figure 4.16 shows the appearance of heat-treated EWL from 50 to 60^{o}C. A noticeable visual change was seen when EWL was heated at above 59^{o}C due to protein denaturation and aggregation of denatured proteins. The extent of the protein aggregation between samples could be confirmed by measuring the optical density of EWL, which represents the clarity (turbidity) of EWL (Figure 4.17). Initially, the turbidity increased slowly at a temperature of 53^{o}C and then increased sharply at 59°C for 2 minutes. No differences were observed among the samples treated at temperatures below 50^{o}C.

The aggregation of protein due to heat treatment occurs through intra- and intermolecular bonds (i.e. disulfide bonds and hydrophobic interactions) between denatured protein molecules due to the unfolding of the conformational structure causing the exposure of reactive amino acids (Visschers & de Jongh, 2005). Unfolding of the native structure of proteins can be caused by applying high temperature, high pressure and shear stress or addition of some other components (Visschers & de Jongh, 2005).

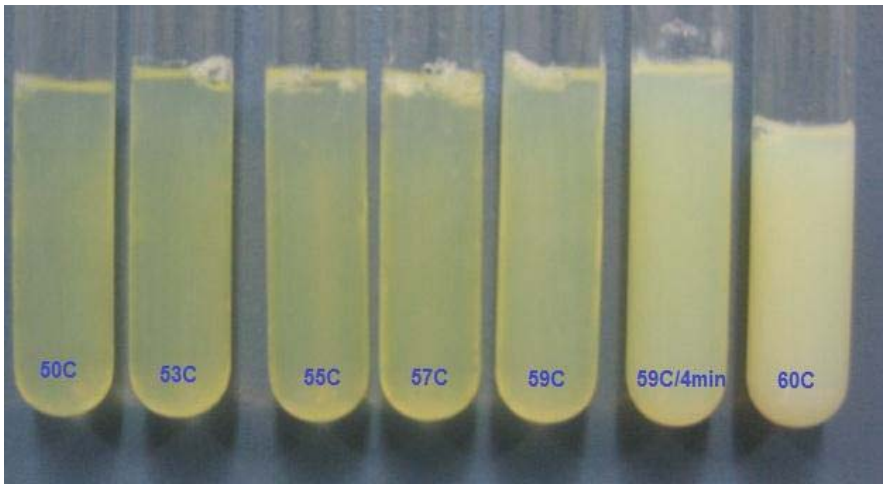

Figure 4.16 Egg white liquid (EWL) solutions containing 10% protein after heat treatment at different temperatures.

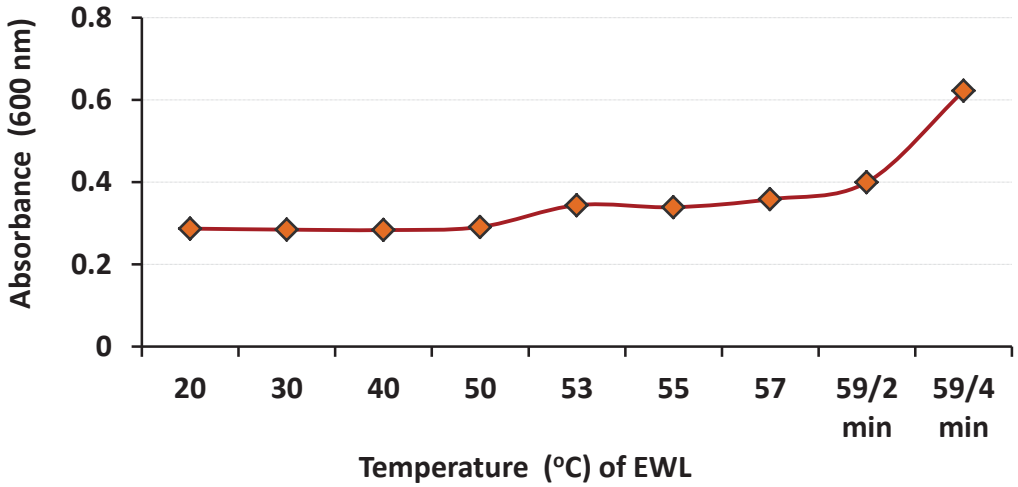

Figure 4.17 Changes in the turbidity of egg white liquids after heat treatment at different temperatures which was determined by measuring absorbance at 600 nm.
Each data point is mean ± SD for n=3.

The effect of thermal treatment of egg white solution was further studied using egg white liquid (EWL), not egg white powder (EWP). A solution of EWL (10 wt% protein) at 20^{o}C was heat-treated at 58^{o}C for 3.5 min, 60^{o}C for 2 min and 63^{o}C for 2 min, respectively as shown in Section 4.2.5. Briefly, denaturation of egg white causing a dramatic change in the physicochemical properties of egg white proteins occurs at around 60^{o}C. At 58^{o}C, egg white proteins are however stable to denaturation unless the

holding time for heat treatment at that temperature is long enough. It should also be mentioned that the temperature and time for pasteurisation of fresh egg white liquid (EWL) being used in the egg industry is 58^{o}C for 3.5 min. At 63^{o}C, denaturation of egg white proteins and aggregation and coagulation of denatured proteins occur more rapidly compared to that at 60^{o}C. These results are shown in Figure 4.18. It can be seen that the EWL treated at 60^{o}C had protein denaturation with the formation of soluble protein complexes while the EWL heat-treated at 63^{o}C had considerable protein coagulation which precipitated rapidly at the bottom of the test tube but the aggregates could be breakdown, suspended and partly dispersible when it was agitated as it did not form a gel. Therefore, the sample could still be passed through a nozzle of the cream whipper used in the experiment to form foam.

Figure 4.18 Pictures of egg white liquid (EWL) containing 10% protein after heat treatment at different temperatures; (A) 58°C for 3.5 min, (B) 60°C for 2 min and (C) 63°C for 2 min.

4.3.7.1 Foamability

Overall, the heat treatment of EWL had some effect on foamability, depending on the extent of protein denaturation and aggregation (Figure 4.19A). The sample heat-treated at 63°C for 2 min showed low foamability with 270 ± 0% compared to non-heat treated sample (at 20°C) of 325 ± 7%. No significant differences were however observed among the samples heat-treated at 58 and 60^{o}C which had the foamaibility as 310 ±14% and 335 ± 21%, respectively. The results indicate that egg white proteins were not affected for their foamability by the heat treatment pasteurisation at 58^{o}C for 3.5 min. Also, protein denaturation causing the formation of soluble and/or dispersible protein

complexes, as shown in the sample heat-treated at 60°C, does not affect the functional properties of egg white proteins as a foaming agent.

The mechanism responsible for a rather significant reduction in foamability observed for the sample heated at 63°C can be attributed to severe protein denaturation and coagulation, leading to a formation of large insoluble aggregates that precipitate readily out of the solution. Thus, the rate of protein adsorption at the air-water interface is low and film thinning cannot occur readily, resulting in fewer bubbles formed at the air-water interface and hence low foamability (Kinsella, 1981). Similar results were also reported by Ibanoglu and Ercelebi (2007) with a reduction in the foamaility of an egg white powder solution (0.01% w/v protein) after heat treatment at 65, 70, 75 and 80°C for 2 min.

4.3.7.2 Foam stability

Overall, foam volume stability did not differ between samples, except for the control sample (20°C) and the sample heat-treated at 58°C that had a sharp reduction in foam volume stability between 10 and 20 min after foam formation compared to the other samples heat-treated at 60 and 63°C (Figure 4.19B). Kinsella (1981) and Belitz et al. (2004) stated that a limited thermal treatment of proteins without causing thermal coagulation of proteins can delay foam volume collapses through a partial unfolding of globular proteins by increasing their rate of adsorption at the air-water interface and the rigidity of bubble walls due to enhancement of protein film strength. This may be applied to the sample heat-treated at 58°C in this experiment or maybe the one treated at 60°C but the results did not show any indication of enhancement of the foam stability in comparison to the control sample prepared at 20°C. This may be due partly to the sparging method used in this study which produced highly condensed, creamy foams.

Foam liquid stability showed a similar pattern for all the samples, except a few cases including the control sample at 20°C with a relatively fast rate of liquid drainage during the first 10 min compared to the other samples (Figure 4.19C). The whole liquid of egg white used for foam preparation from the samples heat-treated at 20, 58 and 60°C drained after 15 min of foam preparation while the sample treated at 63°C drained completely after 20 min.

Although there were some variations amongst samples, the results indicated the two samples heat treated at 60 and 63°C prior to foaming had a relatively higher stability to liquid drainage similar to the results of foam volume stability. Although the results

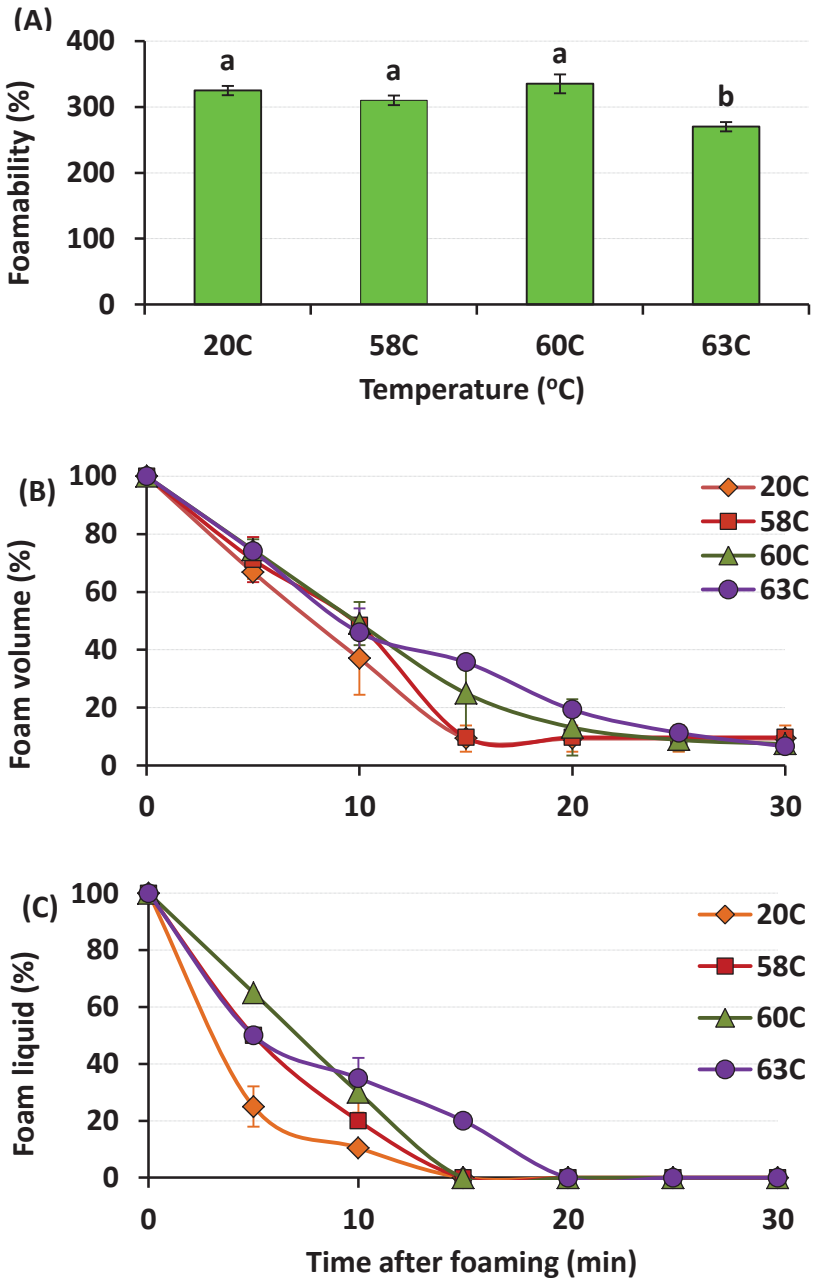

Figure 4.19 Foamability and foam stability of foams produced from EWL solutions after heat treatment at 20, 58, 60 and 63°C which were shaken for 20 times; (A) foamability, (B) foam volume stability and (C) foam liquid stability.

In Figure A, [a-b] Means followed by the same letter within a column are not significantly different ($p < 0.05$). Each data point is mean ± SD for n=2.

cannot be compared directly because of different methods and conditions used, several researchers showed a significant enhanced foam volume and foam liquid stability through a decrease in the rate of liquid drainage from egg white foams by using EWP pre-heated at 70 or 80°C (Talansier et al., 2009; Nicorescu et al., 2011), suggesting protein denaturation can have a desirable effect on foam stability.

In summary, the results suggest that egg white proteins can be manipulated by thermal treatment to induce partial denaturation to enhance their functional properties as a foaming agent.

4.3.8 Heat treatment of EWL solutions containing hydrocolloids and sucrose

In the results shown in Section 4.3.6 (hydrocolloids addition), the foam stability of egg white prepared from the EWP solution was significantly improved, without compromising its foaming ability, by the addition of hydrocolloids. The experiment was further extended with some modifications in formulations by adding other ingredients (20% sucrose, 0.04% locust bean gum, 0.05% citric acid), in addition to 0.04% XG, 0.04% GG and 2% GA, into the EWL solution and then heating the mixture at different temperatures (20, 58, 60 and 63°C). The pH of the EWL was measured before and after mixing with hydrocolloids and sucrose which was not changed drastically (pH 8.99-9.03). The results were compared to the control samples (EWL) prepared without adding any other ingredients but heat-treated under the same conditions which are those described in Section 4.3.7 and shown in Figures 4.19.

Figure 4.20 shows the images of EWL solutions containing those ingredients after heat treatment, including the pictures for those samples already shown in Figure 4.18 which did not contain the ingredients. One notable finding was that the addition of ingredients to EWL prevented the formation of soluble protein aggregates when it was heat-treated at 60°C for 2 min unlike it occurred in the absence of those ingredients (hydrocolloids, sucrose and citric acid). However, the same sample treated at 63°C for 2 min showed the appearance similar to that with no added ingredients, in terms of the formation of protein coagulum. The reason for no occurrence of protein aggregation for the sample at 60°C may be due to a slight increase in the denaturation temperature of egg white proteins due to the presence of hydrocolloids and sucrose which can absorb thermal energy.

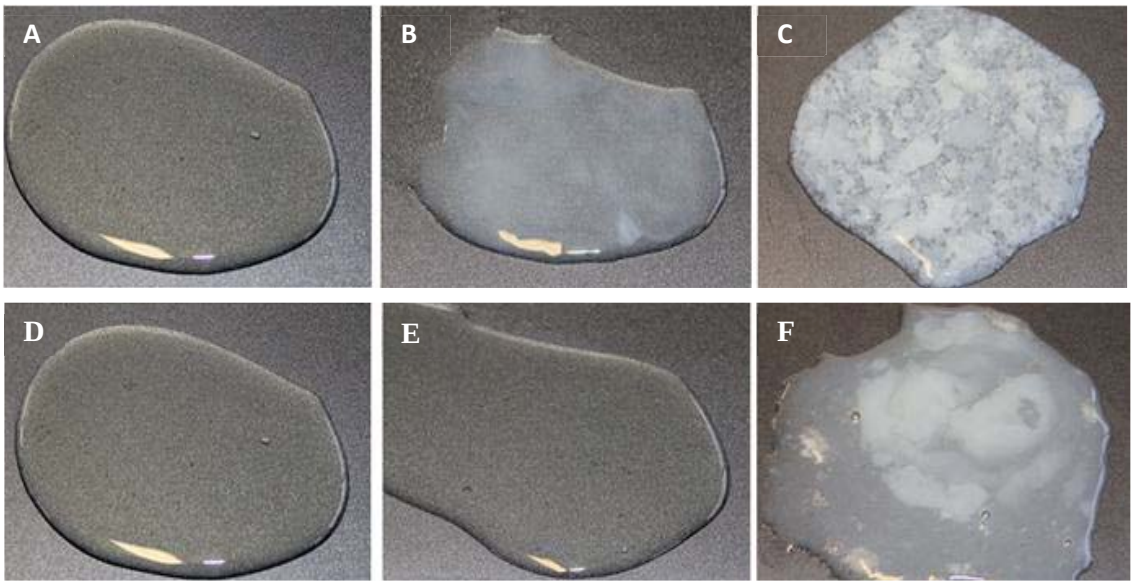

Figure 4.20 Images of EWL samples taken 1 hr after heat treatment at 58^{o}C for 3.5 min (A and D), 60°C for 2 min (B and E) and 63°C for 2 min (C and F) in the absence (A, B and C) and presence of ingredient mixture (sucrose, hydrocolloids, citric acid) (D, E and F).

4.3.8.1 Foamability

The foamability between samples containing the mixture of ingredients that were heat-treated at different temperatures did not differ from each other compared to the sample at 20^{o}C (Figure 4.21A). Also, it was found that the foamability was not significantly different from the EWL samples with no added mixture of ingredients but heat-treated at the same temperatures (Figure 4.22).

4.3.8.2 Foam stability

Heat treatments at all three different temperatures between 58 and 63^{o}C resulted in considerable enhancements in foam volume stability compared to the sample prepared at 20^{o}C (Figure 4.21B). However, this phenomenon was remarkable at the higher temperatures of 60 and 63°C than at 58°C. It is notable that increasing the temperature of EWL in the presence of the mixture of ingredients used in this study does not impair the functional properties of egg white proteins. It instead provides a dramatic increase in the foam stability in both foam volume and foam liquid by reducing the rate of foam collapse and liquid drainage, in particular at 60 and 63^{o}C, compared to the non-heat-

treated sample. This was not however observed as shown in Figure 4.22 when the same sample with no added ingredients was heat-treated.

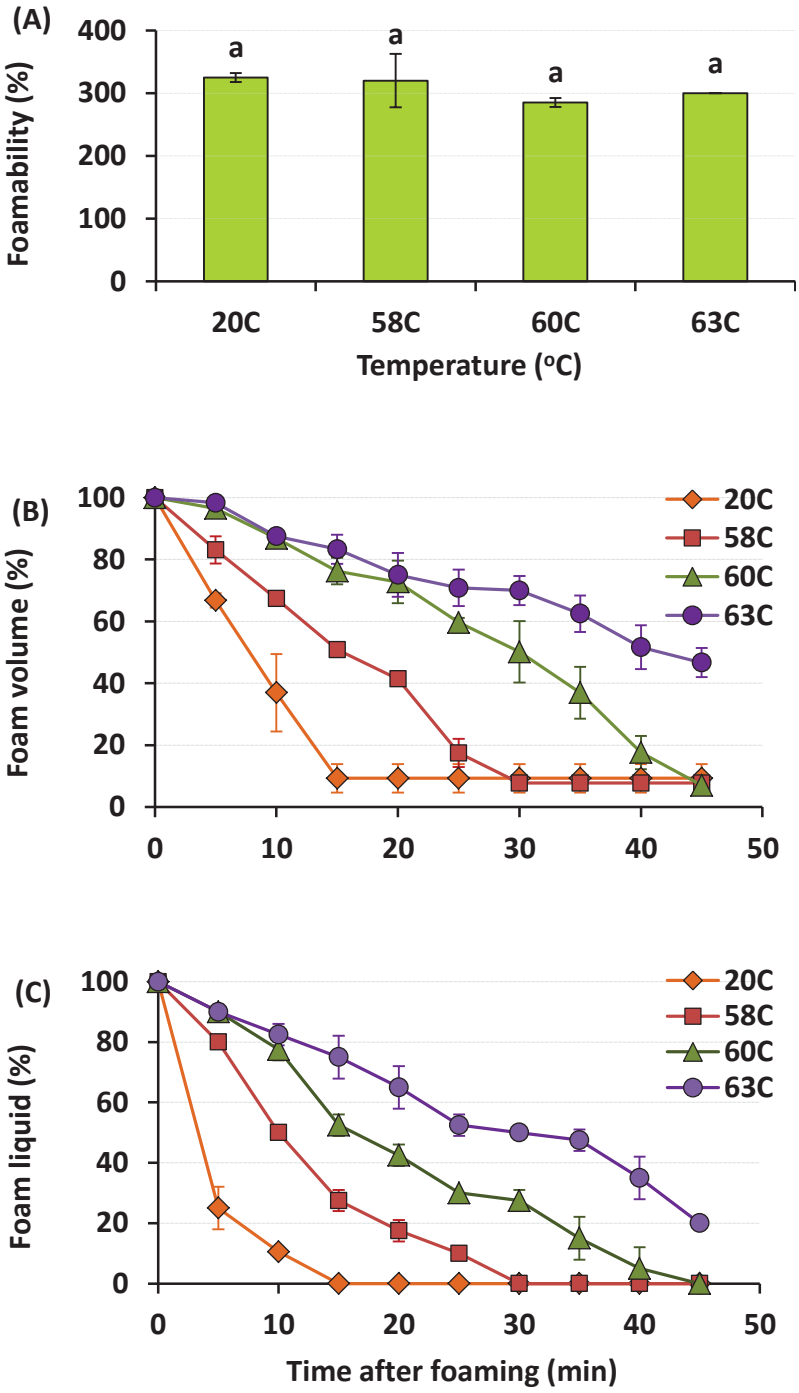

Figure 4.21 Effect of heat-treatment of EWL containing ingredients (sucrose, hydrocolloids, citric acid) at different temperatures (20, 58, 60 and 63°C) on (A) foamability, (B) foam volume stability and (C) foam liquid stability.

[a] Means followed by the same letter within a column are not significantly different ($p < 0.05$). Each data point is mean ± SD for n=2.

It is clear that thermal treatment provided high volume stability over time and the foam required a longer time to collapse (more than 45 min) for the samples treated at 60 and 63^{o}C, while the non-heat-treated sample and the sample heated to 58°C showed foam collapses much faster after foam formation. The same phenomenon was observed regarding the liquid stability. Heat treatment resulted in producing foam with the high ability to conserve its liquid over time (Figure 4.21C). For the non-heat-treated sample, all the liquid drained 15 min after foam formation. In comparison, the liquid drainage rate was slow in the heat-treated samples; liquid drainage was complete after 30 min, 45 min and 50 min for the samples treated at 58, 60 and 63°C, respectively (Figure 4.21C). The results conclude that the effect of ingredients on foam stability was significantly different before and after heat treatment.

Figure 4.22 illustrates the impact that the mixture of ingredients used in this experiment had on egg white foams. The differences in the foam stability between the two sets of samples with and without added ingredients were dramatic, especially at 60 and 63°C. The effect was less pronounced at 58°C and was not observed at 20^{o}C.

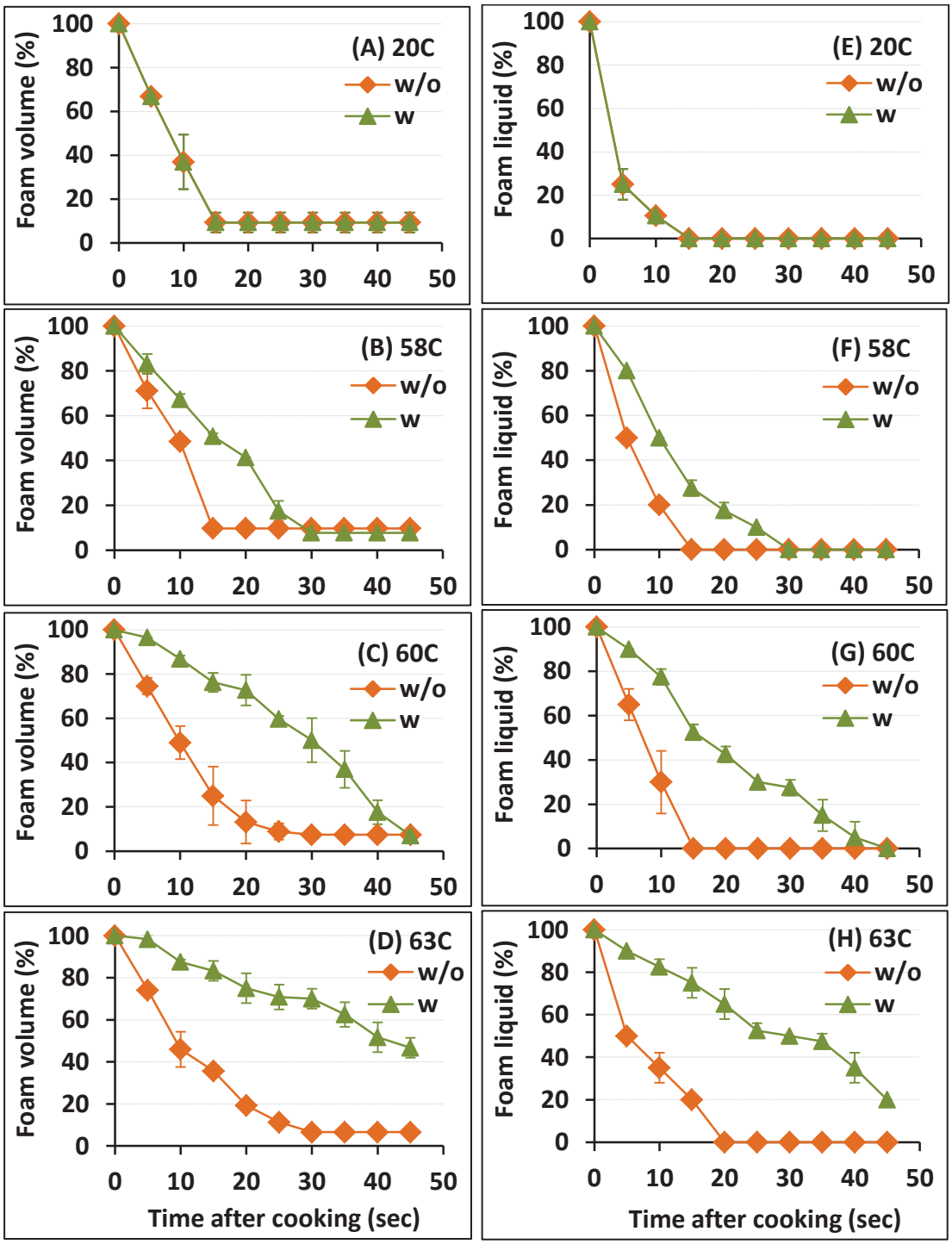

Figure 4.22 Foam stability of egg whites with and without added ingredients. The egg white solutions mixed with ingredients were heat-treated at different temperatures (20, 58, 60 and 63°C) prior to foaming. Foam volume stability (A, B, C and D) and foam liquid stability (E, F, G and H).
Abbreviations w and w/o represent with and without added mixture of ingredient, respectively. Each data point is mean ± SD for n=2.

4.3 Conclusions

Interestingly, the properties (e.g., appearance, foamability and foam stability) of foams produced by using the whipped cream dispenser were very different from the foams generated using the standard mixer beater shown in Chapter 3. The appearance of foams was thick and creamy. The foam volume produced and the stability of foam against foam collapse and liquid drainage were much low compared to the foams prepared using the standard mix beater shown in Chapter 3. Unlike the significant proportion of foam remaining stable for several hours, the foam produced using the whipped cream dispenser had foam collapse and liquid drainage occurring rapidly within 30 min or less.

The effects of various factors tested in Chapter 3 were also examined, including some other variables, such as shaking time of egg white solutions after charging with NO_2 gas. It was found that numbers of shakes had no significant effect on the foamability and foam stability of EWP whereas its impact on the visual appearance of foam produced was considerable. When shaking time was 10 or 20 times, the foam produced was creamy and thick. On the other hand, shaking for longer times (> 30 times) resulted in a type of liquid foam with some big air bubbles present.

The results of effects of addition of sucrose and hydrocolloids and changes in protein concentration were similar to the results shown in Chapter 3. Overall increasing the protein concentration raises the foam stability while the addition of sucrose has no positive effect on foam stability. The addition of both XG and GG, especially at 0.04% concentrations, increased both foam volume and foam liquid stability, due to their increase in liquid viscosity.

Another important factor studied in this chapter was the effect of heat treatment of EWL solution at 58^{o}C for 3.5 min, 60^{o}C for 2 min and 63^{o}C for 2 min prior to making foam which induced partial aggregation of proteins at both 60 and 63^{o}C. The foamability was not different between the samples heat-treated at 58 and 60^{o}C compared to the control sample at 20^{o}C but the sample heat-treated at 63^{o}C was lower in foamability. The stability of foams prepared from heat-treated EWL solutions seemed to be higher than the control sample, particularly for the liquid volume stability. This foam stability effect was found to be significantly enhanced and distinguishable when the EWL solutions were heat-treated in the presence a mixture of ingredients (sucrose, hydrocolloids and citric acid). It effects on both foam volume and foam liquid stability were substantially

higher at higher heat-treatment temperature with significant enhanced stability compared to the control sample (20^{o}C) containing the mixture of ingredients without heat treatment.

Overall the results of heat treatment of EWL provide meaningful information for the application of partial denaturation of egg white proteins, especially in the presence of other ingredients for producing enhanced foam stability. Also, the results indicate that the whipped cream dispenser is highly efficient in making egg white foams although the foam volume and foam stability produced were lower than those produced by the standard mixer beater.

Chapter 5 Microwave cooking of egg white foams

5.1 Introduction

Cooked egg white foam is widely used in foods such as meringues, angel cakes and nougats. The preparation steps and methods for making these desserts differ but they all depend to a certain extent on the functional properties of egg white as a main ingredient to form and stabilise bubbles (foams) during preparation and after cooking in a baking oven.

This research investigated a new method of egg white foam cooking using a microwave oven unlike traditionally it has been done in an oven. Nowadays there is a growing trend that more foods become available to be prepared using a microwave oven to meet an increased demand for consumers due to changes in their life-style. Only limited research has been conducted to examine the effect of cooking egg white foam using microwave. Some experiments in cooking egg white foam using microwave oven were carried out by Hervé This who is a French scientist working mainly in area of molecular gastronomy (Hervé This, 2006). He used the functional properties of egg white to take advantage of it in making a Vauquelin which is a French dessert named after Nicolas Vauquelin (1763-1829), the scientist who first developed it (Hervé This, 2006). Then, after adding ingredients such as citric acid, he cooked it using a microwave oven. However, no studies have been reported for the effect of microwave heat treatment on the foamability and stability of egg white foam.

This chapter studies changes in egg white foams before and after heat treatment in a microwave oven. Also, the impacts of various factors including cooking times, protein concentration, thermal treatment of egg white solution before whipping and cooking, and the addition of different ingredients, which were used as variables in Chapters 3 and 4, were also examined to determine how they influence the properties of egg white foams after microwave heat treatment.

5.2 Materials and Methods

5.2.1 Sample preparation and microwave cooking

Egg white foams were produced using the whipped cream dispenser as described in Section 4.2.1 for Chapter 4. Foams produced were cooked immediately using a microwave oven (Menumaster commercial microwave, RMS510D, UK) with 1000 watt and 25.5 litre capacity. Egg white solutions used for this experiment were EWL and EWP solutions and prepared as described in Section 3.2.2 for Chapter 3.

The initial volume of egg white solutions used for the foam preparation with the whipped cream dispenser was 100 g and the shaking time applied was 20 times. After shaking, foam was dispensed into a glass beaker (700 ml) and then cooked in the microwave oven for different cooking times ranging from 5 s to 40 s to determine its influence on the foam properties.

5.2.2 Analysis of foamability and foam stability

In all foams produced after microwave cooking, the volume of foams was measured immediately and calculated using Equation 1 below. The foam volume stability and foam liquid stability were also measured at 30 s intervals for a total of 5 min immediately after cooking and calculated following Equations 2 and 3.

$$\text{Foamability } \% = \frac{V}{V_I} \times 100 \qquad (1)$$

$$\text{Foam volume stability } \% = \frac{V_T}{V} \times 100 \qquad (2)$$

$$\text{Foam liquid stability } \% = \frac{V - V_{LD}}{V} \times 100 \qquad (3)$$

Where, V is the volume of foam after microwave cooking,
V_I is the volume of initial liquid used for foam preparation,
V_T is the volume of foam measured over time after cooking of foam, and
V_{LD} is the volume of liquid drained over time after cooking of foam.

5.2.3 Effects of some factors on egg white foam after microwave cooking

5.2.3.1 Microwave cooking time

As indicated above, the effect of microwave cooking times (10, 20, 30 and 40 s) was initially determined with EWL solution (10% protein, w/v).

5.2.3.2 Types and concentration of egg white (EWL and EWP)

Effects of type and protein concentration of egg white were examined using EWL (10% protein) and EWP solutions (10 and 20% protein).

5.2.3.3 Effect of heat treatment of EWL before foam preparation

EWL was heat-treated prior to foam preparation at different temperatures; 58°C for 3.5 min, 60°C for 2 min and 63°C for 2 min and then cooled down as described in Section 4.2.5 for Chapter 4. The effect of heat treatment on the foam volume and foam stability of foams after cooking in the microwave oven for 30 s was examined and compared with the control sample prepared from EWL (20±1^{o}C) without heat treatment.

5.2.3.4 Addition of single ingredient to EWL

Egg white foam properties after microwave cooking were studied after the addition of each of different ingredients (sugar, citric acid or hydrocolloid) into 100 g of EWL (10% protein). The concentration of these ingredients used is shown in Table 5.1.

After dissolving ingredients in EWL solution at 20±1^{o}C, the solutions were stored at 4±1°C overnight to allow full hydration of hydrocolloids. Before the foam formation, the temperature of all EWL solutions was warmed up to 20±1°C and the foams were produced by shaking the whipped cream dispenser for 20 times as described above.

Table 5.1 Formulations of EWL with single ingredient at different concentrations.

Ingredients	EWL (g)	Ingredient (g)	Total (g)	Ingredient (%)	Protein (%)	pH
Sugar (S)	100	20	120	17	8.3	9.00
Citric acid (CA)	99.95	0.05	100	0.05	10	7.45
Xanthan gum (XG)	99.96	0.04	100	0.04	10	8.90
Guar gum (GG)	99.96	0.04	100	0.04	10	8.88
Locust bean gum (LBG)	99.96	0.04	100	0.04	10	8.80
Gum arabic (GA)	98	2	100	2	9.8	8.72

5.2.3.5 Addition of mixtures of different ingredients to EWL

Five different combinations of different ingredients were added to EWL solution to examine their effect on the EWL foams after microwave cooking for 30 s (Table 5.2). After adding each set of seven different ingredient formulations to EWL, the mixtures were adjusted to pH higher than pH 7 at 20±1^{o}C

Foams were produced from 100 g of each formulation of EWL mixed with ingredients after shaking 20 times and dispensing into a glass beaker (700 ml). The foams produced were cooked for 30 s in the microwave oven. The cooking time of 30 s was chosen after determining the optimal cooking time that does not cause full gelation of egg white foam.

Table 5.2 Seven different formulations of EWL solutions with different ingredients.

Sample code	Ingredients	Ingredient (g)	EWL (g)	Total (g)	Ingredient (%)	Protein (%)
	Citric acid	0.05			0.04	
1	Sugar	20	100	120.05	16.7	8.33
	Citric acid	0.05			0.04	
2	Xanthan gum	0.04	99	99.16	0.03	9.98
	Guar gum	0.04			0.03	
3	Xanthan gum	0.04			0.03	
	Locust Bean gum	0.04			0.03	
4	Sugar	20	99	119	16.8	8.32
	Xanthan gum	0.04			0.03	
	Guar gum	0.04			0.03	
5	Sugar	20	99	119	16.8	8.32
	Xanthan gum	0.04			0.03	
	Locust bean gum	0.04			0.03	
6	Sugar	20	99	119	16.5	8.32
	Citric acid	0.05			0.04	
	Gum arabic	2			1.65	
7	Sugar	20	100	122.17	16.4	8.19
	Citric acid	0.05			0.04	
	Xanthan gum	0.04			0.03	
	Guar gum	0.04			0.03	
	Locust bean Gum	0.04			0.03	
	Gum arabic	2			1.64	

5.2.3.6 Heat treatment of EWL solution with ingredients before foaming

A total of 100 g of EWL solution was mixed with ingredients denoted as sugar (20%), citric acid (0.05%), xanthan gum (0.04%), guar gum (0.04%), locust bean gum (0.04%) and gum arabic (2%), which was the same as the sample 7 formulation described in Table 5.2. Then, the solution was heat-treated at different temperatures (58°C for 3.5 min and 60°C and 63°C for 2 min) as described in Section 5.2.3.3. After heat treatment and cooling down, the foam was prepared and cooked under the same condition described above in Section 5.2.3.5.

5.2.4 Statistical data analysis

All experiments for the sample preparation and analysis were carried out at least in duplicate. The results were reported as average and standard deviation. The data were statistically analysed using a Minitab statistical software version 16 (Minitab Inc., USA). A one-way ANOVA using a Turkey method was used to determine the significance of means at a 95% confidence level ($p < 0.05$).

5.3 Results and Discussion

5.3.1 Microwave cooking time

The ability of egg white foam to maintain its original volume and hold its liquid against drainage after foam preparation and also during and after the subsequent treatment (e.g. thermal treatment) can be affected by various factors, including ingredient composition and the formulation used for making foam, foaming methods and cooking conditions (e.g. methods, temperature and time). In this experiment, egg white foams prepared from EWL solution by using the whipped cream dispenser after shaking for 20 times and were cooked in a microwave oven (1000W). The effect of microwave cooking time was studied in this experiment.

5.3.1.1 Foam volume

Egg white foam was cooked in a microwave oven for different times (10, 20, 30 and 40 s) after it was produced from EWL solution. The heat treatment of egg white foam by using the microwave oven was found to further increase its foam volume, dependent on the microwave cooking times. Figure 5.1 shows that the volume of foam prepared by using the whipping cream dispenser was increased further by 200 ± 22%, 501 ± 21% and 737 ± 88% after cooking for 10, 20 and 30 s, respectively using high heat (1000W). However, the sample that was cooked for 40 s did not show any further increase than what was observed for 30 s heating (725 ± 99%).

When the increase in foam volume after cooking was calculated compared to the initial EWL volume used to make the foam, the final foam volume was determined to increase up to 525 ± 22%, 826 ± 21%, 1062 ± 88% and 1050 ± 99% at cooking times of 10, 20,

30 and 40 s, respectively. This implies that the foam volume of EWL solution increased after foaming prior to cooking in the microwave was 325%.

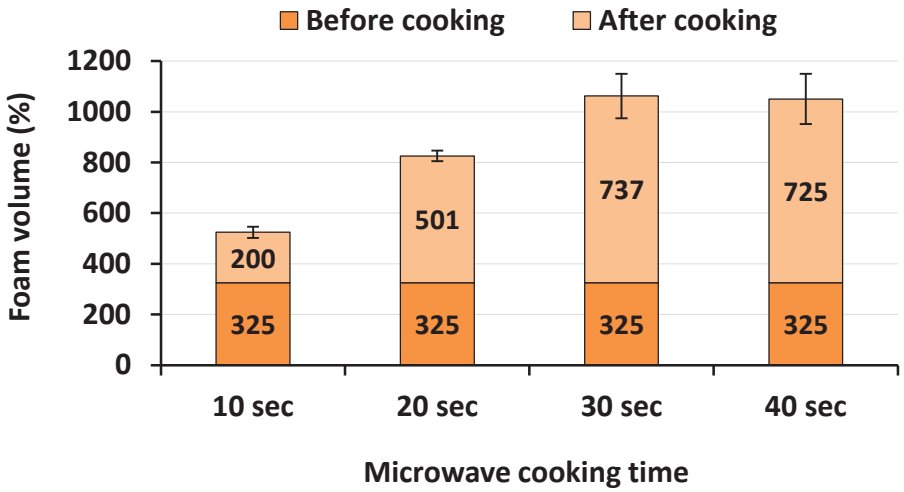

Figure 5.1 Effect of microwave cooking on the foam volume of egg white foam produced from EWL as a function of cooking times (10, 20, 30 and 40 s).
In the stacked bar graph, the lower part represents the foam volume increased after foam preparation using the whipping cream dispenser while the upper part represents the foam volume increased after cooking in the microwave oven. Each data point is mean ± SD for n=2.

The enhancement in foam volume among three samples that were cooked for 10, 20 and 30 s was found to be linearly proportional to the cooking time (R^2= 0.9851) (Figure 5.2). A loss of water during cooking in the microwave oven was observed by measuring the weight of foam before and after cooking. The percentage loss of water was 0.33 ± 0.06%, 1.74 ± 1.20%, 2.93 ± 0.255% and 5.23 ± 0.19% after 10, 20, 30 and 40 s, respectively. A plot of cooking time versus % water loss is shown in Figure 5.3. The results show a high correlation coefficient (R^2 = 0.978) between the cooking time and loss of water.

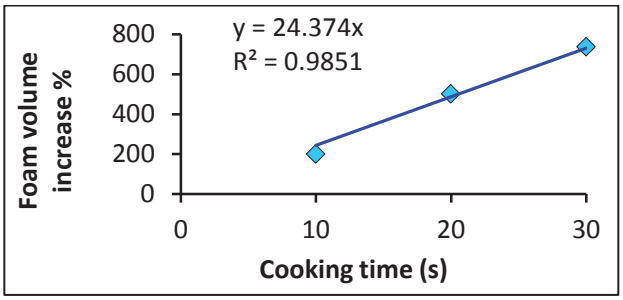

Figure 5.2 Correlation between microwave cooking time and foam volume increase.

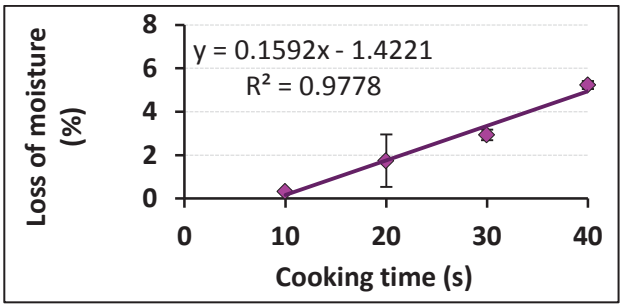

Figure 5.3 Correlation between microwave cooking time and loss of moisture from EWL foam due to evaporation.

5.3.1.2 Foam stability

Figure 5.4 illustrates that overall all foams collapsed rapidly within 3 min after cooking as their foam volumes were reduced to 44±2.83, 26±1.57%, 32±0.24% and 31±2.3% for the samples cooked for 10, 20, 30 and 40 s, respectively. Among these samples, the foam cooked for 10 sec showed the highest foam volume stability and this difference was significant compared to the other cooking times (Figure 5.4A). It was also shown that a 20 s cooking time had a similar trend with a slight reduction while 30 and 40 s cooking times had the lower foam volume stability, particularly during the first 2 s after cooking.

In contrast, foam liquid stability showed the opposite trend to the foam volume stability. The foam cooked for 40 s had a significantly higher liquid stability than the other samples, particularly those cooked for 10 and 20 s (Figure 5.4B). This may be due to the formation of a more rigid gel-like matrix due to protein denaturation and aggregation and evaporation of some moisture as described earlier. The high foam liquid stability of this sample was maintained even 5 min after cooking despite the fact that the foam

volume stability had a significant decrease after 4 min with no notable difference from the other samples. The foam cooked for 30 s also exhibited a similar pattern in its liquid stability to the foam cooked for 40 s but with a slight reduction.

In contrast, the foams cooked for 10 or 20 s showed the lowest liquid stability, and almost all the liquid was drained after 4 min cooking. The results suggest that there was some difference in the structure of foam matrix after cooking between samples resulting from a difference in the degree of protein denaturation and aggregation.

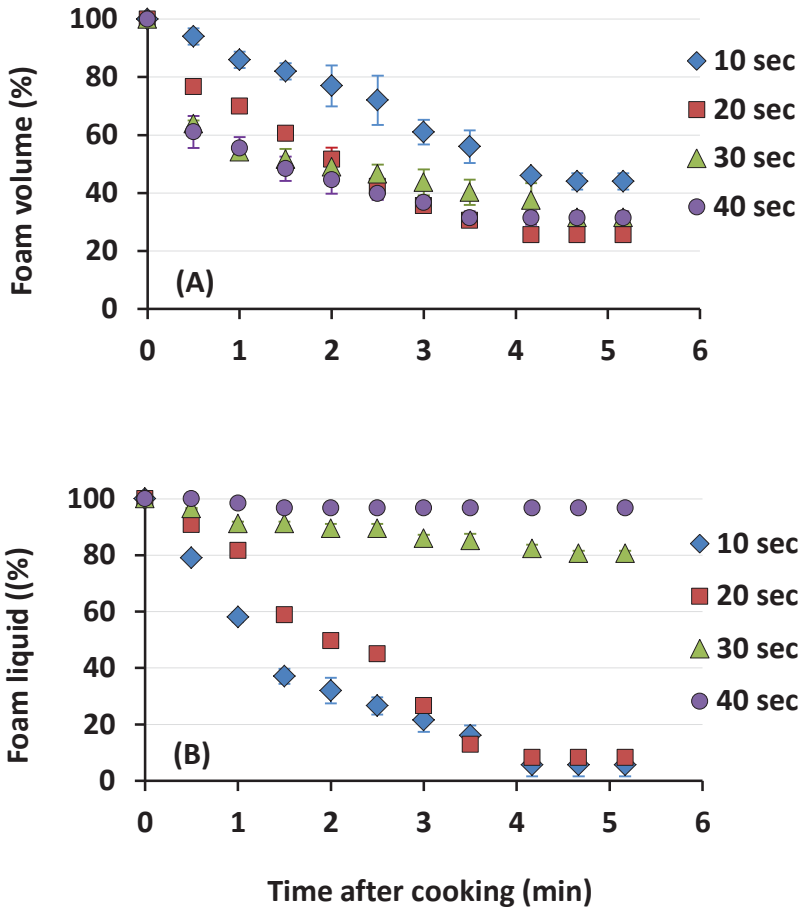

Figure 5.4 Effect of microwave cooking on the foam stability of egg white foam produced from EWL as a function of cooking times (10, 20, 30 and 40 s); (A) foam volume stability and (B) foam liquid stability.
Each data point is mean ± SD for n=2.

5.3.1.3 Foam appearance

The digital images of egg white foams were taken after cooking in the microwave oven for different times (Figure 5.5). It was observed that cooking time for 10 and 20 s resulted in under-cooked foam with low foam volume and more rapid liquid drainage. On the other hand, cooking for 30 and 40 s, particularly 40 s which may be considered as a relatively long cooking time, resulted in a loss of the foamy structure and the formation of a gel matrix that remained stable for some time. The main difference between the foams cooked for different times was that 30 and 40 s cooking produced the highest foam volume.

Based on the overall results of foam volume, foam stability and foam appearance, it was concluded that an optimal microwave cooking time to cook 100 g of foam produced by a whipped cream dispenser was around 30 s.

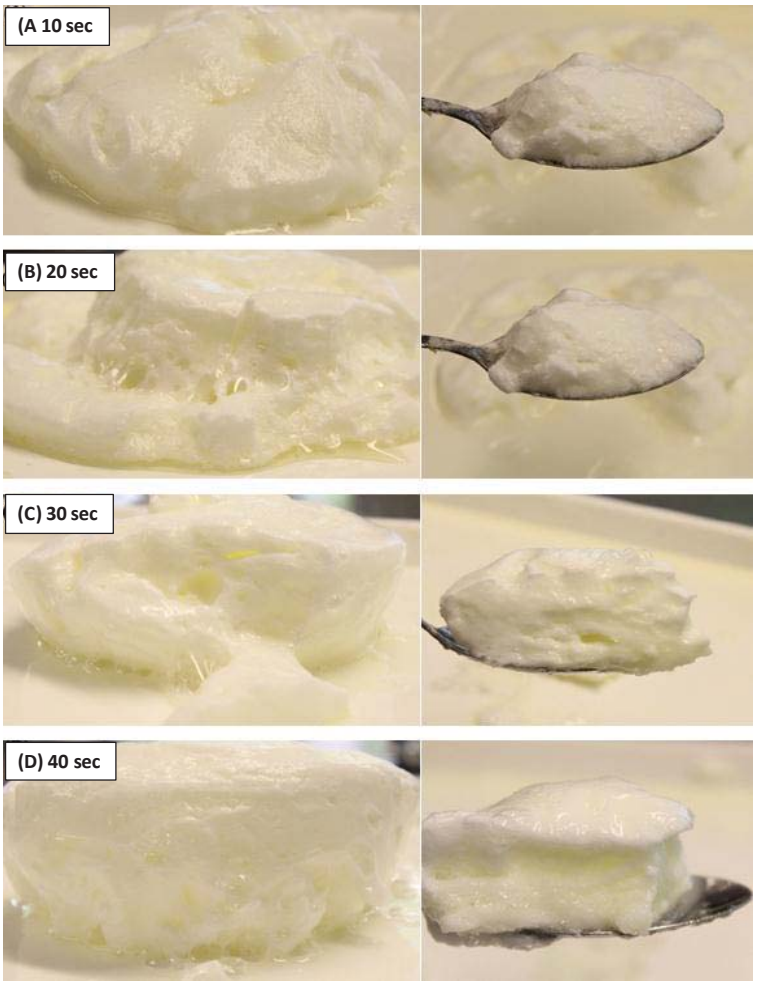

Figure 5.5 Digital images of egg white foams prepared from EWL solutions and then cooked in the microwave oven for different times (A) 10 s, (B) 20 s, (C) 30 s and (D) 40 s.

5.3.2 Types and concentration of egg white (EWL and EWP solutions)

Egg white foams containing 10 and 20% protein were also prepared from spray dried egg white powder (EWP) solution and then cooked in the microwave for 10, 20, 30 and 40 s. The results of foam volume and foam stability were compared to the results of foams prepared from EWL containing 10% protein.

5.3.2.1 Foam volume

The overall foam volume trend for EWP foams showed that after heating in the microwave, the foam volume increased for all samples, with the exception of the EWP foam containing 10% protein cooked for 40 s which showed a decline (Figure 5.6). It is unclear why there was a decrease in foam volume for the EWP sample at 10% protein concentration after cooking for 40 s. For the EWL and EWP foams (100 g), the optimum time for cooking in the microwave oven, in terms of foam volume, was 30 s for all samples. The EWL foam had a slight high in foam volume at all cooking times but the increase was found to be not significant ($p > 0.05$) (Appendix 12).

The loss of moisture from foams during cooking in the microwave oven for different times is shown in Figure 5.7 for the two samples of EWP foams contacting 10 and 20% protein. Again the loss of moisture was linearly proportional to the microwave cooking time (Figure 5.8).

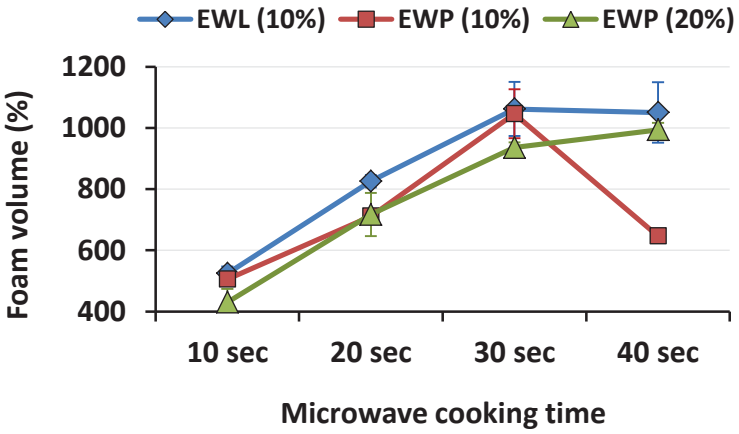

Figure 5.6 Foam volume for different types of egg white solutions at different protein concentrations.
Each data point is mean ± SD for n=2.

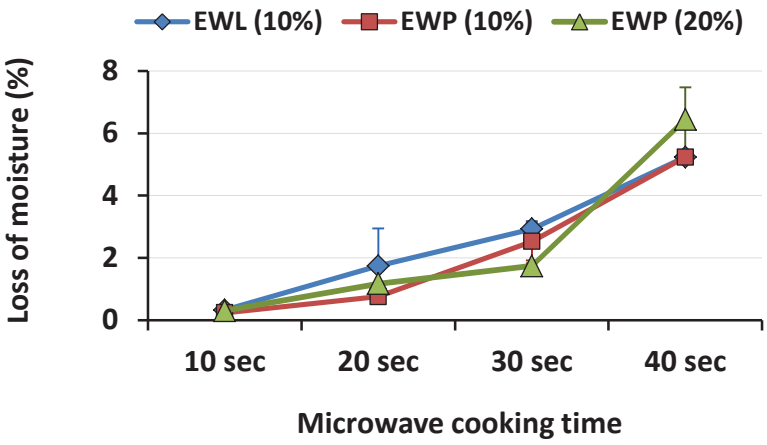

Figure 5.7 Loss of moisture from egg white foams prepared from EWL (10% protein) and EWP (10 and 20% protein) during cooking in the microwave for different times (10, 20, 30 and 40 s).
Each data point is mean ± SD for n=2.

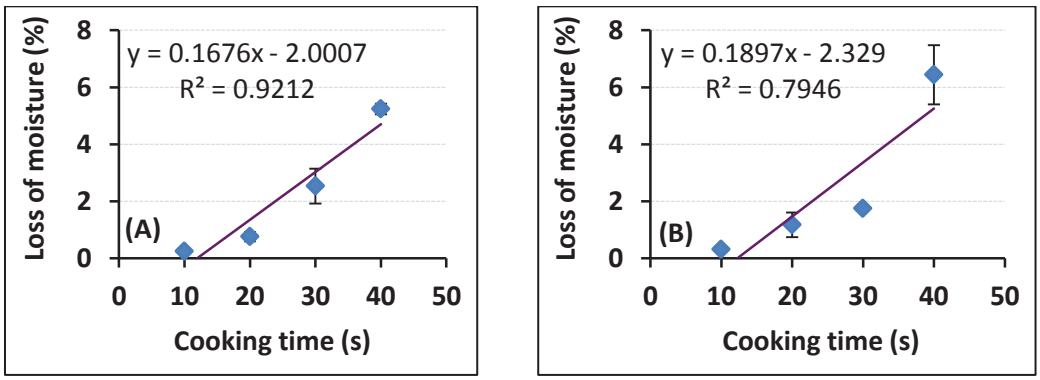

Figure 5.8 Correlation between microwave cooking time and loss of moisture from foam during cooking in the microwave oven due to evaporation; (A) EWP foam at 10% protein and (B) EWP foam at 20% protein.

5.3.2.2 Foam stability

Generally, increasing cooking time led to the enhancement of foam liquid stability but weakened foam volume stability (Figure 5.9). For the foams from EWL and EWP at both 10% protein the foam stability was similar (Appendix 13). However, foams produced with 20% protein were showed a reduce rate of foam collapse, thus enhancing the foam volume stability for a longer time. This was found to be more pronounced between the samples cooked for 10 s. This delayed effect on the foam collapse at high

protein concentration may be because the film between water and air bubbles is thicker so the foam is conserved the liquid for a longer time (Kinsella, 1981; Rodriguez Patino et al, 1995).

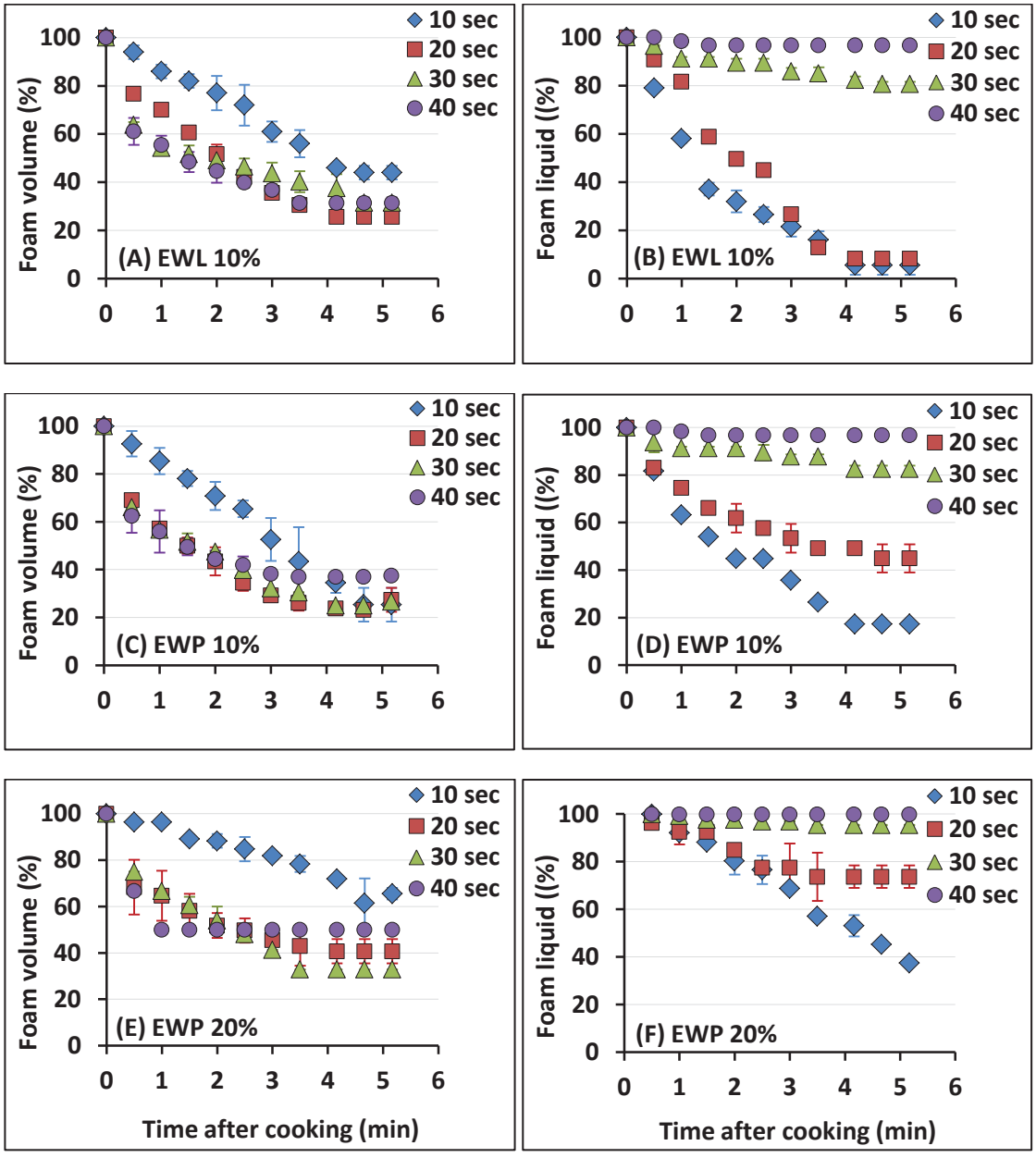

Figure 5.9 Foam stability of egg white foams prepared from EWL (10% w/v protein) and EWP solutions (10 and 20% w/v protein) after cooking in the microwave oven for different times (10, 20, 30 and 40 s). Foam volume stability (A, C and E) and foam liquid stability (B, D and F).
Each data point is mean ± SD for n=2.

Regarding foam liquid stability, the results of increasing liquid stability with increasing cooking time observed from the EWL foams were also seen in the foams prepared from EWP solution but the EWP foams had a higher liquid stability than the EWL foams, notably for the foams cooked for 10 and 20 s (Figure 5.9) (Appendix 14). A possible reason for the high liquid stability at 20% protein concentration could be because there was less water in the foam. Another reason could be the higher viscosity of the foam from 20% protein which enables the foam to retain its liquid more effectively (Sanchez & Patino, 2005). When the foams were cooked for 40 s, there was no difference between any of the samples for foam liquid stability.

5.3.2.3 Foam appearance

The appearances of foams made from EWP solution after cooking in the microwave oven for different times are shown in Figure 5.10. Overall they looked similar in appearance to the foams prepared from EWL which are shown in Figure 5.5 but seemed to be firmer and denser with less liquid drainage. This effect became more notable with 20% protein concentration.

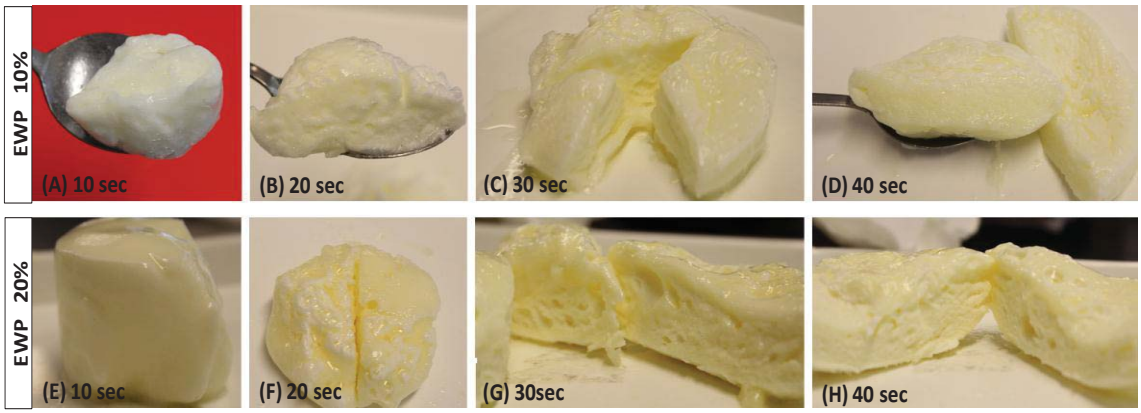

Figure 5.10 Foam appearance after cooking in the microwave oven for different times (10, 20, 30 and 40 s). Egg white foams prepared from EWP solutions containing 10% protein (A, B, C and D) and 20% protein (E, F, G and H).

5.3.3 Effect of heat treatment on EWL before foam preparation

Heat treatment of the initial EWL at different temperatures (20, 58, 60 and 63°C) was examined for its effect on the foam volume and foam stability after cooking in the microwave oven for 30 s.

5.3.3.1 Foam volume

The foam volume increase after cooking was considerably higher for the EWL that was heat-treated at 58, 60 and 63°C prior to the foam preparation (Figure 5.11A) compared to the control sample heated at 20°C prior to foam formation. The differences between the samples heat-treated at 58, 60 and 63°C were however not significantly different ($p > 0.05$) with the final foam volume increase of 1336 ± 13%, 1282 ± 5% and 1482 ± 84%, respectively.

5.3.3.2 Foam stability

Overall the foam volume stability dropped sharply for all samples within the first 1 min after cooking by decreasing their foam volume by 50-60% (Figure 5.11B). After 1 min, the foam volume decreased slowly and steadily for all samples. No significant difference was observed between the control and the samples prepared from heat-treated EWL.

However, with regard to foam liquid stability, the foam prepared from the EWL heat-treated at 58, 60 and 63°C showed the greater liquid stability than the control sample (20^{o}C) (Figure 5.11C). In particular, the foam prepared from the EWL heat-treated at 58 and 60^{o}C was more stable against liquid drainage than the other samples.

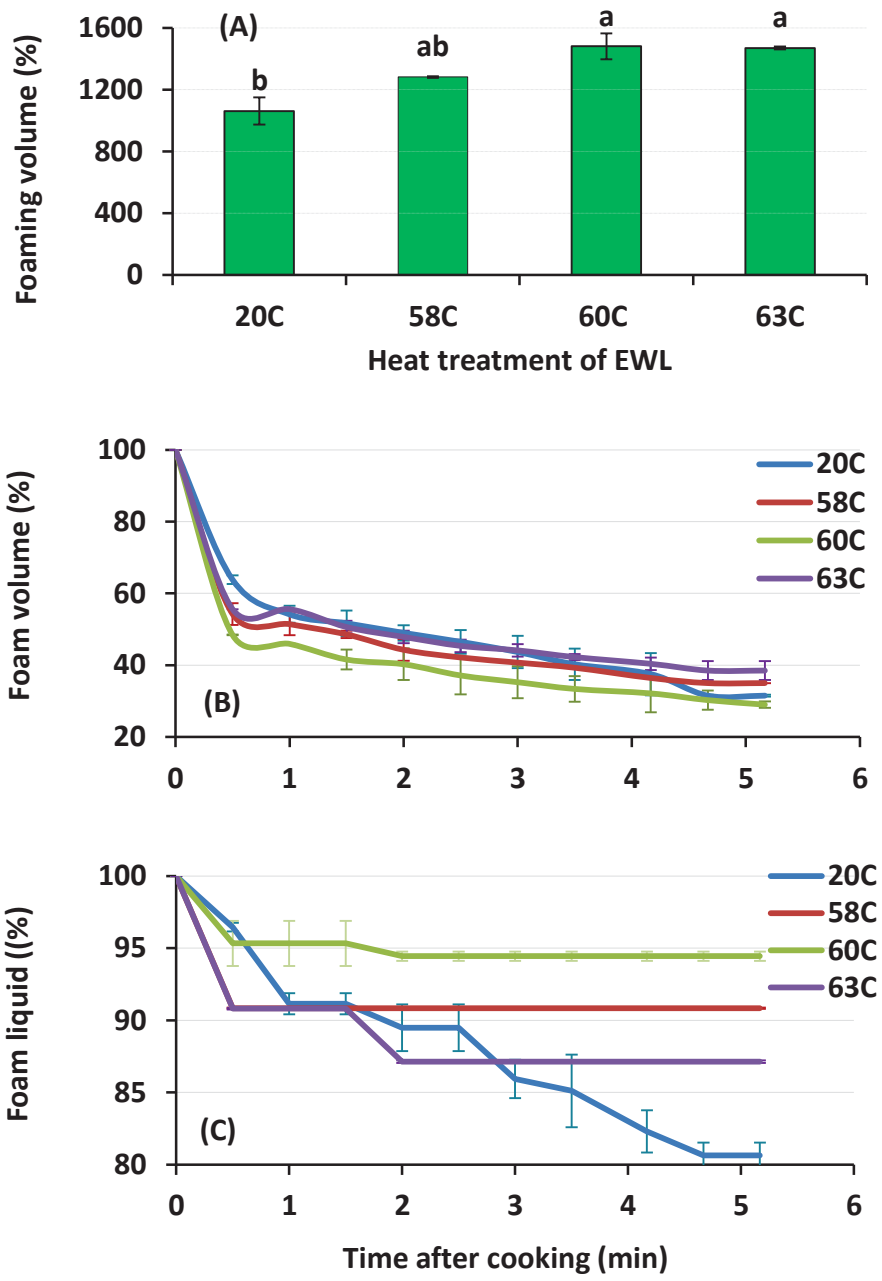

Figure 5.11 Effect of heat treatment of EWL solution at 20, 58, 60 and 63°C, prior to making foam on the foam volume (A), foam volume stability (B) and foam liquid stability (C) of foams after cooking in the microwave for 30 s.

[a] Means followed by the same letter within a column are not significantly different ($p < 0.05$). Each data point is mean ± SD for n=2.

5.3.4 Addition of single ingredient to EWL

In this experiment, each of the following ingredients, sugar, citric acid, xanthan gum, guar gum, gum arabic and locust bean gum, was added individually into EWL to examine their effects on the properties of foam after cooking in the microwave oven for 30 s.

5.3.4.1 Foam volume

All samples containing each of the different ingredients did not show any significant difference in their final volumes after cooking in the microwave for 30 s (Figure 5.12A). The control sample had a final volume increase to 1062 ± 88 % after cooking based on its initial volume of EWL used to make the foam (100 g).

5.3.4.2 Foam stability

The stability of foam volume among all samples had a similar pattern with a rather sharp decline within the first 1 min after cooking (Figure 5.12B). A difference in foam volume stability between samples was not very noticeable but it was observed that the samples containing citric acid, locust bean gum (LBG) or xanthan gum (XG) seemed to be slightly more stable than the other samples (Figure 5.12B). On the other hand, the samples containing sugar or gum arabic (GA) had a slight decrease in foam stability.

Regarding the foam liquid stability, the main noticeable difference was the sample containing sugar which showed a significant decline in the liquid stability compared to all other samples (Figure 5.12C). The samples had GA, LBG or guar gum (GG) displayed a slightly higher foam liquid stability than the others excluding the sugar containing sample.

Overall addition of different individual ingredients did not affect the foamability or foam stability after microwave heating, except for sugar. This may be because of the concentration of individual ingredients used was not large enough to cause a significant difference in the properties of foam when it was cooked in the microwave oven for 30 s.

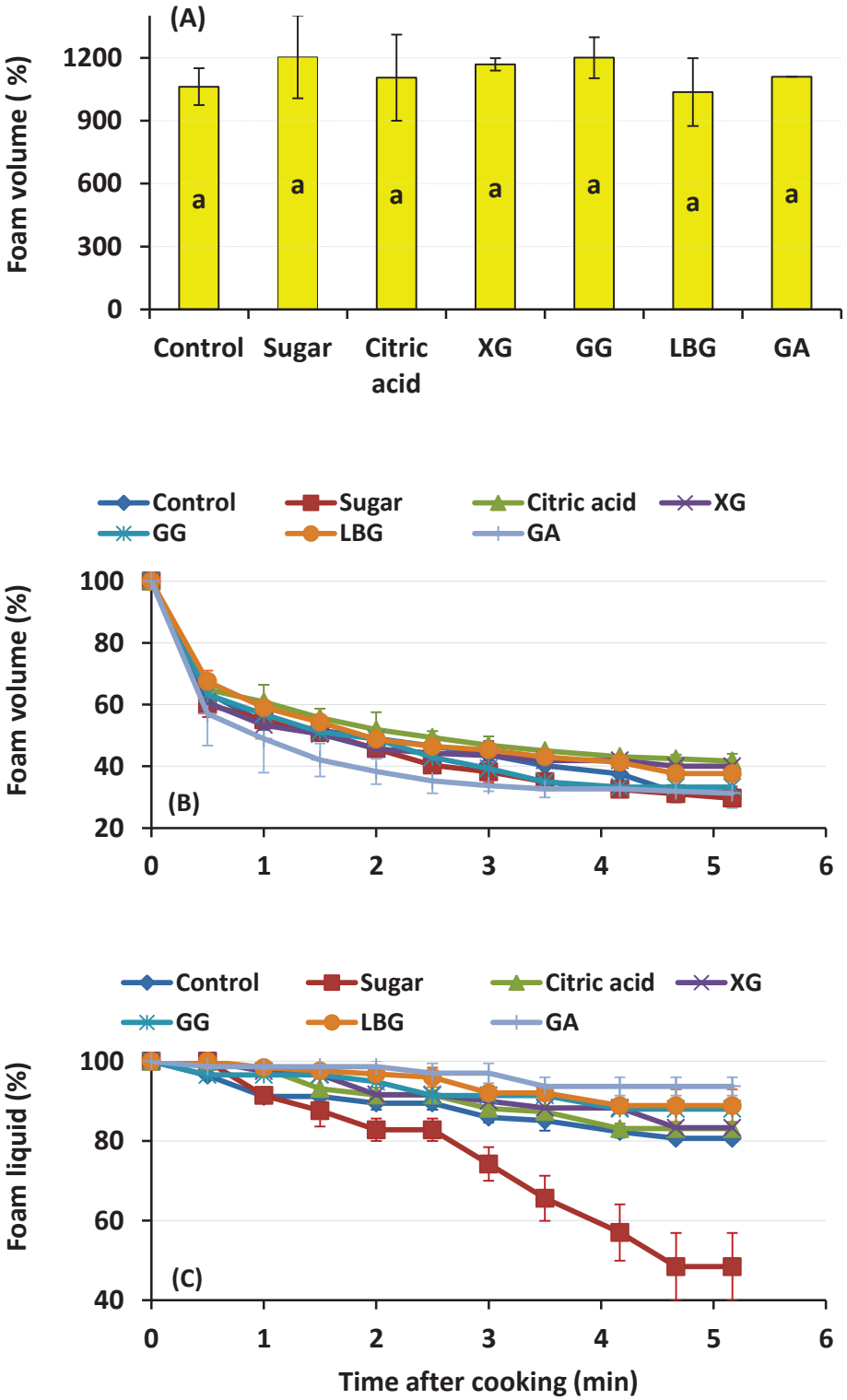

Figure 5.12 Effects of addition of some ingredients on the foam volume (A), foam volume stability (B) and foam liquid stability (C) of EWL foams after cooking in the microwave oven for 30 s.

Abbreviations XG, GG, LBG and GA represent xanthan gum, guar gum, locust bean gum and gum arabic, respectively. [a] Means followed by the same letter within a column are not significantly different ($p < 0.05$). Each data point is mean ± SD for n=2.

Several functional roles of ingredients that can have an impact on the foam volume and/or foam stability are described below based on the literature. Even though the viscosity of egg white solution can increase by adding sugar, this viscosity would be low compared to the viscosity provided by hydrocolloids, thereby the sample containing sugar will be lower in foam liquid stability compared to the ones containing hydrocolloids.

5.3.5 Addition of mixtures of different ingredients to EWL

Foams were prepared from EWL solutions after mixing with seven different combinations of the following ingredients: 20% sugar, 0.05% citric acid and four different types of hydrocolloids (0.04% XG, 0.04% GG, 0.04% LBG and 2% GA). The foams were then cooked in the microwave oven for 30 s.

5.3.5.1 Foamability

Figure 5.13A shows that the foam volume of samples reached up to 1027-1104% after cooking compared to the initial EWL weight (100g). However, there was no difference between the different between the samples with different ingredient combinations.

5.3.5.2 Foam stability

Foam volume stability was also not significantly different between all samples except sample 1 (sugar/citric acid) and sample 6 (sugar/citric acid/GA) (Figure 5.13B). Especially, sample 1 (sugar/citric acid) had lower volume stability than the other samples as no hydrocolloid was added to sample 1. In the case of sample 6 (sugar/citric acid/GA), the ingredient composition was similar to sample 1 although it contained GA as hydrocolloid. GA doesn't increase viscosity particularly 2% GA concentration unlike the other types of hydrocolloids (XG, GG and LBG) which can provide high viscosity at much lower levels. Therefore, it could be concluded that the relatively low volume stability observed for samples 1 and 6 could be possibly related to the lower liquid viscosity compared to the other samples formulated with XG, GG and/or LBG.

The results for foam liquid stability were also in agreement with the foam volume

stability (Figure 5.13C). Sample 1 and 6 had rapid liquid drainage than all other samples, such as samples 2 (XG/GG), sample 3 (XG/LBG), sample 4 (sugar/XG/GG) and sample 7 (all ingredients added). Among all samples, the highest liquid stability was observed from sample 2 (XG/GG) followed by sample 3 (XG/LBG). It is known that XG gives a synergistic effect for the formation of higher viscous solution when used together with GG or LBG through their binding along the region of their backbone chain with no branches (Philips et al., 1996). The ratio for the optimal synergistic effect is 5:5 of XG and LBG and 8:2 of XG and GG (Lachke, 2004).

On the other hand, it was observed that when sugar was added together with XG/GG or XG/LBG, the foam liquid stability, such as sample 4 (sugar/XG/GG) and sample 5 (sugar/XG/LBG), declined compared to their corresponding sample 2 (XG/GG) and sample 3 (XG/LBG) with no added sugar.

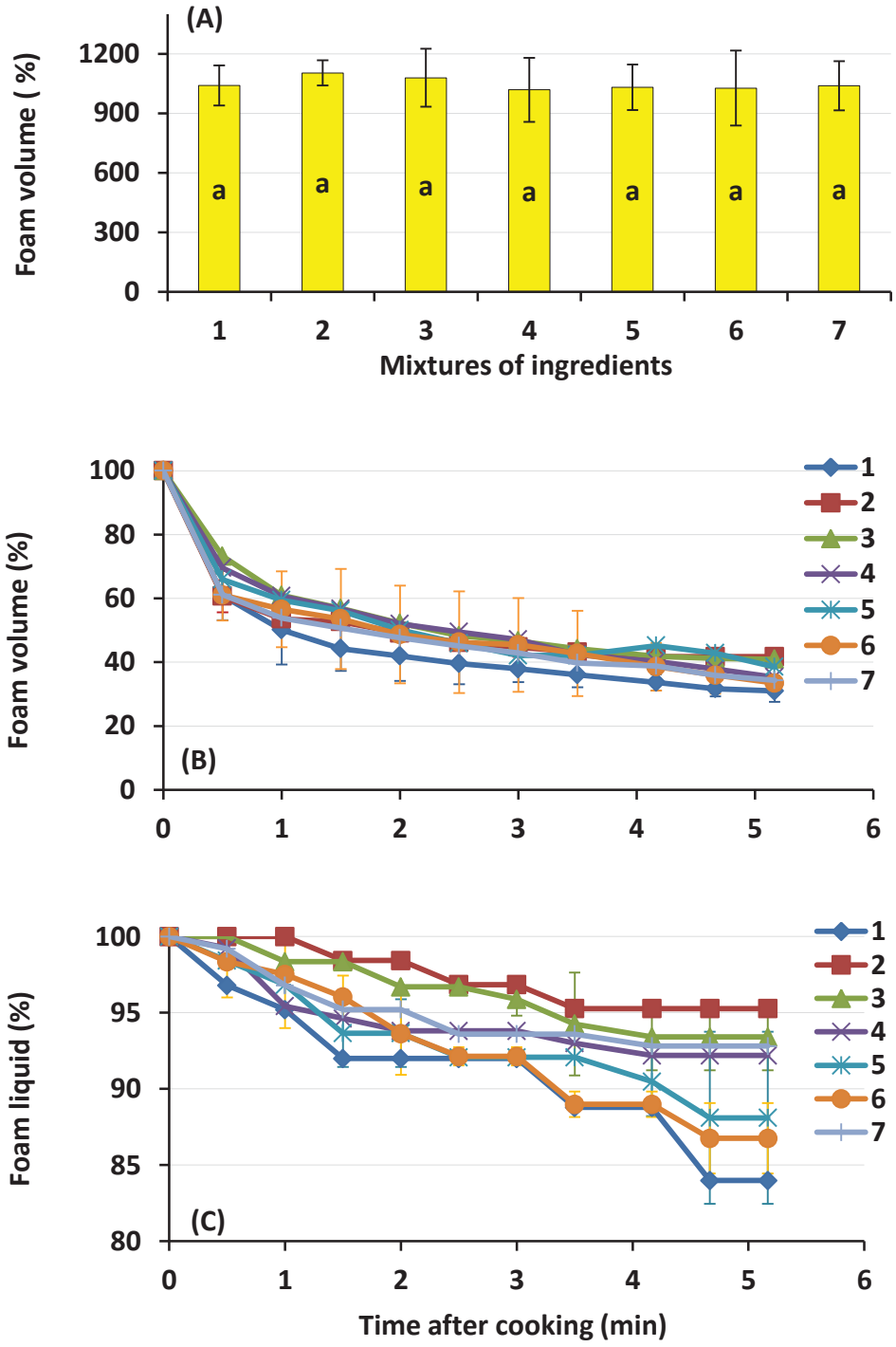

Figure 5.13 Effect of addition of mixtures of different ingredients into EWL solution on the foam volume (A), foam volume stability (B) and foam liquid stability (C) of foams after microwave cooking for 30 s.

Numbers represent mixture of ingredients; (1) sugar (20%)/citric acid (0.05%). (2) xanthan gum (0.04%)/guar gum (0.04%), (3) xanthan gum (0.04%)/locust bean gum (0.04%), (4) sugar (20%)/xanthan gum (0.04%)/guar gum (0.04%), (5) sugar (20%)/xanthan gum (0.04%)/locust bean gum (0.04%), (6) sugar (20%)/citric acid (0.05%)/gum arabic (2%) and (7) all ingredients. In Figure A, [a] Means followed by the same letter are not significantly different ($p < 0.05$). Each data point is mean ± SD for n=2.

5.3.6 Heat treatment of EWL solution with ingredients before foaming

In this experiment, the EWL solution was mixed with a combination of all ingredients, which was the same as the ingredient formulation used in sample 7 described in the previous Section 5.2.3.5, and then the EWL solution containing ingredients was heat-treated at different temperatures 58, 60 and 63°C as shown in Section 5.2.3.6. After the thermal treatment, foams were prepared by using the gas sparging method and then cooked in the microwave oven for 30 s. The results were compared to the foam prepared from the control sample containing ingredients at 20°C without heat treatment.

5.3.6.1 Foam volume

The foam prepared from non-heat treated EWL solutions containing ingredients at 20°C produced the lowest foam volume with 1039 ± 124% increase after microwave cooking. On the hand other, foams prepared from heat-treated EWL solutions containing ingredients at 58, 60 and 63°C showed the higher foam volume in the range of 1487-1530% but there was no significant difference between them ($p > 0.05$) (Figure 5.14A).

There was no significant difference ($p > 0.05$) in the majority of samples for the heat treatment of EWL solution with no added ingredients as shown in Figure 5.11A for Section 5.3.3. It should be pointed out that the sample heat-treated at 58°C prior to making foam was higher in its foam volume in the presence of ingredients (1478 ± 10%) compared to that in the absence of ingredients (1282 ± 5%), the reason for the higher foam volume is not clearly understood as this was not observed from the other samples containing ingredients that were heated at 60 or 63°C.

In summary, the foam volume was found to increase when foam was produced from the heat-treated EWL solution, especially at 60 or 63°C, regardless of the addition of ingredients, compared to the foam prepared the EWL solution at 20°C. Again, it was interestingly to observe that overall there was no considerable difference in the foam volume prepared between the heat-treated EWL solutions with and without added ingredients. However, it should also be pointed out that the EWL solution with added ingredients had a lower overall protein content of around 8.2% due to the addition of ingredients (20%sucrose, 0.05% citric acid, 0.04% XG, 0.04% GG, 0.04% LBG and 2% GA) compared to the protein content of 10% in the EWL solution with no added ingredients. Also, the total solids content between the EWL with and without added

ingredients differed by approximately 16%. These differences should also be taken into account when comparing the samples with and without containing ingredients as it can have a significant influence on the formation and properties of foam.

5.3.6.2 Foam stability

Figure 5.14B shows that the heat-treated sample at 58^{o}C produced a foam with the lowest volume stability whereas the unheated control sample at 20^{o}C had the higher volume stability than the others although it was not very different from the other heat-treated samples at 60 and 63^{o}C.

For foam liquid stability, the control and heat-treated sample at 63^{o}C showed the higher stability than the others (Figure 5.14C). In contrast, the samples heated at 58 or 60^{o}C produced a foam with a less liquid stability, particularly for the sample treated at 60^{o}C. These differences between samples were observed around 3 min after cooking.

There was no correlation between foam stability and heat treatment temperatures. Also, the pattern of changing the stability of foams prepared from heat-treated EWL solutions with no added ingredients as a function of temperature (which was described in Section 5.3.3 and shown in Figure 5.11), could not be observed in this experiment. Nevertheless, the results indicated that the addition of all ingredients with no heat treatment (i.e. control sample at 20^{o}C) did enhance the foam liquid stability considerably compared to the control sample without containing ingredients shown in Figure 5.11 for Section 5.3.3. The other samples heat-treated at 60 or 63^{o}C with and without ingredients showed the opposite results and interestingly, the samples treated at 58^{o}C were observed to have a similar pattern in their stability with no difference, regardless of the presence or absence of ingredients.

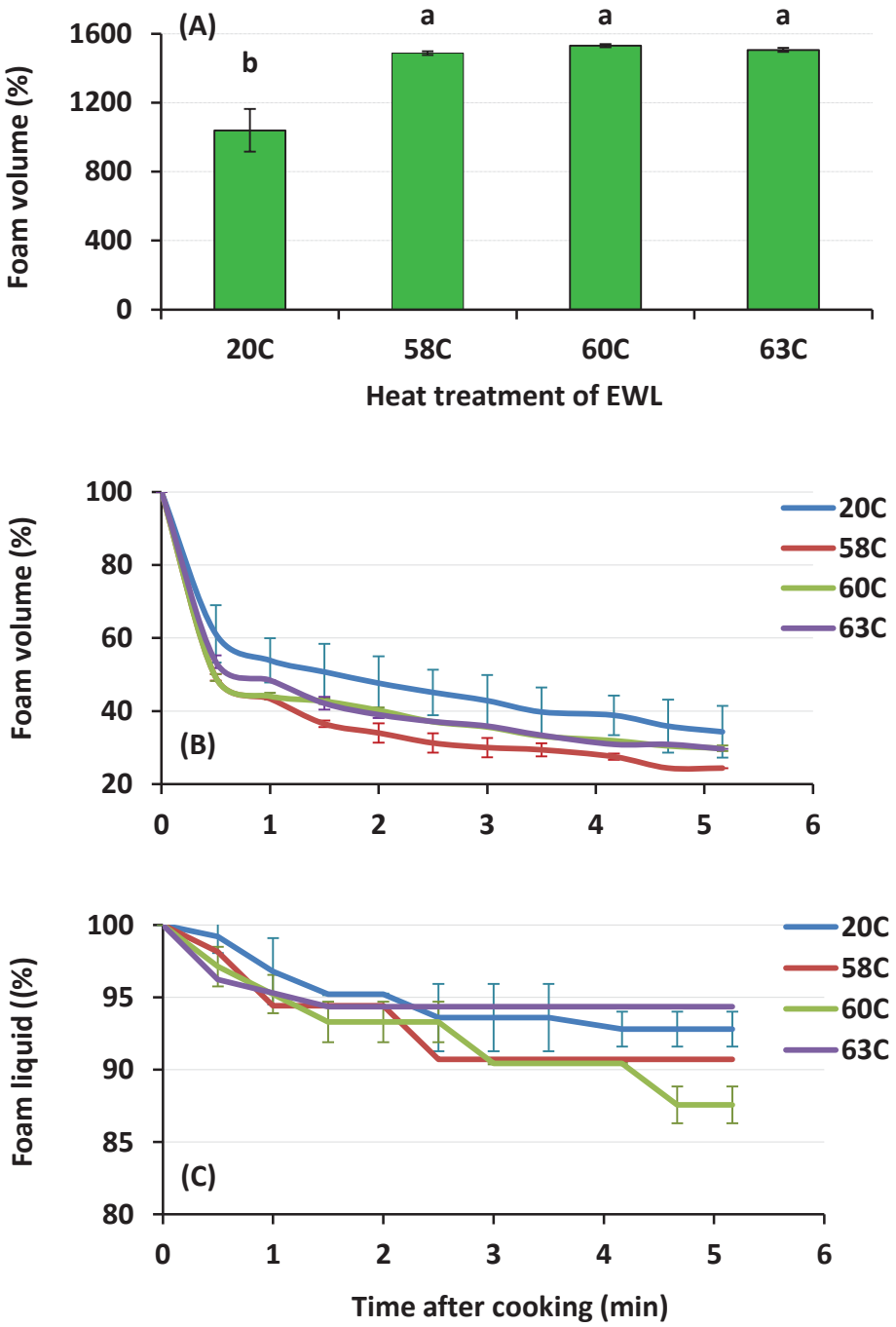

Figure 5.14 Effects of heat treatment at 20, 58, 60 and 63°C of EWL solution containing ingredients (20%sucrose, 0.05% citric acid, 0.04% XG, 0.04% GG, 0.04% LBG and 2% GA) on the foam volume (A), foam volume stability (B) and foam liquid stability (C) of foams after microwave cooking for 30 s.

In Figure A, [a] Means followed by the same letter are not significantly different ($p < 0.05$). Each data point is mean ± SD for n=2.

5.4 Conclusions

Heat treatment of egg white foams in a microwave oven was studied. Overall the foam volume was found to be significantly further increased after cooking in the microwave oven. However, the extent of foam volume increase and the properties of foams after cooking were dependent on the time of microwave cooking applied. The longer cooking time, the firmer and denser foam resulted in, due to protein aggregation and/or gelation coupled with evaporation of water. As a result, foam volume and foam liquid stability were significantly affected by cooking time but its influence was observed to be opposite between foam volume stability and foam liquid stability. The longer cooking time resulted in the higher foam liquid stability but the lower foam volume stability.

Effect of various types of single individual ingredient (sugar, citric acid, XG, GG, LBG and GA) was studied. No pronounced effects on the foam volume and foam stability were observed after microwave cooking between all samples compared to the control sample, except a significant faster liquid drainage observed from a sample added with sucrose (20%). On the other hand, when these ingredients were added in various combinations, although the volume of foams between samples after microwave cooking was not different, the foam liquid stability was observed to be significantly increased for the samples formulated with two different types of hydrocolloids, such as XG/GG, XG/LBG, compared to the control sample, which could be due partly to a synergistic effect for the enhancement of liquid viscosity.

Effect of heat treatment of EWL solution at 60 and 63^{o}C prior to foam preparation was also found to significantly enhance the volume of foams produced after microwave cooking in comparison to the control sample. Also, the liquid stability of foams from the heat-treated EWL solutions was higher than the control sample. However, this positive effect of heat treatment was not seen when the EWL solutions containing a mixture of all ingredients (sugar, citric acid, XG, GG, LBG and GA) were heated.

The results showed that egg white foams produced with the whipped cream dispenser were able to be cooked in a microwave oven. The appearance of cooked egg white foams had a fluffy foam network or semi-gel like structure with foamy layers, suggesting that egg white foams can be converted into a semi solid matrix type of foams that can hold shape and retain its liquid after cooking in the microwave. To enhance the properties of foam or produce foams with desired characteristics, various types of

ingredients at different concentrations need to be further investigate. Some factors tested in the study indicated the foam volume after cooking can be further increased compared to the control sample but in most cases, their effects was more significant in the stability of foam liquid against liquid drainage and was not effective in enhancing the foam volume stability over time after cooking. Overall the results provide significant insights into the potential for the development of a new product based on egg white foams that can be suitable for cooking in a microwave oven.

Chapter 6 Microbial stability of egg white liquid

6.1 Introduction

There are several causes for egg white spoilage which can result from survival of organisms through the pasteurisation process or by the introduction of organisms after the thermal processing which is usually occur during cooling operation and later during storage (Silva et al., 2012). The predominant types of bacteria that are found to grow in egg white liquid (EWL) solution after pasteurisation are gram-negative and rod-shaped bacteria (Tucker & Featherstone, 2011). The most prevalent non-spore forming bacteria that can infect EWL belong to the Enterobacteriaceae family. The optimal growth temperature is 37^{o}C but they can grow in the range of 10-45^{o}C and are destroyed during a pasteurisation process (Nemeth et al., 2011).

Egg white contains a number of proteins with antimicrobial activities, which act as a natural defence system (Macherey et al., 2011). Egg white proteins can be divided into three categories depending on their antimicrobial activities, antibacterial inhibitor (ovalbumin, lysozyme and avidin), antimicrobial ability (ovotransferrin, ovomucin, ovomacroglobulinan and cystatin) and antiviral activity (lysozyme and ovoinhibitor) (Kovacs-Nolan et al., 2005). These antimicrobial effects may be attributed to bacterial cell lysis, metal binding and vitamin binding (Kovacs-Nolan et al., 2005).

EWL should meet a microbiological specification for aerobic plate counts of less than 25,000 per gram (Browns & Ito, 2001). The aerobic plate count of unpasteurised egg white liquid is generally in the range of 10^{3}-10^{6} CFU/gram (Browns & Ito, 2001). Therefore, aerobic plate counts with more than 10^{7} may indicate poor egg breaking process, poor sanitation or improper storage of EWL (Browns & Ito, 2001). Aerobic plate count does not differentiate between the different types of bacteria, but the incubation temperature and agar nutrient content will modify the type of organisms that will grow.

The objective of this project was to determine the microbial stability of EWL with and without added ingredients during storage at 4^{o}C for 8 weeks, In this study, plate count agar was used and the incubation temperatures used after inoculation of samples in agar plates were 38°C (aerobic bacteria) and 55°C (thermophilic and spore forming bacteria).

6.2 Materials and Methods

6.2.1 Sample preparation

The bacterial stability of a frozen pasteurised egg white liquid (EWL) purchased from Eggcel (Eggcel, New Zealand) as described in Section 3.2.1 for Chapter 3 was tested. This was carried out after thawing to a liquid state. Although the EWL had been produced through pasteurisation by the manufacturer, the EWL solution was heat-treated again in this study at two different temperatures (58°C for 3.5 min and 60°C for 2 min) without and with the addition of ingredients (sugar, citric acid, xanthan gum, guar gum, locust bean gum and gum arabic) at the concentrations of each ingredient as shown in Section 5.2.3.4. After preparation of samples for heat-treated (pasteurised) EWL solutions at two different temperatures and unheated EWL solution both with and without added ingredients, 10 ml aliquots of samples were taken and transferred to sterilized centrifuge tubes (15 ml size). The tubes were stored at 4±1°C for 8 weeks for the determination of microbial growth.

6.2.2 Analysis of microbial growth

All samples were analysed for microbial growth at 0 day (control) and after 1, 2, 4, 6 and 8 weeks during storage. Briefly, samples were first diluted 10 times by transferring 1 ml of samples into 9 ml of peptone water (0.1%) (Merck Chemicals, Microbiology, Germany). Then, serial dilutions were made from the stock solution via successive dilutions up to 10^{-6} (i.e. 6 fold dilution, v/v). These dilutions were used to enable to count the bacterial growth numbers on an agar plate between 30 and 300 CFU (colony forming unit) per ml sample. A 0.1 ml of each dilution was added and inoculated into a petri dish containing a plate count agar (PCA) (Merck Chemicals, Microbiology, Germany) using a surface spread method with a sterilised glass rod (first in 70% ethanol and then in flame).

The inoculation of samples was carried out in duplicate for each dilution. After spreading, the petri dishes were closed with lids and allowed to stand at room temperature (20^{o}C) for 10-20 min to smear the inoculum onto the surface of agar. The petri dishes were inverted and incubated at 38±1°C for 48 hr for the growth of aerobic bacteria (if present). For thermophilies and spore forming bacteria that are resistant to

the pasteurisation temperature of egg white (58^{o}C for 3.5 min), another set of plates inoculated with the heat-treated samples was incubated at 55±1°C for 48 hr which is the optimal temperature for their growth.

6.2.3 Counting colonies

Following incubation, all colonies on petri dishes with between 30 and 300 CFUs were counted. If plates from all dilutions had no colonies, the result was expressed as less than 1x10^{-1} CFU per ml. When the number of CFU per plate exceeded 300 or could not be counted, the count was recorded as too numerous to count (TNTC). If plates contained colonies which had spread, a representative portion of the plates free from spreaders was selected and then the total count of the entire plate was estimated or recorded as Spreader (SPR).

For pasteurized egg white liquid samples, thermophiles and spore forming bacteria were detected and counted. Plates with between 25 and 250 colonies were counted. If the colony count on a plate exceeded 250, the counts were recorded as too numerous to count (TNTC). The actual number in both plates of a dilution was counted as per the formula (1). This formula is designed to count the number of thermophilic bacteria using aerobic count plate.

$$N = \mathrm{C} \times \mathrm{D} \qquad (1)$$

Where N is the colony forming unit (CFU) per ml of sample (CFU/ml), C is the number of colonies on the plate and D is the dilution factor (e.g. 10, 10^2, 10^3, etc)

6.3 Results and Discussion

6.3.1 Non-heat treated EWL solutions

EWL samples without heat treatment either in the presence or absence of ingredients (sugar, citric acid, xanthan gum, locust bean gum, guar gum and gum arabic) were tested for bacterial stability during storage for 8 weeks at 4^{o}C. The results are shown in Table 6.1. EWL samples without added ingredients were free from any bacterial growth during 8 weeks of storage, while the EWL samples added with ingredients had a growth of microorganisms which was however observed only from the samples stored for

longer than 6 weeks. The number of microbial growth in samples after 6 weeks exceeded the acceptable number for an aerobic plate count of unpasteurised EWL which is in the range of 10^3 to 10^6 CFU/ml (Browns & Ito, 2001). The results indicate that the mixture of EWL and ingredients can be refrigerated for up to four weeks before spoilage occurs.

Table 6.1 Aerobic plate counts in non-heat treated EWL samples with and without added ingredients.

	Week	Count (CFU/ml)				
		10^{-1}	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
Without ingredients	0 day	<10	<10	<10	<10	<10
	1	<10	<10	<10	<10	<10
	2	<10	<10	<10	<10	<10
	4	<10	<10	<10	<10	<10
	6	<10	<10	<10	<10	<10
	8	<10	<10	<10	<10	<10
With ingredients	0 day	<10	<10	<10	<10	<10
	1	<10	<10	<10	<10	<10
	2	<10	<10	<10	<10	<10
	4	<10	<10	<10	<10	<10
	6	TNTC	TNTC	$4.0x10^4$	<10	<10
	8	TNTC	TNTC	TNTC	TNTC	$3.3x10^6$

6.3.2 Heat treated EWL solutions

EWL solutions with and without added ingredients were also heat-treated at two different temperatures (58°C for 3.5 min and 60°C for 2 min) and then stored at 4°C for 8 weeks. The results shown in Table 6.2 indicated that there was no growth of microorganisms during 8 weeks. Usually, egg is pasteurised at 58°C for 3.5 min in order to kill the most prevalent food pathogens in egg white including *Salmonella enteritidis* (Tucker & Featherstone, 2011). In general, heat treatment kills all bacteria, except thermophilic bacteria and spore forming bacteria. Therefore, the growth of these microorganisms was determined in this study by incubating egg white samples at 55°C. As indicated above, no growth was seen in any of the samples for 8 weeks, suggesting that heat treatment carried out at two different temperatures was effective in preventing these microorganisms.

Thermophiles are one of the most heat resistant microorganisms (Tucker & Featherstone, 2011). Among many different thermophilic bacteria, the most important type which can infect egg white liquid is *Geobacillus stearothermophilus* which used to be known as *Bacillus stearothermophilus.* It is a gram-positive bacterium with a rod shape. The temperature range that they can grow in is between 30°C and 75°C but optimally at 55°C. Other groups of bacteria concerned are spore-forming bacteria. An example of these bacteria is mesophiles which can survive after heat treatment process (Tucker & Featherstone, 2011). They are aerobic spore-formers and belong to the genera *Bacillus* and *Sporolactobacillus* (Browns & Ito, 2001). The optimal growth temperature of mesophiles is 40°C.

Some prorteins in egg white have antimicrobial functions, thus enabling egg to protect from bacterial growth. They include lsozyme disrupting the bacterial cell wall, ovotransferrin chelating metal ions (e.g. iron) thus causing iron depletion required for cell growth (Kovacs-Nolan et al. 2005) and conalbumin and avidin destroying bacterial cells and inhibiting their proliferation in egg white (Nemeth et al., 2011). In addition, ovomucin and ovomucin-derived peptides have antiviral activity besides their physical roles in the structure and viscosity of egg white (Satdelman & Cotterill, 1995; Kovacs-Nolan et al. 2005; Macherey et al., 2011). In summary, due to the presence of these antimicrobial proteins along with heat treatment, EWL is highly stable against microbial growth during storage for 8 weeks.

Table 6.2 Plate counts for thermophiles during 8 weeks storage at 4°C from EWL samples heat-treated at two different temperatures (58°C for 3.5 min and 60°C for 2 min).

	Storage (week)	Ingredient addition	Count (CFM/ml)		
			10^{-1}	10^{-2}	10^{-3}
58°C for 3.5 min	0 day	No	<10	<10	<10
		Yes	<10	<10	<10
	1	No	<10	<10	<10
		Yes	<10	<10	<10
	2	No	<10	<10	<10
		Yes	<10	<10	<10
	4	No	<10	<10	<10
		Yes	<10	<10	<10
	6	No	<10	<10	<10
		Yes	<10	<10	<10
	8	No	<10	<10	<10
		Yes	<10	<10	<10
60°C for 2 min	0 day	No	<10	<10	<10
		Yes	<10	<10	<10
	1	No	<10	<10	<10
		Yes	<10	<10	<10
	2	No	<10	<10	<10
		Yes	<10	<10	<10
	4	No	<10	<10	<10
		Yes	<10	<10	<10
	6	No	<10	<10	<10
		Yes	<10	<10	<10
	8	No	<10	<10	<10
		Yes	<10	<10	<10

6.4 Conclusions

Heat treatment of EWL at 58°C for 3.5 min or 60°C for 2 min showed the stability of EWL solutions against microbial growth, including thermophilic and spore-forming bacteria, during storage for 8 weeks at 4°C. However, the addition of ingredients without heat treatment resulted in microbial spoilage after four weeks, suggesting the importance of heat treatment to prolong the microbial stability of egg white.

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Chapter 7 Overall conclusions and recommendations

The comprehensive data collected in this research focussed on the characterisation of egg white foams produced by using two different foaming methods, whipping method and gas sparging method. Also, a microwave oven was used for cooking egg white foams. The whipping method using a standard mix beater produced a foam volume of egg white seven or eight times greater than the original volume. In contrast, the foam volume produced was much lower when the gas sparging method, using a whipped cream dispenser with NO_2 gas charger, was used to produce foams.

The effects of various factors on egg white foams were studied using two different types of egg white, such as egg white liquid (EWL) and egg white powder (EWP) by using two different foaming methods described above. The results obtained indicated that in general, factors causing an increase in the viscosity of egg white solution tended to decrease its foaming ability while it enhanced the stability of foam against foam collapse and/or liquid drainage. Throughout the systematic experimental approaches, some factors affecting egg white foams could be analysed and identified. For example, the higher foamability was observed when the temperature of egg white solution was at 20^{o}C than 4^{o}C, when its protein concentration was at 5% and 10% than at 20% and when its pH was very low or high, such as pH 3 or pH 10. In regard to the foam stability, some factors (e.g. 20^{o}C and pH 3) exhibited the same positive effect as for the foamability, while some other variables had the opposite effect on foamability and foam stability. For instance, the higher concentration of egg white protein decreased the foaming ability but increased the foam stability. In the case of sucrose, it caused a decrease in both foamability and foam stability. On the other hand, hydrocolloids produced more stable foams without compromising the foamability of egg white.

The production of egg white foam using a whipped cream dispenser was a new technique, which has not been reported anywhere else. The use of this equipment was simple and easy to operate. Foam can be produced readily at any time. Numerous factors were studied, including shaking times and types of egg white (EWL and EWP). It was found that the appearance of foams was affected by the shaking time, with excessive shaking tending to result in liquid foams whereas the optimal shaking time produced thick and creamy foams. The two different types of egg white raw materials had no remarkable effect on both foamability and foam stability but their foam

appearance differed from each other.

One of the important factors studied in this project was the effect of heat treatment of EWL solution at 58^{o}C for 3.5 min (i.e. egg white pasteurisation temperature) and 60^{o}C for 2 min prior to making foam. The volume of foam (foamability) was not affected by these heat treatments, compared to the control sample at 20^{o}C. On the other hand, the foam stability of heat-treated EWL solutions was higher than the control sample. Also, the addition of ingredients (sugar, citric acid, xanthan gum, guar gum, and gum arabic) before the heat treatment of EWL solution enhanced the foam stability by further reducing the rate of foam collapse and liquid drainage. These heat treatment results have a significant implication that partial protein denaturation and protein aggregation can be applied to further improve the functional properties of egg white proteins as foaming agents. However, this needs to be further investigated to characterise its mechanisms by analysing the extent of protein denaturation and aggregation via chemical analyses (e.g. SDS-PAGE and size of protein aggregates) and instrumental techniques (e.g. interfacial tension and viscoelastic properties of interfacial layers, rheological properties and viscosity of solution, and structural features of foams using microscopic examinations).

The foam properties, including foam volume, foam stability and foam appearance, produced by the whipped cream dispenser were different from the foam properties produced using the standard mix beater. Especially, the appearance of foams produced by the whipped cream dispenser was denser, thicker and creamier. The foam could hold its shape although it had a fast liquid drainage compared to the foam prepared using the standard mix beater. This was a result due to its high foam density due to a relatively small volume of foam formed. The low volume of foams produced with the whipped cream dispenser needs to be further investigated as well as to explore its impact on the foamability and foam stability of egg white. In addition, the standard identity of egg white foam created by using the whipped cream dispenser needs to be defined and established clearly in detail. Based on a standard identity defined, its foaming properties and foam stability will need to be manipulated to further improve its characteristics and expand its applicability for its wider applications in various food systems by formulating egg white with other ingredients (e.g. stabiliser and hydrocolloids) and/or pre-treatments (e.g. thermal or high pressure) of egg white before the foam preparation.

Some other attractive aspects of egg white foams produced by using the whipped cream dispenser are their textural properties and appearance being soft, dense, viscous and

creamy as mentioned above. These rheological properties enabled the foam to maintain its shape and conferred unique textural properties that can be spreadable without flowing. This attribute has the high potential for its use in a variety of food applications, including desserts, appetisers, toppings, sauces, dips, etc. In order to better achieve this, various product prototypes need to be explored and developed systematically and logically through more extensive studies by using a variety of other ingredients (e.g. stabilisers, colourings, flavours and hydrocolloids) at different combinations and concentrations. The properties, stability and consumer acceptability of products also need to be explored.

Another interesting result obtained was that the foams created using the whipped cream dispenser as well as using the whipping method can be further processed by heat treatment or cooking in a microwave oven. The egg white foams after cooking in the microwave oven vary in their properties and appearance, depending on the microwave cooking times used and the types and concentrations of ingredients (hydrocolloids, sugar, citric acid) incorporated into egg white prior to foaming. After microwave cooking, some foams appeared to be fluffy, some had a rigid foamy structural network, some semi-solid-gel-like structure and etc. This is another key area to be further studied through systematic approaches. The amount of moisture being lost due to evaporation which also causes thermal expansion of foam volume during the microwave cooking has a significant impact on the final properties of foams after cooking. The temperature of egg white foams being exposed and reached during cooking in the microwave oven also needs to be measured according to the microwave cooking time associated with the quantity of foams being cooked and their initial water contents. The relationships between all these parameters need to be standardised and established.

For the microbiological safety and stability of egg white during storage until its use in the preparation of foam, egg white liquid (EWL) was tested for its shelf life with and without added ingredients (e.g. hydrocolloids, sugar and acid). The EWL samples were heat-treated at the pasteurisation temperature of egg white (58^{o}C for 3.5 min) or a slightly higher temperature (60^{o}C for 2 min) and then stored at 4^{o}C for 8 weeks. The heat treatment resulted in no growth of bacteria including thermophilic and spore forming bacteria during storage for 8 weeks, suggesting the effectiveness of heat treatment against the microbial contamination and growth, therefore, the egg white liquids are stable at 4^{o}C for at least 2 months.

Overall the research project was carried out successfully and the project goals were achieved. However, there are some areas that may need to be further investigated to allow the research outcomes to be more effectively commercialised.

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Appendices

Appendix 1 Foamability (%) for EWL foams produced at different whipping speeds (3 and 5) using the whipping method.

Whipping speeds	Foamability (%)	P
3	714 ± 5.7[a]	0.274
5	723 ± 7.2[a]	

[a] Means followed by the same letter within a column are not significantly different ($p < 0.05$). Results are expressed as the means ± SD for n=2. Section 3.3.2.1

Appendix 2 Foamability (%) for egg white foams prepared from EWL solutions at two different temperatures (4°C and 20°C) using the whipping method.

Solution temperature (°C)	Foamability (%)	P
4	682 ± 5.9[b]	0.024
20	723 ± 7.2[a]	

[a–b] Means followed by the same letter within a column are not significantly different ($p < 0.05$). Results are expressed as the means ± SD for n=2. Section 3.3.3.1

Appendix 3 Foam volume stability (%) for egg white foams prepared from EWP solutions with different protein concentrations (5, 10, 15 and 20%) using whipping method.

Time after foam formation	Protein conc. (%)				
	5	15	15	20	p
30 minutes	98.1±1.2[b]	98±1.3[b]	99.5±0.7[ab]	99.69±0.5[a]	0.008
1h	95.8±0.8[ab]	93.7±1.6[b]	96.4±1.2[a]	95.6±1.8[ab]	0.023
2h	86.6±6.9[a]	86.7±3.4[a]	91.7±0.8[a]	89.5±2.4[a]	0.114
3h	82.3±5.9[a]	81.7±4.1[a]	84.8±5.2[a]	86.6±1.8[a]	0.249
4h	81.7±6.3[a]	81.3±4.1[a]	83.8±5.2[a]	85.7±2.4[a]	0.363
5h	81.7±6.3[ab]	77.6±0[b]	83.7±5.2[ab]	85.7±2.4[a]	0.021

[a–b] Means followed by the same letter within a row are not significantly different ($p < 0.05$). Results are expressed as the means ± SD for n=2. Section 3.3.5.2

Appendix 4 Foamability (%) of egg white foams prepared from EWL solutions with different pH values using the whipping method.

pH	Foamability (%)	p
3	960 ± 26[a]	
4	806 ± 24[ab]	
5	781 ± 74[ab]	
6	776 ± 60[b]	0.014
7	709 ± 41[b]	
8	771 ± 6.0[b]	
8.82 (original)	735 ± 2.0[b]	
10	844 ± 72[ab]	

[a–b] Means followed by the same letter within a column are not significantly different ($p < 0.05$). Results are expressed as the means ± SD for n=2. Section 3.3.6.1

Appendix 5 Foam volume stability (%) of egg white foams prepared from EWL solutions with different pH values using the whipping method.

Time	pH of EWL solution								
	3	4	5	6	7	8	8.82	10	p
30 minutes	98.3±0.9^{a}	97.6±1.5^{a}	97.9±1.7^{a}	95.8±2.6^{a}	98.1±2.3^{a}	95.1±2.1^{a}	96.2±1.3^{a}	94.7±2.7^{a}	0.010
1h	95.6±0.7^{a}	94.4±0.9^{ab}	94.5±1.6^{ab}	92.5±1.6^{abc}	93.7±2.5^{ab}	90.4±1.2^{c}	91.9±1.2^{bc}	92.3±2.3^{b}	<0.05
2h	93.1±0.4^{a}	91.4±0.3^{ab}	90.4±1.4^{abc}	94.7±2.3^{e}	89.7±2.4^{bc}	85.7±0.9^{de}	83.8±1.5^{e}	87.9±1.8^{c}	<0.05
3h	91.9±0.7^{a}	87.9±2.6^{ab}	88.7±1.3^{ab}	73.2±4.8^{de}	80.3±2.9^{c}	72.8±1.2^{e}	77.4±0.3^{cd}	85.6±2.1^{b}	<0.05
4h	90.7±0.1^{a}	87.0±2.6^{ab}	87.9±1.3^{ab}	68.7±4.9^{d}	76.6±1.6^{c}	71.9±1.1^{d}	76.7±0.5^{c}	84.3±1.4^{b}	<0.05
5h	90.6±0.1^{a}	86.8±2.4^{b}	87.6±1.1^{ab}	66.4±4.2^{e}	76.4±1.9^{c}	71.7±0.9^{d}	76.4±0.8^{c}	83.9±1.6^{b}	<0.05

$^{a–e}$ Means followed by the same letter within a row are not significantly different ($p < 0.05$).
Results are expressed as the means ± SD for n=2. Section 3.3.6.2

Appendix 6 Foam liquid stability (%) for egg white foams prepared from EWL solutions with different pH values using whipping method.

Time	pH of EWL solution								
	3	4	5	6	7	8	8.82	10	p
30 min	86.2±8.5^{ab}	85.3±10.9^{ab}	87.8±11.7^{ab}	83.2±7.3^{ab}	90.6±8.5^{a}	75.1±10.2^{ab}	72.0±9.8^{b}	74.2±11.2^{ab}	0.012
1h	60.3±6.9^{ab}	62.8±7.8^{ab}	64.1±9.8^{a}	67.5±6.4^{a}	66.0±8.2^{a}	48.5±7.9^{bc}	45.8±7.3^{c}	55.4±8.0^{abc}	<0.05
2h	37.2±2.8^{bc}	39.8±5.8^{ab}	35.7±3.1^{abc}	30.5±3.8^{bcd}	39.9±5.0^{a}	23.7±3.8^{d}	25.8±7.9^{d}	29.5±4.5^{cd}	<0.05
3h	28.2±3.1^{ab}	30.3±4.5^{a}	22.4±1.5^{bc}	16.9±2.5^{cd}	24.7±3.6^{ab}	12.9±2.9^{d}	12.2±4.5^{d}	17.0±5.5^{cd}	<0.05
4h	18.8±6.1^{a}	22.4±4.2^{a}	16.8±1.6^{a}	5.7±2.4^{b}	7.5±4.2^{b}	5.5±2.2^{b}	7.3±2.2^{b}	8.1±1.9^{b}	<0.05
5h	17.0±4.1^{ab}	20.9±5.7^{a}	14.0±2.9^{b}	5.7±2.3^{c}	5.4±1.9^{c}	4.2±1.2^{c}	5.5±0.2^{c}	5.2±0.4^{c}	<0.05

$^{a–e}$ Means followed by the same letter within a row are not significantly different ($p < 0.05$).
Results are expressed as the means ± SD for n=2. Section 3.3.6.2

Appendix 7 Zeta *p*otential for EWL solutions at different pH values using Zetasizer Nano ZS90.

pH Values of EWL solution	Zeta potential values
3	26.65
4	19.28
5	2.60
6	-8.55
7	-11.73
8	-12.55
8.82 (original)	-21.45
9	-15.50

Results are expressed as the means ± SD for n=2. Section 3.3.6.2

Appendix 8 Foamability (%) of egg white foam prepared from EWL solutions after mixing with sodium chloride (NaCl) and calcium chloride ($CaCl_2$) at different concentrations by using whipping method.

	Salt concentration (mM)						
	0	**10**	**50**	**100**	**200**	**400**	**p**
NaCl	790±23[a]	681±17[a]	796±53[a]	709±8.7[a]	663±55[a]	731±51[a]	0.064
$CaCl_2$	774±6.4[a]	-	708±67[a]	666±24[a]	-	-	0.162

[a–b] Means followed by the same letter within a row are not significantly different ($p < 0.05$). Results are expressed as the means ± SD for n=2. Section 3.3.7.1

Appendix 9 Foam volume stability (%) for egg white foams prepared from EWL solutions with sodium chloride (NaCl) at different concentrations by using the whipping method.

Time after foam formation	**Sodium chloride concentration**						
	0	**10**	**50**	**100**	**200**	**400**	**p**
30 minutes	95.5±1.2[a]	95.31.3 [a]	95.01.2 [a]	93.9±1.0[ab]	92.2±1.5[b]	92.3±1.8[ab]	0.001
1h	93±0.9 [a]	92.3±0.8abc	92.6±0.8[ab]	91.0±1.4[bc]	89.0±0.6[d]	90.6±1.5[cd]	<0.05
2h	89.3±1.0[a]	88.1±1.1 [ab]	88.2±0.3[ab]	87.9±0.7[ab]	86.8±0.3[b]	88.1±1.5[ab]	0.005
3h	87.3±0. 1[a]	86.2±0.2 [ab]	87.4±0.4 [a]	96.7±0.6 [a]	85.1±0.1[b]	86.7±1.6 [a]	<0.05

[a–b] Means followed by the same letter within a row are not significantly different ($p < 0.05$). Results are expressed as the means ± SD for n=2. Section 3.3.7.2

Appendix 10 Foam volume stability (%) for egg white foam prepared from EWP solutions with different volumes (50, 100, 200 and 400 ml), which were shaken for different times (10, 20, 30, 40 & 50 times) using the sparging method.

Shaking time	Time after shaking	Volume of EWP solution (ml)				
	(min)	50	100	200	400	p
10	5	58.0±12.6^{bc}	53.3±1.9^{c}	71.7±4.7^{ab}	76.7±3.0^{a}	0.010
	10	32.4±9.3^{a}	22.1±15.1^{a}	43.4±8.6^{a}	44.1±15.2^{a}	0.178
	20	18.3±15.4^{a}	16.0±15.1^{a}	9.7±4.0^{a}	8.8±5.7^{a}	0.683
	30	0±0^{b}	0±0^{b}	9.7±4.0^{a}	6.5±4.^{ab}	0.007
20	5	68.9±10.2^{a}	66.8±1.6^{a}	67.5±7.5^{a}	83.5±2.9^{a}	0.041
	10	31.2±5.6^{b}	35.8±9.0^{b}	38.2±11.6^{b}	63.7±10.2^{a}	0.011
	20	31.2±5.6^{a}	9.7±3.3^{ab}	5.8±2.6^{b}	16.9±14.9^{ab}	0.023
	30	0±0^{b}	0±0^{b}	5.3±1.9^{ab}	8.2±3.7^{a}	0.003
30	5	58.3±8.8^{b}	64.7±4.9^{ab}	69.1±4.2^{ab}	78.2±4.4^{a}	0.019
	10	27.4±12.1^{a}	27.4±7.9^{a}	40.6±13.7^{a}	45.7±3.4^{a}	0.145
	20	26.2±10.1^{a}	8.4±1.6^{b}	8.5±1.5^{b}	8.7±3.3^{b}	0.009
	30	0±0^{b}	0±0^{b}	8.5±1.5^{a}	8.7±3.3^{a}	<0.05
40	5	52.8±6.3^{b}	61.9±8.3^{ab}	74.8±2.7^{a}	74.6±1.9^{a}	0.003
	10	34.8±3.8^{a}	27.3±8.1^{a}	45.4±5.1^{a}	44.2±11.8^{a}	0.066
	20	34.8±3.8^{a}	8.4±1.7^{b}	3.9±1.9^{b}	8.2±3.1^{b}	<0.05
	30	0±0^{b}	0±0^{b}	3.9±1.9^{ab}	8.2±3.1^{a}	0.002
50	5	63.9±5.5^{a}	65.1±3.1^{a}	70.1±1.6^{a}	82.4±13.1^{a}	0.053
	10	33.3±6.4^{a}	31.2±3.7^{a}	49.7±3.3^{a}	49.2±14.1^{a}	0.039
	20	33.3±6.4^{a}	8.4±1.9^{b}	5.4±0.8^{b}	9.9±3.7^{b}	<0.05
	30	0±0^{b}	0±0^{b}	5.4±0.8^{a}	5.7±1.6^{a}	<0.05

$^{a–c}$ Means followed by the same letter within a column are not significantly different ($p < 0.05$). Results are expressed as the means ± SD for n=3. Section 4.3.2.2

Appendix 11 Foam liquid stability (%) for egg white foam prepared from EWP solutions with different volumes (50, 100, 200 and 400ml), which were shaken for different times (10, 20, 30, 40 and 50 times) using the sparging method.

Shaking time	Time after shaking	Volume of EWP solution (ml)				
	(min)	50	100	200	400	p
10	5	23.3±5.8^{b}	29.3±1.2^{b}	71.7±4.7^{ab}	37.7±12.7^{a}	0.011
	10	23.3±5.8^{a}	0.7±0.6^{b}	14.0±9.6^{ab}	26.7±2.9^{a}	0.002
	20	0.7±1.2^{ab}	0±0^{b}	0±0^{b}	4.2±2.9^{a}	0.031
	30	0.7±1.2^{ab}	0±0^{b}	0±0^{b}	4.2±2.9^{a}	0.031
20	5	33.3±5.8^{bc}	26.7±5.8^{c}	45.0±5.0^{b}	61.2±5.3^{a}	<0.05
	10	0±0^{b}	10.7±0.6^{b}	28.3±5.8^{a}	30.9±8.3^{a}	<0.05
	20	0±0^{a}	1.7±2.9^{a}	10.8±6.3^{a}	10.7±5.6^{a}	0.030
	30	0±0^{b}	1.7±2.9^{a}	9.2±7.2^{a}	5.8±6.3^{a}	0.185
30	5	36.7±5.8^{bc}	23.3±5.8^{c}	53.3±5.8^{a}	46.3±6.9^{ab}	0.002
	10	7.3±2.3^{b}	8.3±7.6^{b}	37.5±10.9^{a}	17.9±9.5^{ab}	0.007
	20	4.0±3.5^{b}	0±0.6^{b}	28.3±5.8^{a}	9.2±7.2^{b}	0.001
	30	4.0±3.5^{b}	0±0.6^{b}	28.3±5.8^{a}	9.2±7.2^{b}	0.001
40	5	20.0±10.0^{b}	23.3±5.8^{b}	61.7±3.8^{a}	74.6±1.9^{a}	<0.05
	10	0.7±1.2^{b}	8.3±7.7^{b}	40.8±6.3^{a}	17.5±9.0^{b}	<0.05
	20	0.7±1.2^{b}	0.3±0.6^{b}	28.3±2.9^{a}	3.3±1.4^{b}	<0.05
	30	0.7±1.2^{b}	0.3±0.6^{b}	28.3±2.9^{a}	3.3±1.4^{b}	<0.05
50	5	30.7±9.0^{b}	38.3±2.9^{ab}	51.8±2.8^{a}	40.4±6.2^{ab}	0.014
	10	3.33±5.8^{c}	15.0±5^{bc}	34.2±5.2^{a}	20.0±4.5^{b}	0.001
	20	3.33±5.8^{b}	3.7±5.5^{b}	18.3±5.8^{a}	7.9±4.7^{ab}	0.032
	30	3.33±5.8^{b}	3.7±5.5^{b}	18.3±5.8^{a}	5.8±3.8a^{b}	0.025

$^{a–c}$ Means followed by the same letter within a column are not significantly different ($p < 0.05$). Results are expressed as the means ± SD for n=3. Section 4.3.2.2

Appendix 12 Foamability (%) for egg white foam prepared from different types of egg white solutions using the sparging method and cooked in a microwave oven for different times (10, 20, 30 and 40 sec); solutions were EWL (10% w/v protein) and EWP solutions (10% & 20% w/w protein).

Cooking Times	Type of solution			
(sec)	EWL (10%)	EWP solution (10%protein)	EWP solution (20% protein)	p
10	524.6 ±22.2 [a]	505.7±20.5 [a]	430.3±43.7 [a]	0.104
20	825.5 ±20.5[a]	711.4±0.9 [a]	717.3±70.6[a]	0.123
30	1062.5±88.4 [a]	1046.0±80.3 [a]	936±17.2 [a]	0.286
40	1051±99 [a]	647±9.5 [b]	994.1±22.1[a]	0.012

[a] Means followed by the same letter within a row are not significantly different ($p < 0.05$). Results are expressed as the means ± SD for n=2. Section 5.3.2.1

Appendix 13 Foam volume stability (%) for egg white foam prepared from different types of egg white solutions using the sparging method and cooked in a microwave oven for different times (10, 20, 30, & 40 sec); solutions are EWL (10% w/v protein) and EWP solutions (10% & 20% w/w protein).

Cooking time	Time after cooking	Type of solution			
Sec	Min	EWL (10%)	EWP (10% protein)	EWP (20% protein)	p
10	**30 sec**	94.00±2.8[a]	82.7±5.3[a]	96.4±0.3[a]	0.615
	1 min	86.0±2.8[a]	85.4±5.5[a]	96.4±0.3[a]	0.091
	2 min	11.0±7.1[a]	70.8±5.9 [a]	88.3±2.9 [a]	0.110
	3 min	61±4.2[ab]	52.6±8.9 [b]	81.9±1.2 [a]	0.031
	4 min	46.0±0[b]	24.5±4.3[c]	71.9±1.7 [a]	0.002
	5 min	44.0±2.8 [b]	25.4±7.1 [b]	65.6±2.5 [a]	0.007
20	**30 sec**	76.7±0 [a]	69.0±0 [a]	68.3±11.8 [a]	0.487
	1 min	70.0±1.6 [a]	57.1±1.7 [a]	64.6±10.8 [a]	0.273
	2 min	51.7±3.9 [a]	43.5±5.9 [a]	51.8±5.3 [a]	0.315
	3 min	35.4±0[ab]	29.2±0.8 [b]	45.4±4.9 [a]	0.024
	4 min	25.6±1.6 [b]	23.8±0 [b]	40.7±5.3 [a]	0.023
	5 min	25.6±1.6[b]	23.2±0.8 [b]	40.7±5.3 [a]	0.022
30	**30 sec**	64.0±1.4 [b]	66.1±0.4 [b]	75.0±0 [a]	0.002
	1 min	54.5±2.1 [b]	56.7±1.2 [b]	66.7±0 [a]	0.006
	2 min	49.5±2.1 [a]	47.5±0 [a]	53.9±6.0 [a]	0.338
	3 min	44.0±4.2 [a]	32.2±0 [b]	41.3±0[ab]	0.034
	4 min	38.0±5.7 [a]	25.0±0 [a]	32.8±0 [a]	0.062
	5 min	32±0 [b]	25±0 [c]	32.8±0 [a]	*
40	**30 sec**	61.5±4.9 [a]	62.5±7.1 [a]	66.7±0 [a]	0.602
	1 min	65.0±4.2 [a]	56.0±8.9 [a]	50±0 [a]	0.545
	2 min	45.0±4.2 [a]	44.5±3.5 [a]	50±0 [a]	0.304
	3 min	37±1.4 [b]	38.3±1.8 [b]	50±0 [a]	0.004
	4 min	31.5±2.1 [c]	37±0 [b]	50±0 [a]	0.001
	5 min	31.5±2.1 [c]	37±0 [b]	50±0 [a]	0.001

[a–c] Means followed by the same letter within a row are not significantly different ($p < 0.05$). Results are expressed as the means ± SD for n=2. Section 5.3.2.2

Appendix 14 Foam liquid stability (%) for egg white foam prepared from different types of egg white solutions using the sparging method and cooked in a microwave oven for different times (10, 20, 30, & 40 sec); solutions are EWL (10% w/v protein) and EWP solutions (10% & 20% w/w protein).

Cooking times	Time after cooking	Type of solution			
Seconds	Min	EWL	EWP (10% protein)	EWP (20% protein)	p
10	30 sec	79.0±0.9 [c]	81.6±0.3 [b]	100±0 [a]	<0.05
	1 min	58.0±1.8 [c]	63.2±0.5 [b]	92±0 [a]	<0.05
	2 min	31.9±4.5 [b]	44.8±0.8 [b]	80.4±6.0 [a]	0.003
	3 min	21.5±4.1 [c]	35.7±0.9 [b]	68.7±0.8 [a]	0.001
	4 min	5.6±4.0 [c]	19.3±1.2 [b]	53.1±1.1 [a]	0.001
	5 min	5.6±4.0 [c]	17.3±1.2 [b]	37.5±1.5 [a]	0.003
20	30sec	90.82±0.2 [ab]	83.1±0.0 [b]	96.5±4.9 [a]	0.041
	1 min	81.6±0.5 [b]	74.6±0.0 [c]	92.5±0.7 [a]	<0.05
	2 min	49.6±5.2 [a]	52.7±19 [a]	85±0 [a]	0.091
	3 min	26.6±1.8 [b]	53.4±6.1 [ab]	77.5±10.6 [a]	0.013
	4 min	8.2±2.3 [c]	49.2±0.1 [b]	73.5±4.9 [a]	0.001
	5 min	8.2±2.3 [c]	44.9±5.9 [b]	73.5±4.9 [a]	0.002
30	30sec	97±0 [a]	93.8±4.2 [a]	100±0 [a]	0.171
	1 min	91.5±0.7 [b]	91.3±0.7 [b]	99±1.4 [a]	0.007
	2 min	90±1.4 [b]	91.3±0.7 [b]	98±1.4 [a]	0.014
	3 min	86±1.4 [b]	87.8±0.9 [b]	97±0 [a]	0.003
	4 min	93 ±1.4 [b]	82.6 ±1.3 [b]	95±0 [a]	0.003
	5 min	81±1.4 [b]	82.6 ±1.3 [b]	95±0 [a]	0.002
40	30 sec	100 ±0	100 ±0	100 ±0	*
	1 min	99 ±0 [b]	98.3±0 [c]	100±0 [a]	<0.05
	2 min	97±0 [b]	97.8±0.04 [c]	100±0 [a]	<0.05
	3 min	97±0 [b]	96.8±0.04 [c]	100±0 [a]	<0.05
	4 min	97±0 [b]	96.8±0.04 [c]	100±0 [a]	<0.05
	5 min	97±0 [b]	96.8±0.04 [c]	100±0 [a]	<0.05

[a–c] Means followed by the same letter within a row are not significantly different ($p < 0.05$). Results are expressed as the means ± SD for n=2. Section 5.3.2.2