Characterization and Functional Potential of Tofu Whey Wastewater as a Food Ingredient

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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2025

Abstract

Tofu whey, a liquid by-product of tofu production, is often discarded as wastewater, leading to environmental and economic concerns. However, its nutritional composition and bioactive properties suggest potential applications as a functional food ingredient. This study aims to characterize the physical and chemical properties of tofu whey liquid (TWL) and tofu whey powder (TWP), evaluate their functional properties, including foaming and emulsifying abilities, and investigate their bioactive properties. Additionally, their potential as an egg white substitute in meringue formulations was assessed. TWL is the liquid waste by-product generated during tofu-making and TWP is the powder obtained by removing most of the moisture from TWL.

Chemical composition analysis revealed that TWL contained 97.34 ± 0.23% moisture, 0.38 ± 0.05% crude protein, 0.10 ± 0.06% fat, 0.43 ± 0.05% ash and 1.76 ± 0.23% carbohydrates, while TWP had 5.23 ± 1.04% moisture, 15.08 ± 0.35% crude protein, 1.54 ± 0.29% fat, 15.09 ± 1.74% ash and 62.88 ± 2.27% carbohydrates. The primary sugar identified was sucrose, and the proteins were mainly degraded subunits of glycinin and conglycinin. Mineral analysis showed significant levels of copper, chloride, iron, magnesium and potassium.

Functionality testing demonstrated that TWL exhibited limited foaming properties, while TWP showed improved foam stability at a 10% concentration, maintaining over 50% stability after 30 minutes. When adjusted to pH 4 from pH 5.8, foam stability further improved, exceeding 60%. Emulsification studies indicated that TWL had poor stability at higher oil concentrations (20%) but performed better at lower oil concentrations (5%). In contrast, TWP exhibited enhanced emulsifying properties across various oil concentrations, particularly at pH 2 and pH 7–9, likely due to improved zeta potential. The addition of 500 mM NaCl further increased emulsion stability by promoting Na^+-water interactions, enhancing electrostatic repulsion.

Th bioactive properties of TWL and TWP were also analysed, including their antioxidant, antimicrobial, and anti-browning activities. The results indicated that both TWL and TWP contained decent levels of total phenolics, flavonoids, and saponins. These bioactive

compounds suggest potential applications in extending food shelf life and enhancing overall product stability.

As an egg white substitute in meringue, TWL and TWP produced mixed results. The best performance showing similar texture and rheological to egg white, was observed when TWL or TWP was incorporated with egg white, with minimal impact on meringue texture. Batter made with TWP alone showed no significant differences in density and ϕ (air incorporation) compared to egg whites, whereas TWL resulted in higher density and lower ϕ, correlating with increased batter viscosity.

Overall, these findings highlight the potential of TWP as a sustainable functional ingredient with foaming, emulsifying, antioxidant, antimicrobial, and anti-browning properties. Future studies should explore further applications in food formulations and optimize processing conditions to enhance its bioactive and functional properties in various food systems.

Acknowledgements

During my studies at Massey University, School of Food and Advanced Technology, I would like to express my deepest appreciation to Dr Sung Je Lee for his supervision and guidance throughout my research project. Despite some setbacks during the project period, he remained calm and patient, helping me through challenging times. Without his dedication and support, this thesis would not have been possible.

I would also like to thank the School of Advanced Food Technology Laboratory managers, Mr Arthur Xu and Ms Rachel Lui, for their invaluable assistance in providing training on laboratory equipment and facilitating the ordering of chemicals.

Additionally, I am grateful to my friends and colleagues, Lan Luo, Charles Liu, Vina Zhang and Sinong Wu, for their support and for sharing their expertise, which greatly helped me develop essential laboratory techniques.

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Chapter 1. Introduction

1.1 Background

Tofu is a soybean-derived product made by coagulating soy milk into a gel. It is widely consumed worldwide, particularly in East Asia, where it serves as a key protein source in the daily diets of over a billion people. With globalization and tofu's versability in various cuisines, including its potential as a meat analogue, the tofu industry has experienced significant growth. In 2018, the global tofu market was valued at 2.31 billion USD, with an estimated compound annual growth rate (CAGR) of 5.2% from 2019 to 2025 (Anjum et al., 2023; Golbitz & Jordan, 2006). However, the increasing demand for tofu has also led to a rise in tofu production, generating substantial waste by-products, including tofu whey.

Tofu whey is a liquid by-product that is considered a waste in industry. It is obtained during tofu production when it is pressed out of coagulated soy curds to shape the tofu. Tofu whey powder (TWP) is a concentrated version of tofu whey liquid (TWL) after it has been dried in this study freeze drying was used because it was the most time effective, equipment for freeze drying available was there and lack of evidence base research on the effect on freeze drying on tofu whey's structure and functionality.

TWL has high biological oxygen demand (BOD) of 4.1-6.8 g/L and chemical oxygen demand (COD) of 7.5-12 g/L (Hongyang et al., 2011; Wang & Serventi, 2019), making its treatment costly. An alternative to wastewater treatment is upcycling, i.e. reusing the nutrients in tofu whey without requiring full purification into potable water. The upcycling of food waste has been increasingly explored in the food industry, with promising results from similar legume-derived wastewater. For instance, Liluva (legume wastewater) has been successfully upcycled due to its strong emulsification and foaming properties, providing a cost-effective alternative to wastewater treatment (Mustafa et al., 2018; Serventi & McNeill, 2024).

The functionality of tofu whey, whether in liquid or powdered form, is important in determining its potential for valorization as a food ingredient. Its functional properties, particularly its ability to act as an emulsifier and foam agent, are highly valued in the food industry due to their role in enhancing the texture and appearance of food products (Norn,

2015). In this study, meringues were chosen as a model food application to evaluate the foaming ability of tofu whey. Meringues are voluminous foams formed by incorporating air through whisking, with sugar and proteins stabilizing the air bubbles within the structure (Vega & Sanghvi, 2012). Additionally, the antioxidant potential of tofu whey was explored to promote its use as a functional food ingredient, aligning with current global trend toward the consumption of superfoods and health-oriented products (Franco Lucas et al., 2022). This dual role as both a functional and health-promoting ingredient could increase tofu whey's value in the food industry.

Despite its potential, there is a lack of evidence-based practical use of tofu whey's functional properties and its possible applications in the food industry. This project aims to explore the functionality of tofu whey as a functional food ingredient. It is hypothesized that tofu whey will demonstrate strong emulsifying and foaming properties, given that TWL is naturally foamy upon collection. The objectives of this study are:

- To determine the chemical and physical properties of tofu whey, including its nutritional composition, bioactive components (e.g. total phenolic, flavonoid, and saponin content), and functional health benefits (e.g., antioxidant, antimicrobial, and anti-browning properties).
- To evaluate its functional properties, such as foaming and emulsification capacity, under different conditions.
- To investigate its potential incorporation into food products, specifically in meringues, by assessing whether tofu whey can partially or fully replace egg whites without negatively affecting the physical and textural properties of the final product.

1.2 Overview of Thesis

This thesis is structured as follows:

- Chapter 1 introduces tofu and tofu whey, highlighting the importance of upcycling tofu whey. It outlines the research objectives and goals.
- Chapter 2 presents a literature review, including various types of legume wastewater (e.g. liluva) and their applications. It also discusses the tofu production process, tofu whey composition, and the known nutritional and antinutritional components. The

chapter further examines previous studies on the functional properties of tofu whey, including the effects of different treatments.

- Chapter 3 is the first experimental chapter, analyzing the composition and characteristics of tofu whey liquid (TWL). The aim is to determine whether unprocessed tofu whey can be used as a functional food ingredient.
- Chapter 4 is the second experimental chapter, focusing on tofu whey powder (TWP), produced through freeze-drying. The chapter evaluates whether freeze-drying improves the functionality of tofu whey for potential food applications.
- Chapter 5 is the third experimental chapter, investigating the incorporation of tofu whey into meringues. The study assesses whether tofu whey (liquid or powder) can partially replace egg whites while maintaining desirable textural and structural properties.
- Chapter 6 summarizes the main findings of the study, discusses the overall outcomes and implications, and provides recommendations for future research.

Chapter 2. Literature review

2.1 Introduction

Soybeans have been known to be essential legumes in Asian countries as they have been used for centuries as a protein source (Zhang et al., 2018). As technology evolves and the world becomes globalized (Boerema et al., 2016), the consumption of soybeans and soy products increases globally due to the nutritional value and health benefits provided (Xiao et al., 2018). Bioactive compounds in soybeans and soy products, such as isoflavones, saponins, phytosterols, and peptides (Chua et al., 2018; Isanga & Zhang, 2008), have been shown to provide a certain degree of health benefits against cancer, hypertension, osteoporosis (Benedetti et al., 2015; Diana et al., 2014; Singh et al., 2017). However, soybean and soy products also contain antinutrient compounds such as lectin and Kunitz trypsin inhibitors, which could lead to a leaky gut (Popova & Mihaylova, 2019).

Soy products are divided into two categories: fermented soy products (for example, miso, soy sauce and soybean paste)(Jang et al., 2021) and unfermented soy products (soymilk, soy nuts and tofu). The most popular soybean product is tofu, processed globally and consumed in high quantities in East and Southeast Asian countries. It has also become more accepted by a western consumer base due to its health benefits and globalization (Ali et al., 2021). In Tofu production, the two generated waste streams are okara (the pulp of the soybean) and tofu whey (the liquid waste squeezed out of tofu) (Ali et al., 2021). There have been many studies on using okara as a food and nutritional ingredient (Vong & Liu, 2016). While there is little or limited research about tofu whey, it seems that the utilization of tofu whey is not limited to food compared to okara (Chua & Liu, 2019).

Additionally, tofu whey as waste has ample nutrient content that must be treated, as the biological and chemical oxygen demand of wastewater from the soy industry can reach up to 6.8 and 12 g/L and seemingly cost 130 USD to treat per cubic meter (Wang & Serventi, 2019) which must be done before being discharged into sewage. Therefore, utilization of tofu whey is vital, and the upcycling of tofu whey is the start of an exemplary circular economy that reduces environmental pollution and food waste whilst also providing the soy industry with a way to reduce monetary loss from their waste.

Tofu production is reviewed to understand essential processes and production elements that could affect the composition of tofu whey. The main review examines the current understanding of the chemical composition, physicochemical properties, stability, functional properties and current application of tofu whey.

The scope of this review looks mostly at tofu whey, and only references to soy whey are appropriate. This is because tofu whey is referred to as the whey produced from tofu production, and soy whey is produced from the production of soy protein isolate, which has different processing conditions that can affect the tofu whey composition.

2.2 Soy/Tofu whey

Soy whey and tofu whey are similar as wastewater produced from soymilk after coagulant is added and curdling of the soymilk has occurred. The two main producers of this wastewater are the tofu production industry, which refers to it as tofu whey, and the soy protein isolate production industry, which refers to soy whey. The main difference between soy and tofu whey, stems from the soy protein isolate's defatting process, which makes soy whey have 0% lipids in its content while tofu whey in comparison has a low level of lipid content in it (Chua & Liu, 2019). Furthermore, recent research has shown applications for wastewater like aquafaba to be used as a functional food ingredient. Tofu whey, a wastewater which is further down the production line, might have similar or different applications that can be upcycled into food systems or other purposes (Chua & Liu, 2019; Wang et al., 2018).

2.3 Tofu production process

Tofu or bean curd production has been happening for many years and has a long history of being produced in Asia, mainly China, as it can be dated back to the 965 CE, the first record of tofu was first seen (Shurtleff & Aoyagi, 2013). Tofu manufacturing is a multi-step process with many different outcomes depending on what type of tofu will be made. However, the multi-step process is generally the same, with minor adjustments during production to give a different kind of tofu (Shurtleff & Aoyagi, 2013; Zhang et al., 2018). The general outline of tofu production (Figure 2.1) starts with the selection of soybeans as different soybeans can give different chemical and biological components which can affect the flavours, textures, and physicochemical properties of tofu. The next step is to soak the soybeans, allowing

moisture uptake into the soybeans. This is followed by grinding the soybeans and filtering the slurry into soymilk and okara. Okara, which is a soy pulp or tofu dregs, consists mostly of insoluble compounds in soybeans that remain after the filtration of the soybean slurry. The soymilk is then cooked and coagulants are then added to curdle the soymilk into curds which are then broken and filtered into tofu molds which allow the tofu to be pressed and further molded into the correct shape into which they can be cut and packaged (Ali et al., 2021; Zhang et al., 2018; Zheng et al., 2020). Although tofu manufacturing has years of history, there is still currently research going on the tofu manufacturing process as it is such a widely eaten delicacy.

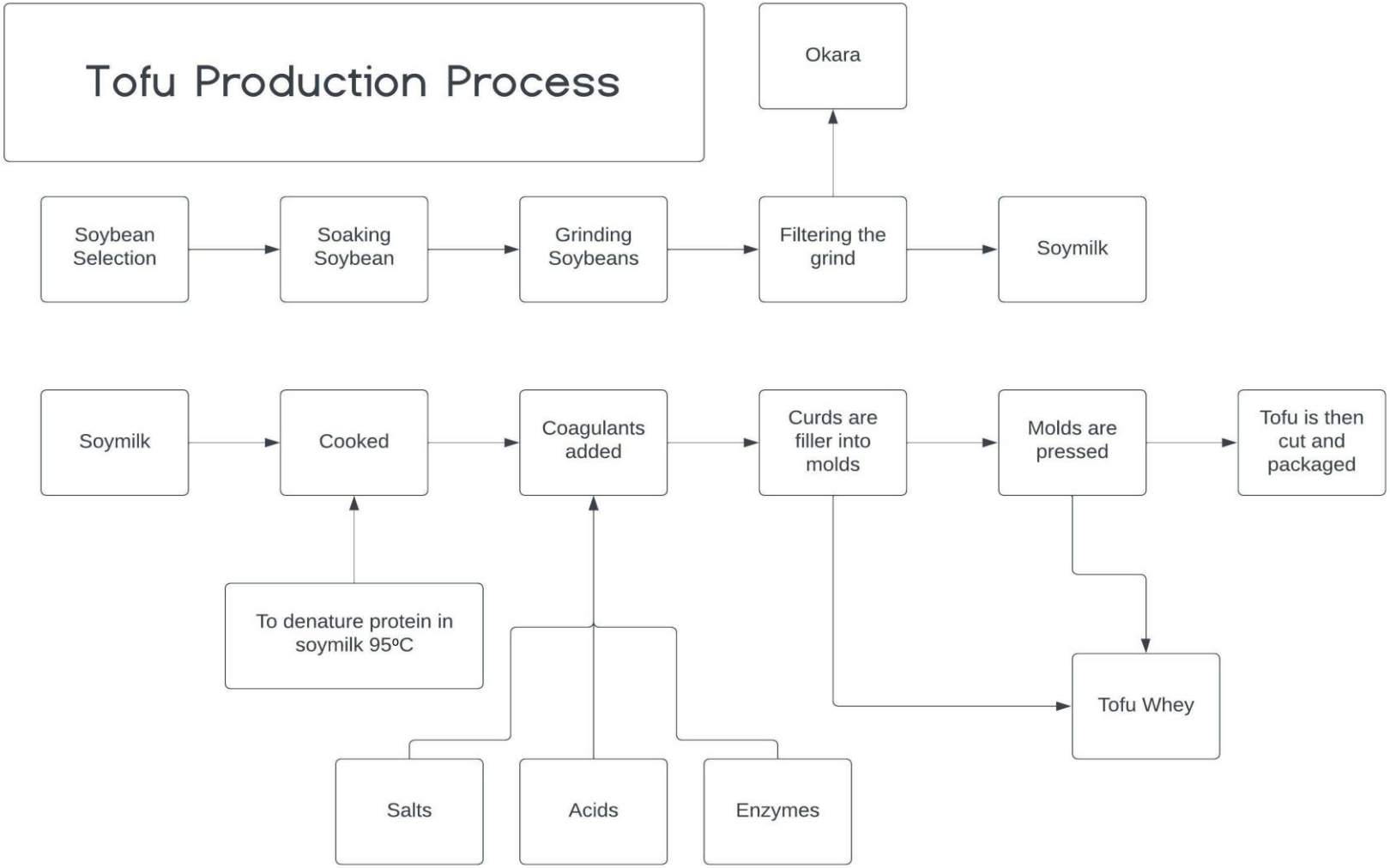


Figure 2.1: A flow chart displaying the multi-step process in tofu production (Zheng et al., 2020).

2.4 The production of soymilk

2.4.1 Soybean selection

The first step of making tofu, as shown in Figure 2.1, is to make soymilk. Many attributes of the soybean can affect the production of tofu. Such selection of soybeans is essential as different variants and cultivars can have different chemical compositions in the soybean content. One of the main components that influence tofu production is the amount of protein in the soybean, as higher protein content has been shown to give higher yield and quality of tofu. There are two main types of storage protein in soybean, such as glycinin (11S) and β-conglycinin (7S) which relate to the texture of tofu as 11S is known to affect the hardness of the tofu and 7S effects the springiness of the tofu (James & Yang, 2016; Zheng et al., 2020). These proteins will effect on how coagulants will react to make the final tofu gel based on the amount of protein percentage found in the soybean content (Zhang et al., 2018). While it is clear that proteins and starch, such as stachyose, have a positive effect on tofu production, other components like lipids and sugars, have been shown to affect the yield of tofu and negatively affect the quality of tofu (Zhang et al., 2018). However, further research is required on the different components of soybeans to find more concrete evidence to give more insight.

2.4.2 Soaking the soybean

The next crucial step in tofu manufacturing is soaking the soybeans, which is vital as soaking changes the soybean's chemical and physical properties, influencing their structural characteristics and how they will be processed further down the production line. In a study on how soaking conditions can affect the characteristics of soybean was conducted (Pan & Tangratanavalee, 2003), the researchers reported that reducing the size of soybean could be more suitable for the grinding process and that increasing temperature would reduce the time required for soaking to get up to the moisture content needed but would increase the loss of solid content in the soybeans. This suggests that soaking conditions such as soybean-to-water ratio, pH, temperature, and time are significant for tofu processing as these factors can modify the properties, allowing the soybean to be processed quicker and easier. Furthermore, decreasing the solid content could alter the yield and quality of the resulting

tofu due to the loss of protein content (Guan et al., 2021; Zhang et al., 2018). However, it is also a critical step as it has been shown that soaking soybeans decreases soymilk extraction time while also increasing the amount of protein in the soymilk (James & Yang, 2016; Pan & Tangratanavalee, 2003), which would also affect the final yield and quality of the tofu.

2.4.3 Grinding and filtering the soymilk

The final step is grinding the soybean into a soymilk slurry and filtering it into okara and soymilk. The process can be done in three ways: uncooked, cooked, and mixed hot water (Zhang et al., 2018). In the uncooked slurry method, the soymilk from the slurry is just filtered, while the cooked slurry method involves the heat-treatment of the soymilk slurry for a certain amount of time before the soymilk was filtered out. In case of the hot water mixed slurry, hot water is added into the slurry and then filtering the soymilk at a temperature above 65^{o}C (Guan et al., 2021; Zhang et al., 2018). The tofu production in all three methods gives different yields. The final quality of tofu produced for uncooked slurry produces harder and more adhesive tofu. In comparison, cooked slurry tofu produces a softer and higher yield due to the cooked method. Cooking the slurry at high temperatures (65 ^{o}C) allows some proteins and polysaccharides to leak from the okara into the soymilk. Polysaccharides increase the water-holding capacity in the tofu, allowing soft tofu from the cooked slurry method (Zhang et al., 2018). The soymilk is filtered from the okara, as okara can impact the gel network in tofu during coagulation due to the fibers' size as it is generally removed. However, in recent research, the addition of okara into tofu for a more nutritional food has been studied as reducing okara particle size has been shown to minimize the okara from disrupting the gelling ability during coagulation (Lan et al., 2021; Ullah et al., 2023).

2.5 Tofu production from soymilk

2.5.1 Protein denaturation in soymilk

Soymilk is turned into tofu via a two-step process of heat treatment and coagulation of protein using coagulants. The first step of this process is very important as the filtered raw soymilk is relatively stable where the hydrophobic group is inside the protein, and the hydrophilic group is on the surface, which allows them to interact with the water and oil in soymilk making them stable again separation. Heat is used to disrupt this stability by

providing energy into the system, promoting hydrophobicity and sulfhydryl content, causing the breaking of bonds and changing of the protein molecular structure, which leads to promoting aggregation of protein as there are more hydrophobic groups exposed to water and due to thermodynamic law the proteins would want to aggregate to reduce exposure of hydrophobic group to water (Guan et al., 2021; Zhang et al., 2018). This process also provides other benefits (Zheng et al., 2020) as "destroying trypsin inhibitors, saponins, and other harmful substances." However, some studies reported by several researchers (Liu et al., 2004; Wang et al., 2007) show that using a two-step heating method has more impact on tofu production than the traditional one-step heating method does, as the two-step heating method looks at targeting down and denaturing two main proteins 11S and 7S, which has been shown to have different denaturation temperatures of 11S at 85-95^{o}C and 7S at 65-75^{o}C for 5 minutes each, while one-step heating method use 95^{o}C to try inhibit both proteins at 10 minutes. The studies also show that using the two-step heating method will increase tofu yield, reduce syneresis and increase the product's final quality.

On the other hand, recent works show the application of new nonthermal methods to denature the proteins, such as ultrasound and ultra-high-pressure homogenization as ultrasound and ultra-high-pressure homogenization has cavitation effect which can change protein structure as they can stretch protein structure and expose hydrophobic group(Ali et al., 2021; Guan et al., 2021). The research (Fan et al., 2021; Huang & Kuo, 2015) on the production of tofu using ultrasound and ultra-high-pressure showed that the methods also cause the exposure of hydrophobic groups and sulfhydryl content, thus promoting the use of new technology in commercial tofu manufacturing. However, more research is still required as both these works show that the final tofu product has decreased in textural properties, and ultra-high-pressure homogenization showed structural differences from heat-treated structure, promoting further investigation before this method might be used commercially.

2.5.2 Coagulation of soy protein

Coagulation of the tofu comes after the soymilk has been denatured and can be done in general via three different mechanisms of salt, acid, or enzymatic (Table 2.1). Salt-induced gelation is done using cations such as calcium (Ca^{2+}) and magnesium (Mg^{+}) to aggregate the

protein due to charge-charge interactions pulling them closer and making gel networks. Acid gelation is done by decreasing the pH of soy proteins to the isoelectric point (pH is generally 4.5 but can be between 4 to 5) at which there is a net zero charge of soy proteins in solution, thus decreasing their repulsive charge forces, and increasing their attraction force causing their aggregation and precipitation, which makes a protein gel network (Kohyama et al., 1995). Lastly, enzymatic coagulation is done using enzymes (microbial transglutaminase) by catalyzing cross-linking reactions between the proteins to form intermolecular and intra-molecular bonds to form a gel network (Tang et al., 2007). Among the different types of coagulant, the most common coagulant is still salt, which gives the standard tofu flavor and aroma known worldwide. While acids might give a smoother and softer texture tofu, there may be sour off-flavors and aromas due to the low pH compared to salts and enzymatic coagulation.

However, on the other hand, enzymatic coagulation has the potential to do well as it gives a better aroma than acid-induced tofu and when compared to salt, has a softer texture and modified taste due to the degrading of the soybean proteins (Zhang et al., 2018; Zheng et al., 2020), but will require more research on the sensory property to find out acceptability before its possibility of commercialization.

2.5.3 Standard industrial practices and composition changes

In tofu production, standard industrial practices are illustrated in Figure 2.1. While the overall process is consistent, production methods can vary between companies, particularly in three main aspects: the soybean-to-water ratio, coagulation methods, and curd handling. These three factors significantly influence the final characteristics of tofu, and manufacturers typically adjust them based on the desired tofu type. The soybean-to-water ratio is a key determinant of the total solid in the final product. A higher soybean ratio results in higher total solids, while a low ratio yields tofu with lower total solids.

Coagulation methods also vary and typically involve either salts or acids. Salt coagulants generally produce tofu with higher protein yield due to ionic cross-linking of proteins, while acid coagulants tend to form weaker gel structures via protein aggregation (Ibrahim et al., 2022). Among salt coagulants, the type of salt used can further affect the gel strength. For instance, calcium sulfate (commonly known as gypsum) exhibits better solubility and ion

release compared to magnesium chloride (nigari), which enhances ion-protein interactions and results in a stronger gel network (Prabhakaran et al., 2006). Curd handling, however, is the most important step as it determines the final moisture content and composition of tofu. This step has a more significant impact than the soybean-to-water ratio or coagulant type. For example, soft tofu generally contains 80-90% moisture due to minimal pressing, whereas firm tofu contains approximately 50-60% moisture due to extended pressing durations (Liu, 2012).

Table 2.1: Type of coagulants used to produce tofu.

Type of Coagulant	Concentration	Mechanism	Reference
Calcium Chloride (Salt)	0.4 or 0.5% based on the amount of soymilk. 10,15,20,30 or 40 mmol/L of calcium in the soymilk	The use of Calcium Cation is used to bridge the protein together to form a Gel	(Prabhakaran et al., 2006). (K. Chen et al., 2021)
Calcium Sulfate (Salt)	0.4 or 0.5% based on the amount of soymilk. 10,15,20,30 or 40 mmol/L of calcium in the soymilk	The use of Calcium Cation is used to bridge the protein together to form a Gel	(Prabhakaran et al., 2006). (K. Chen et al., 2021)
Trimagnesium Citrate (TMC)	0.35% based on the dry bean weight	Magnesium Cation is used to bridge the protein together to form a Gel. Additionally, the due molecule contains citrates, which should drop the pH after magnesium has been used to bridge proteins to form a good gel network	(Joo & Cavender, 2020)
Magnesium Sulfate (Salt)	0.4 or 0.5% based on the amount of soymilk	The use of Magnesium Cation is used to bridge the protein together to form a Gel	(Prabhakaran et al., 2006)
Magnesium Chloride (Salt)	0.4 or 0.5% based on the amount of soymilk. 0.5% and 1.0% w/v	The use of Magnesium Cation is used to bridge the protein together to form a Gel	(Prabhakaran et al., 2006). (Ibrahim et al., 2022)
Glucono-delta-Lactone (GDL) (Acid)	0.5% and 1.0% w/v. 0.6% based on the amount of soymilk	Glucono-delta-lactone, slowly provide hydration causing the pH value to drop to the isoelectric point of soy protein which promotes isoelectric precipitation of the protein	(Grygorczyk & Corredig, 2013). (Ibrahim et al., 2022)

Bilimbi and Lime Juice (Acid)	6 and 7% Lime juice (2.35 pH) and 20 and 22% bilimbi Juice (1.45pH)	A similar mechanism where acid is used to drop pH to the isoelectric point of soy protein which promotes the precipitation of protein	(Sitanggang et al., 2020)
Microbial Transglutaminase (Enzymes)	100 unit/100 mL of Soymilk. 5 U/g of protein	The enzymes help the cross-linking of lysine isopeptide bonds	(Tang et al., 2007). (J. Zhu et al., 2019)
Grape Pomace (Acid)	2.5% and 5% (pH 3.56 and 4.09)	Purpose mechanism where acid is used to drop pH to the isoelectric point of soy protein, which promotes precipitation of protein	(Zeppa et al., 2021)

Table 2.2: Effect of Production factors on tofu composition and texture.

Production Factor	Moisture	Protein	Fat	Mineral	Texture	Reference
Calcium Sulfate (Gypsum)	Low	High	High	High Ca	Dense, firm	(Prabhakaran et al., 2006). (K. Chen et al., 2021)
Magnesium Chloride (Nigari)	Low	High	High	High Mg	Soft, smooth	(Prabhakaran et al., 2006). (Ibrahim et al., 2022)
High bean ratio	Low	High	High	Moderate	Firm	(Liu, 2012)
Low bean ratio	High	Low	Low	Low	Soft	(Liu, 2012)
GDL	High	Medium	Low	Low	Soft, smooth	(Grygorczyk & Corredig, 2013). (Ibrahim et al., 2022)

2.6 Chemical composition of tofu whey

2.6.1 Proximate analysis of tofu whey

The composition of tofu whey from tofu production is shown in Table 2.3. The data in this table shows that tofu whey still contains an essential amount of nutrients in them and that based on the countries or locations/regions where the cultivar of soybeans is from, or their processing methods can affect the given chemical composition of the tofu whey. In the south American region, tofu whey has more fat/lipid content than Chinese tofu whey by 0.6 g per 100 ml or 100 g. In comparison, protein in Chinese tofu whey was substantially higher by 0.35 g per 100 ml or 100 g. At the same time, both regions' ash and moisture contents are similar. While no dietary fiber analysis is provided, this is probably due to okara being removed from the soymilk taking away much dietary fiber, as stated (Li et al., 2012), "Okara contains about 50% dietary fiber, 25% protein, 10% lipids, and other nutrients." However, on the other hand, this does not mean that there is no dietary fiber within the tofu whey, and could be data that is missing in the literature as most of these analyses are done for data on the component, which will be helpful to their studies, but data on dietary fiber due to their health benefits will help provide further insight on the health benefits which tofu whey can give helping with the valorizing of tofu whey in the food systems. Additionally, there is a gap in the literature regarding how tofu production methods affect tofu whey, despite the well-documented effects these methods have on tofu itself.

Table 2.3: Proximate analysis of tofu whey from different literatures

Country	Argentina	Brazil	China	China	China	China
Lipids	Not given	1.00g per 100g	0.39g per 100g	0.39g per 100ml	0.40g per 100ml	0.42g per 100ml
Protein	0.32 ± 0.05g per 100ml	0.35g per 100g	0.82g per 100g	0.71g per 100ml	0.65g per 100ml	0.69g per 100ml
Carbohydrates	0.87 ± 0.04g per 100ml	0.85g per 100g	Not given	Not given	Not given	Not given
Dietary Fiber	Not given	Not given	Not given	Not given	Not given	Not given
Ash	0.44 ± 0.04g per 100ml	Not given	0.46g per 100g	0.39g per 100ml	0.44g per 100ml	0.56g per 100ml
Moisture	97.9 ± 1.0g per 100ml	Not given	95.9g per 100g	Not given	Not given	Not given
Total Solid	2.10 ± 1.0 g per 100ml	Not given	4.1g per 100g	Not given	Not given	Not given
Reference	(Ossa et al., 2020)	(Benedetti et al., 2016)	(Zhang et al., 2012)	(Tu, Tang, et al., 2019)	(Tu, Azi, et al., 2019)	(Li et al., 2017)

2.6.2 Mineral composition

Not much study has been done on the general mineral compositions of tofu whey wastewater, as looking at the mineral composition and the loss of minerals from soybean to soymilk (Nti et al., 2016), further processed foods or waste might have no mineral value left in them. However, from the literature that is present, what is believed to be the main mineral composition of tofu is calcium, phosphate, and chloride (Chua & Liu, 2019). In comparison, another study (Belén et al., 2013) shows that the mineral content of magnesium is 500mg/L and calcium is 10mg/L and states that the high amount of magnesium was due to the use of magnesium chloride salts as a coagulant, which suggests that mineral composition of tofu whey will always change based on the batch as a different coagulant and amount of coagulants will have a significant effect on the mineral composition of tofu whey. On the other hand, no data is reported on trace minerals in tofu whey which are essential for human health and development. These minerals will most

likely not change based on the type of coagulants used, and research on them could provide insight into the valorization of tofu whey in food systems.

2.6.3 Protein composition

2.6.3.1 Glycinin and β-conglycinin

The two significant proteins affecting tofu's structural and textural identity are glycinin and β-conglycinin, which can be found in soymilk and soybeans. They are both considered to be a type of protein classified as globulins based on the solubility of protein and are also known as major storage proteins for many different legume seeds. They have been known to provide emulsifying, gelling and foaming properties (Kimura et al., 2008; Rickert et al., 2004). Soybean glycinin belongs to the 11S globulin family which consists of six subunits as hexamer proteins with a molecular mass of 300-360 kDa with six acid and basic polypeptide chains each that are paired together by disulfide bonds, the acid units are 34-45 kDa, and the basic units are 17-20 kDa. While β-conglycinin or 7S globulins are considered trimers with a molecular weight of about 180 kDa with three subunits of α' with 57-72 kDa, α with 57-68 kDa, and β with 45-52 kDa in different configurations and combination of each other as they are linked via hydrophobic and hydrogen bonding interaction (González-Pérez & Arellano, 2009; Kao et al., 2003; Yaklich, 2001). These two proteins could be found in tofu whey as a study by Kao et al. (2003) suggested that proteins in tofu whey were mainly low molecular weight proteins. However, the data from Kao et al., 2003 SDS-PAGE showed that tofu whey contains high molecular weight protein bands that were in similar regions to β-conglycinin or 7S globulins. Further studies confirming glycinin and β-conglycinin in tofu whey are essential as they can be used as a functional food ingredient and ensure that they are present in tofu whey, and their concentration could be vital to the valorization of tofu whey in different food systems, depending on the type of its food application.

2.6.3.2 Lipoxygenase, β-amylase, lectin, and Kunitz trypsin inhibitor

Lipoxygenase, β-amylase, lectin, and Kunitz trypsin inhibitor are found in soy whey produced from the manufacturing process of soy protein isolate via Alkalinization method followed by acid precipitation (Li et al., 2014), and other methods (Zhang et al., 2014). Lipoxygenases are an enzyme that catalyzes the oxidation of polyunsaturated fatty acids. This enzyme needs to

be inactivated within tofu production as soymilk and tofu made without lipoxygenase has an increase in overall acceptability for consumers (Obatolu, 2008; A. Yang et al., 2016). The lipoxygenase isomers found in plants are larger than isomers found in animals, with 900 amino acids and a molecular weight of about 105 kDa.

β-amylase is an enzymatic protein that catalyzes the hydrolysis of the second α-1,4-glycosidic linkages in starch molecules, resulting in the production of maltose (Das & Kayastha, 2019). Besides the functionality of turning starch into sugar, they provide no functional interaction in tofu production or as a functional ingredient. However, β-amylase has an optimal temperature of 50^{o}C which is attributed to the cysteinyl residues as they provide the thermal stability (Totsuka et al., 1994), which makes it highly possible for these proteins to stay in the tofu whey. The β-amylase in soybeans consists of 494 amino acids with a molecular weight of 57 kDa. They have a structure comprising $(\beta/\alpha)_8$ core barrel domain in the N-terminal with a C-terminal loop region and a sac structure as the catalytic site (Das & Kayastha, 2019; Morita et al., 1976).

Lectins are a group of glycoproteins used for signaling and storage in soybean. They are used for signaling when under stress from environmental changes or pathogenic attacks and are generally used as storage protein when defending against insects and animals (Van Holle & Van Damme, 2015). Unfortunately, lectins are known as antinutrients as they bypass the human defense system to cause disease by disrupting the small intestines' surface and can cause autoimmune diseases in people by stimulating the growth of white blood cells (Popova & Mihaylova, 2019). The lectins in soybeans have a molecular weight of about 25-35 kDa, with a structure generally consists of two or four subunits that are linked to give a raise to a homodimer (Delves & Roitt, 1998; Mishra et al., 2019).

Kunitz trypsin inhibitors are enzymatic proteins that have a function as protease inhibitors against trypsin or chymotrypsin (Bendre et al., 2018). Therefore, Kunitz trypsin inhibitors act as an antinutrient for the human body as it stops the digestion of proteins in the gut and forces excess trypsin synthesis, which burden the body's requirements (Popova & Mihaylova, 2019). The Kunitz trypsin inhibitors in soybeans have a molecular weight of about 20-22 kDa with a typical structure containing β-trefoil of a single peptide with two disulfide bonds (Bendre et al., 2018).

Although these low molecular proteins are found in soy whey, a study to confirm them in tofu whey would be required. While there are a few differences between soy whey and tofu whey production, these proteins exist in soybean and can remain in the whey during production. Furthermore, making the suggestion by Kao et al., (2003) more likely as tofu whey seem to have a higher density of low molecular weight proteins, which could be lectin and Kunitz trypsin inhibitors, as these proteins could be left in the whey during tofu production. However, these enzymes present in tofu whey collected after tofu-making would not have enzymatic activities due to their denaturation caused by heat treatment applied during the production of tofu-making.

2.6.3.3 Peptide chains

The majority of protein composite in tofu whey could be peptide chains., The data provided by Kao et al. (2003) suggested that a lot of proteins found in tofu whey are below 16 kDa, which is quite a low molecular weight and could be represented by peptide chains from soybean. One example of this is a study done by Nieto-Veloza et al., (2021), which showed that bioactive lunasin, with a molecular weight of 5kDa and composed of 43 amino acids, was successfully isolated from tofu whey using ethanolic-isoelectric precipitation and gel filtration chromatography. The study stated that lunasin has been shown to provide "anti-inflammatory, antioxidant, anti-hypertensive, cholesterol-regulating, neuroprotective and immunomodulatory properties." Identifying peptides in tofu whey is a recent study, and further study should look to identify other peptides that could exist in tofu whey as bioactive components.

2.6.4 Carbohydrate composition

2.6.4.1 Main saccharide composition in tofu whey

The main carbohydrate composition in tofu whey is shown in Table 2.4. The main mono- and disaccharides are glucose, fructose, and sucrose, while the main oligosaccharides are raffinose and stachyose. The amount of saccharides will vary due to different variants, and as for why data from (Champagne et al., 2003) showed high value is due to the processing which occurred in the tofu whey before analysis of the saccharides as they heated at 110^{o}C

for 3 minutes and centrifuged at 10,000 x g at 4^{o}C for 10 minutes. However, that ratio for the values is similar to the data from the other two studies and shows some validity.

Table 2.4: Saccharide composition in tofu whey

Sugar			
Glucose	0.04 g/100g	0.46g/L	1.2g/L
Fructose	0.03g/100g	0.36g/L	1.1g/L
Sucrose	0.16g/100g	5.16g/L	11.3g/L
Raffinose	0.04g/100g	1.80g/L	1.6g/L
Stachyose	0.22g/100g	5.48g/L	6.4g/L
Reference	(Sriniworn et al., 2015)	(Fei et al., 2017)	(Champagne et al., 2003)

2.6.4.2 Polysaccharides

The primary type of polysaccharides found in tofu whey would be soluble polysaccharides as tofu whey has a high majority of its component being moisture. One polysaccharide found in tofu whey is soluble soybean polysaccharide SSPS1 which was successfully isolated from tofu whey (Guan et al., 2022). SSPS1 has a molecular weight of 2737 kDa and is composed of 97.3% glucose and 2.7% mannose with a glucan structure that has a majority of → 6)-α-D-Glcp-(1 → linkages with minor branch linkages of α-D-Glcp-(1 → at the third oxygen on the main chain (Guan et al., 2022). On the other hand, a study done by Ossa et al. (2020) suggests that in the concentrated tofu whey, soluble soy polysaccharides from the cotyledon and hull existed in tofu whey due to a net charge equal to zero at a pH range of 2.5 to 3.0 as galacturonic acid residues have a pKa at around 3.0. The polysaccharide that is said to be in the cotyledon and hull of the soybean consists of a molecular mass of 550 kDa and is composed of galacturonan and rhamnogalacturonan backbone with diglycosyl repeating units of → 4)-α-D-Gal-(1 →2)- α-L-Rha-(1→ linkages with arabinan and galactan branches (Chen et al., 2017; Jia et al., 2015; Maeda & Nakamura, 2021). While both polysaccharides could be in the tofu whey, (Guan et al., 2022) have stated that "The structure of SSPS1 extracted from tofu whey is significantly different from other reported soybean polysaccharides." However, they do not claim that the other reported soybean polysaccharides are not found in tofu whey, suggesting that both these polysaccharides could be found in tofu whey. Another possible polysaccharide in tofu whey could be SSPS2,

as identified by Guan et al. (2022), as it has a molecular weight of 14 kDa, and not much else is known about it. Overall further research is required on identifying and characterising the polysaccharides that could be found in tofu whey as there is little research being done on it.

2.6.4.3 Dietary fibre

There is no information about dietary fibre in tofu whey. However, studies done by Ossa et al. (2020); Sun et al. (2022) stated that tofu whey contains 2.21g/100 ml of water-soluble carbohydrates and in concentrated tofu whey there is upwards of 31.8% w/w of total dietary fibre. Therefore it can be concluded that the original tofu whey has dietary fibre in it but there is hardly any study on dietary fiber in tofu whey because okara is mainly made of dietary fibre (42.4-58.1g/100g dry matter) (Redondo-Cuenca et al., 2008; Vong & Liu, 2016). However, it is still possible for tofu whey to contain soluble dietary fibre, as no analytical work on this has been done. Although the dietary fibre in okara and soybean is not well understood as the chemical structure of the dietary fibres is still not clear, what is known is the monomers of the dietary fibres. Glucose makes the majority component in insoluble dietary fibre, followed by a substantial amount of arabinose, xylose, uronic acids, and galactose. Whereas, for soluble dietary fibres, galactose, uronic acids, and arabinose form the majority component and glucose and xylose were found in substantial amount. (Redondo-Cuenca et al., 2007; Redondo-Cuenca et al., 2008). Further analytical studies to show on the characteristic of dietary fibre and the amount in tofu whey are vital as they will provide additional valorization value for tofu whey.

2.6.5 Nutrients and antinutrients in tofu whey

2.6.5.1 Isoflavone

Isoflavones are a polyphenol from legumes, particularly soybeans, thus found in tofu whey. The isoflavones found in soybeans predominantly exist in the glycoside form, including daidzin, genistin, and glycitin (Figure 2.2). Their aglycones are referred to as daidzein, genistein, and glycitein (Benedetti et al., 2015; Chua & Liu, 2019; Chua et al., 2018; Zhao et al., 2020). These Isoflavones have pretty similar structures as the aglycones have a backbone of 3-phenyl-chromen-4-one, while the glycoside has the same backbone with a glucose compound linked to it at the 7^{th} carbon on the oxygen (Hsiao et al., 2020). As shown in Table

2.5, more glucoside form in tofu whey which is a problem as the bioavailability and absorption of glucosides are worse than in aglycones (Gómez-Zorita et al., 2020; Pabich & Materska, 2019). Tofu processing increases the amount of isoflavones overall but also produces more of the naturally low bioavailable isoflavone in the glycoside form, as the soaking process of the soybean releases endogenous β-glucosidase, which converts glucosides to aglycone. While the heating or cooking process of the soybean cause the naturally occurring malonyl compound to convert into glucosides via de-esterification, overall increase the isoflavones with lower bioavailable as seen in Table 2.5(Hsiao et al., 2020). Although the bioavailability of isoflavone is low, there have been studies showing health benefits, as described in Table 2.5. The benefits it provides are bone health maintenance and reducing specific types of cancer. Isoflavones provide bone health by stimulating and promoting insulin-like growth factor 1 production and oestrogenic activity, which in turn increases osteoblastic activity, which increases the rate of bone formation and decreases the rate of bone resorption, overall helping with bone health (Gómez-Zorita et al., 2020; Xiao et al., 2018). Additionally, isoflavones can reduce a specific type of cancer through three known mechanisms. One is binding to α or β estrogen receptors, increasing apoptosis and decreasing cell proliferation. The second mechanism is inhibiting aromatase enzymes, which stop the conversion of androgen to oestrogen, reducing cell proliferation. The last mechanism is that it acts as an antioxidant in malignant cells, reducing cell proliferation (Gómez-Zorita et al., 2020; Pabich & Materska, 2019). Other health benefits, such as reducing cardiovascular risk, are controversial as some suggest that soy isoflavones do not provide an effect on reducing cardiovascular risk (Gómez-Zorita et al., 2020), while other studies indicate that regular consumption of soy isoflavones reduces cardiovascular risk based on population studies (Pabich & Materska, 2019). However, further studies on these health benefits are required, especially clinical trials, as there is still not enough data to make conclusive statements about the health benefits associated with isoflavones. Furthermore, analytical data on isoflavone is required for tofu whey as current studies have indicated various ranges of isoflavone in tofu whey but have confirmed that there are more glucosides than aglycones.

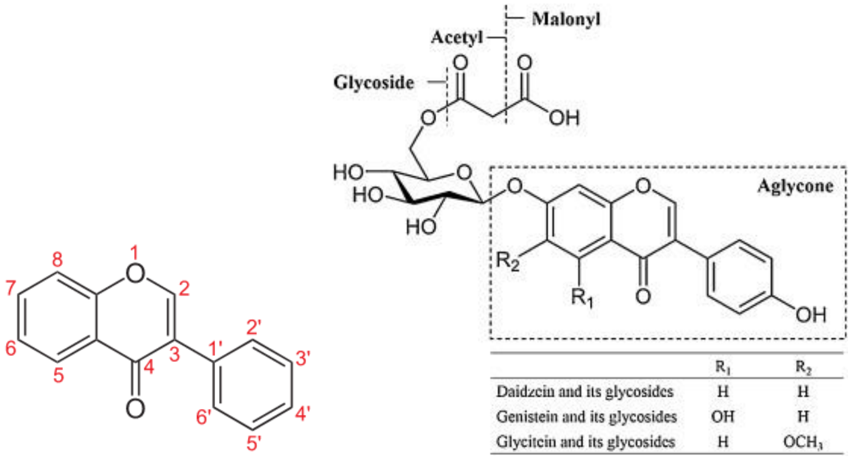


Figure 2.2 Chemical structures of 3-phenyl-4H-chromen-4-one (Left) and isoflavone (Right) in soybean (Lim et al., 2020)

2.6.5.2 Phytic acid

Phytate, or phytic acid, is a compound in plants that is used to store phosphorus and inositol in plant seeds. They are also found in tofu whey in small amounts, as (Chua et al., 2018) state there is 22.40 mg of phytic acid-phosphate per liter. The phytic acid structure is generally referred to as a six-folded dihydrogen phosphate ester of inositol (Bloot et al., 2021) as shown in Figure 2.3. Phytic acid is quite a controversial chemical as some believe it to be an antinutrient, and some believe that it has health benefits and that the antinutrient provided also gives health benefits. The reason why phytic acids are believed to be antinutrients is due to their chelating ability on minerals, reducing the bioavailability and absorption of the minerals into the body, which can affect the human body due to the basic needs of minerals in the regulation of the human body (Abdulwaliyu et al., 2019; Bloot et al., 2021). Iron deficiency is an example of phytic acid chelating a mineral and acting as an antinutrient, which can cause a defect in hemoglobin synthesis and lead to anaemia. However, cheating minerals such as calcium may be considered a health benefit due to preventing kidney stone formation and urolithiasis as it stops the crystallization of calcium with oxalate (Abdulwaliyu et al., 2019). Additionally, it exhibits intense antioxidant activity due to its ability to chelate irons, which form an inactive chelate structure. As a result, it removes an iron catalytic reaction which prevents hydroxyl radical formation and stops the auto-oxidation process from occurring (Bloot et al., 2021). Its antioxidant activity is then further shown to play essential roles in phytic acids' ability to be the anti-cancer agent by

specifically targeting cancer cells (Masunaga et al., 2019) and anti-diabetic agent by reducing the rate of carbohydrate digesting and delaying the onset of diabetes (Abdulwaliyu et al., 2019). However, further studies on phytic acid with the clinical trial are required before a firmer statement can be made on whether it is a nutrient or antinutrient, as there are a limited number of clinical trials with phytic acid and dosage or amount of phytic acid which is required before these effects are seen is also not known. Furthermore, analytical data on phytic acid is required for tofu whey as only (Chua et al., 2018) has confirmed how much phytic acid is in tofu whey.

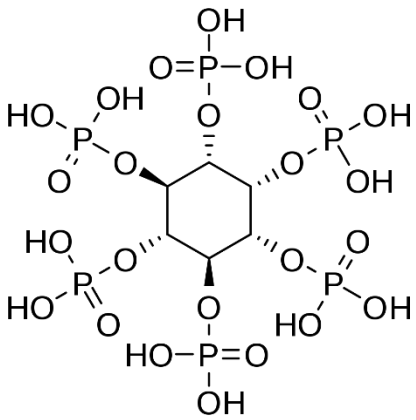


Figure 2.3 Chemical structure of six-folded dihydrogen phosphate ester of inositol (Phytic acid)

2.6.5.3 Gamma-aminobutyric acid

Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter in the mammalian central nervous system and has been found in tofu whey by Chua et al. (2018) as they have stated there is 26.82mg of GABA per liter. The structure of GABA is butanoic acid with an amino substituent at the 4th carbon (Chua et al., 2019) (Figure 2.4). GABA has been widely studied and has shown many health benefits, as it most benefits the physiological disorder, with the majority of its function being neurotransmission, cell regulation, and hormonal regulation (Diana et al., 2014). In recent years it seems that studies show that GABA, which is consumed through foods, fails to cross the blood-brain barrier, which means that GABA consumed through foods no longer plays a significant role in the central nervous region

(Chua et al., 2019), but still show health benefits such as anti-stress, anti-hypertensive, anti-cardiovascular, anti-diabetics and anti-depression. Furthermore, they still play a pivotal role in the central nervous system. Moreover, recent studies by Inotsuka et al. (2020) indicate that even though food consumed GABA is unable to permeate through the blood-brain barrier, there is still a GABA-induced gut-brain interaction and that GABA-activated intestinal cells successfully secreted exosomes which activates the neuronal cells but the mechanisms behind this interaction is still unknown. Overall future studies should look at understanding more about the mechanism by which food consumed GABA works in the body and furthermore, analytical data on GABA is required for tofu whey as only (Chua et al., 2018) have confirmed how much GABA there is in tofu whey.

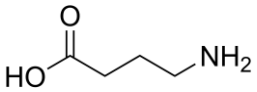


Figure 2.4 Chemical structure of gamma-aminobutyric acid (GABA)

2.6.5.4 Saponins

Saponins are one of the main secondary metabolites in legumes and can be found in tofu whey. The studies done by Sun et al. (2022) have stated that the main saponins found in tofu whey at a concentration of 1.26 mg/ml seem to be that of B-type or B group and are mainly known as soyasaponin 1. Soyasaponin 1 has a triterpenoid structure of lipid soluble aglycone with carbohydrate moiety attached to it (Figure 2.5). Saponins are a quite controversial compound as they have been known to be an anti-nutrient which causes hypoglycaemia and leaky gut (Popova & Mihaylova, 2019) while also providing health benefits such as cytoprotective activity and anti-cancer activity via inhibiting sialytransferase (Singh et al., 2017). The reason some view them to be anti-nutrient is because they are natural toxic substance in plants as they are used in plant defences against insects and bacterial, which leads them to become toxic to humans, as they bind to cholesterol and cause a strong hypocholesterolemic effect in the body as they prevent absorption of the cholesterol from the small intestine, causing leaky gut (Sidhu & Oakenfull, 1986; Zaynab et al., 2021). However more recent studies have shown saponins and especially soyasaponin 1 providing health benefits such as cytoprotection by using their antioxidant defence mechanism against cytotoxicity causing lipid radicals and oxidative stress (Vila-Donat et al.,

2015). As for their ability to be an anti-cancer agent is due to inhibiting sialytransferase which is known for being highly associated with tumour growth (Bachran et al., 2014), and was shown to inhibit the α2,3-sialytransferase activity and stop further mRNA expression on to the cell surface (Guang et al., 2014). Although current studies tend to be showing more of health benefits side for using saponins but there is still toxic capability from them and further studies with clinical trials are required before saponins can be viewed as a more health benefit compound than an anti-nutrient. Also, further analytical work on saponins in tofu whey is required as there is a very limited amount of study on saponins in tofu whey.

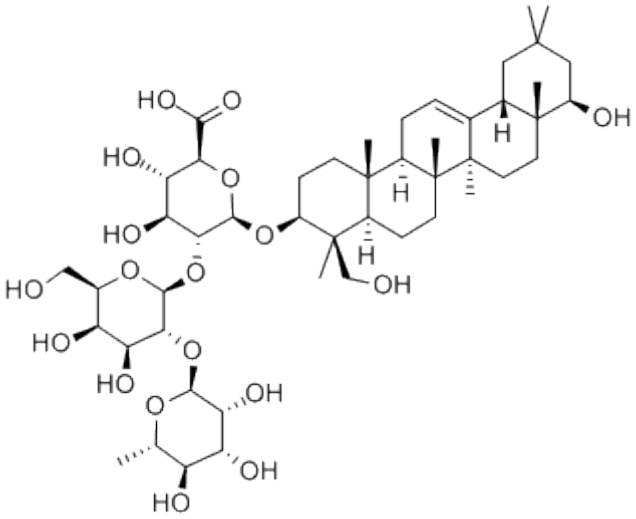


Figure 2.5. Chemical structure of Soyasaponin 1

Table 2.5: Nutrient and antinutrient compounds found in tofu whey/soy whey.

Compound	Amount Found	Benefits/Nutrient provided	Antinutrient	References
Isoflavones: **Daidzin** **Genistin** **Glycitin** **Daidzein** **Genistein** **Glycitein**	Daidzin = 9.72 - 17.66 mg/L Genistin = 13.91 - 25.99 mg/L Glycitin = 3.77 - 11.77 mg/L Daidzein = 1.88 - 4.01 mg/L Genistein = 1.16 - 1.69 mg/L Glycitein = 0.38 - 12.43 mg/L	Maintaining good bone health to prevent osteoporosis. Can be helpful in reducing specific types of cancer such as breast and endometrial cancer.	N/A	(Benedetti et al., 2015; Chua et al., 2018; Pabich & Materska, 2019)
Phytate **Phytic acid**	22.40 mg/L	Antioxidant Urolithiasis Anti-microbial	Absorb minerals	(Abdulwaliyu et al., 2019; Chua et al., 2018)
Gamma-Aminobutyric Acid	26.82 mg/L	Anti-Stress Anti-hypertensive reduce cardiovascular risk Anti-diabetics Anti-depression	N/A	(Chua et al., 2019; Chua et al., 2018)
Saponins	1.26 mg/ml	Hepatoprotective activity Cytoprotective effects Lowering Cholesterol Antioxidant activity Inhibition of sialytransferases	Impair protein digestion hypoglycemia Leaky gut	(Popova & Mihaylova, 2019; Singh et al., 2017; Sun et al., 2022)
Lectin	Unknown	N/A	Autoimmune disease Leaky Gut White blood cell growth	(Li et al., 2014; Popova & Mihaylova, 2019)
Kunitz Trypsin inhibitor	Unknown	N/A	Prevent protein digestion	(Li et al., 2014; Popova & Mihaylova, 2019)

2.7 Physicochemical properties and stability of tofu whey

2.7.1 Physicochemical properties

The physicochemical properties of tofu whey are shown in Table 2.6. Except for pH, limited information is available regarding the physicochemical properties of tofu whey due to the lack of extensive analysis. The observed pH values algin with expectation, as tofu production typically involves acid or salt coagulants. Acid coagulants result in a lower pH range of 5.0 - 5.5 (Cao et al., 2017), while salt coagulants yield a slightly higher pH range of 5.6 – 5.9 (Prabhakaran et al., 2006). Other physicochemical parameters also correspond to expectations. Since tofu whey is almost water, it has high moisture content with low total solids, resulting in specific gravity and density values close to those of water. The low sugar content of tofu whey explains its low Brix value and contributes to the slightly acidic pH, which is further influenced by the presence of organic acids. An interesting observation is the presence of solid suspensions in tofu whey, which requires further investigation. (Wang et al. (2018) reported this phenomenon but did not state the nature of these solid suspensions, which could potentially include proteins, polysaccharides or other components.

Table 2.6 Physicochemical properties of tofu whey

Physicochemical properties	Value	References
pH	5.12 - 6.21	(Chua & Liu, 2020; Chua et al., 2018; Ossa et al., 2020; Sun et al., 2022; Wang et al., 2018; Zhao et al., 2020)
Solid suspension g/L	2.77	(Wang et al., 2018)
Brix	2.5 - 3.7	(Chua & Liu, 2020; Chua et al., 2018; Zhao et al., 2020)
Specific gravity	1.0601	(Chua & Liu, 2020)
Total Organic acid g/L	3.78 – 4.78	(Chua & Liu, 2020; Chua et al., 2018)
Density g/L	1057	(Ossa et al., 2020)

2.7.2 The effects of different treatments or conditions on Tofu Whey

2.7.2.1 The effect of pH on tofu whey

The effect of pH on tofu-making is apparent as it is used to precipitate proteins in soymilk to form a gel-like structure (e.g. solid substance) through covalent bonding forces (Cao et al., 2017; Grygorczyk & Corredig, 2013). However, little is known about the effects of pH on tofu whey. Since tofu whey contains similar components to soybeans and soymilk, its effects, in terms of turbidity, zeta potential, protein solubility, and carbohydrate solubility, are expected to be comparable. Zeta potential showed positive values below pH 4, a value of 0 between pH 4 and 5, and negative values above pH 5 (Ossa et al., 2020). A study by Sriniworn et al. (2015) showed similar results, eluding that proteins in tofu whey play a significant role in controlling ionic charge. This is expected since tofu whey is derived from soybeans and likely exhibits similar properties to soybeans and soymilk. At its isoelectric point (pH 4-5), tofu whey has the highest turbidity due to increased aggregation of biopolymers as electrostatic charge repulsion is weakened. In contrast, at more acidic (pH ~2) or alkaline (pH ~2) conditions, turbidity is lower due to stronger electrostatic repulsion caused by a greater presence of positive and negative charges on the biopolymers (Ossa et al., 2020; Xue et al., 2013). However, carbohydrate solubility does not follow the same trend as protein solubility. The solubility of carbohydrates in concentrated tofu whey varies with temperature. At 50^{o}C, solubility is lowest at pH 3 due to the pKa of galacturonic acid residues in polysaccharides. As the temperature increases to 65 and 80^{o}C, the carbohydrate

solubility pattern changes from higher solubility observed at lower pH to lower solubility at higher pH. This shift is attributed to glycosylation reactions with peptides, as the amount of nonreactive lysine increases as the temperature for the concentrated tofu whey increases (Ossa et al., 2020; Q. Zhang et al., 2021). Nevertheless, the best model for predicting carbohydrate solubility is based on concentrated tofu whey at 50^{o}C, as it minimizes the presence of nonreactive lysine. Although the zeta potential and protein solubility behaviour of tofu whey is similar to that of soy protein isolate (Malhotra & Coupland, 2004), further research is needed to fully understand the effects of pH on the functionality and physicochemical stability of tofu whey.

2.7.2.2 The effect of freezing on tofu whey

Freezing is one of the methods used for food preservation. However, current research suggests that freezing can also concentrate tofu whey as it primarily consists of water (Belén et al., 2013). Concentrating tofu whey increases total solids, enhancing their potential for valorization. However, little is known about how freezing affects tofu whey beyond the findings of (Belén et al., 2013), who studied its freeze concentration using falling-film freezing. This study highlighted that tofu whey, with a concentration between 1.9 and 15.5 Brix, exhibits Newtonian fluid behaviour and has a freezing point ranging from – 0.6 to -2.7^{o}C.

Furthermore, the study also showed that freezing increases protein and calcium concentrations up to the second concentration stage, after which the viscosity of tofu whey rises significantly, likely due to protein conformation changes, significant protein aggregate breakdown, and phase separation driven by depletion forces (Belén et al., 2013). The decrease in calcium concentration means that calcium ions may compete with hydrogen ions for protein binding sites, potentially leading to ionic bonding between proteins and increasing viscosity.

In contrast, total sugar content increases linearly as tofu whey concentration increases/ These changes may modify sugar crystallinity and induce depolymerization, which can alter the behaviour of sugar and promote hydrophilic matrix formation (Belén et al., 2013). Even though this study provides valuable insights into freeze concentration, little is known about how thawing affects the solid structure of tofu whey or how conventional freezing affects its

properties. Further investigation is required to understand the structure and stability changes caused by freezing and thawing.

2.7.2.3 The effect of salt on tofu whey

The effect of salt on tofu whey is similar to that of pH but occurs via a different mechanism. Salts bind tofu protein to form a gel-like structure (K. Chen et al., 2021; Prabhakaran et al., 2006). However, there is a lack of information on the effect of salt on tofu whey. Since tofu whey is mainly water with a low total solid content, its response to salt could resemble either that of water or soymilk. Salt would dissolve and exist as free ions in solution if it behaves like water, forming ionic bonds with water molecules (Dill et al., 2005). However, if it behaves like soymilk, multivalent cations (with a charge of +2 or higher) could reduce electrostatic repulsion against proteins, facilitating aggregation. These cations would act as bridges, forming networks between proteins that trap water within the matrices (Kao et al., 2003). However, some monovalent cations like sodium may block network formation due to their larger ionic radii, which allow them to bind only at single sites rather than bridging between proteins (Arii et al., 2021). Further studies on salts with different valences are necessary to fully understand their impact on the stability and physicochemical properties of tofu whey.

2.7.2.4 The effect of ultrasound on tofu whey

Ultrasound is a novel technology that has gained popularity in recent years due to its diverse applications in the food industry, such as microbial and enzyme inactivation, enhancing bioactive compounds, and homogenization (Awad et al., 2012; Gallo et al., 2018; Kidoń & Narasimhan, 2022; O'donnell et al., 2010). Furthermore, ultrasound has enhanced the total phenolic and flavonoid content, isoflavone (glucosides and aglycones) content, and antioxidant activity in tofu whey (Zhao et al., 2020). This occurs through the formation of cavitation bubbles, which repeatedly compress and decompress the material, ultimately disrupting the cellular structure of tofu whey and releasing bioactive compounds. S Similar effects have been observed in other fruit and vegetable liquid systems (Wen et al., 2018; Zhao et al., 2020).

However, it is unknown whether these cellular structures are essential to the functionality of tofu whey and whether their destruction affects the functional properties, such as emulsifying ability and stability, which tofu whey proteins are stated to possess. Although the functional properties of tofu whey are not yet well understood, its physicochemical characteristics seem to be relatively stable. Studies have shown that despite structural disruption, parameters such as pH, total soluble solids, total protein content, and colour remain unchanged, with no significant differences observed between ultrasound-treated and control samples (Zhao et al., 2020). Nevertheless, further research is required to understand the full impact of ultrasound on tofu whey. While ultrasound treatment enhances bioactive compounds, its effects on the functional properties of tofu whey have yet to be extensively studied, indicating a significant gap in current knowledge.

2.7.2.5 The effect of fermentation on tofu whey

Fermentation has been used for centuries to preserve food via metabolic processes that transform chemical substrates using enzymes (Ross et al., 2002; Steinkraus et al., 2004). However, in recent years, fermentation has been used not only to preserve food but also to enhance food flavours (Medina et al., 2013; Steinkraus, 1994) and provide health benefits such as improving digestion, boosting immune systems and less-studied effects such as antioxidant, anti-cancer and anti-diabetic properties (Marco et al., 2017; Şanlier et al., 2019).

There are many methods by which fermentation can be conducted. Recent studies on tofu whey have primarily focused on fermentation using yeast (Chua et al., 2017, 2018) and lactic acid bacteria (Fei et al., 2017; Y. Zhu et al., 2019). Yeast fermentation of tofu whey has shown that saccharomyces and some non-saccharomyces yeast strains can metabolize sucrose to produce ethanol for beverages, which could cause a pH decrease due to increased malic acid, while others that do not produce ethanol tend to retain high sucrose content with a higher pH due to malic acid degradation (Chua et al., 2017, 2018). However, all tested yeast strains were able to metabolize soluble proteins, endogenous volatile compounds, phytate, γ-aminobutyric acid (GABA) and Isoflavone glucosides to varying degrees, resulting in changes to the physicochemical properties of tofu whey (Chua et al., 2017, 2018).

Yeast fermentation provides several benefits, including increased isoflavone aglycone content and reduced phytic acid, which contribute to the previously mentioned health benefits. Additionally, fermentation reduces unpleasant flavour and aroma compounds associated with tofu whey, producing a more desirable beverage aroma and flavour. However, a potential detriment is decreased soluble protein content, which may eliminate some functional properties attributed to tofu whey proteins.

Similarly, lactic acid fermentation causes a decrease in sugar content over the fermentation period, increased organic acid content, and reduced pH. The effect of lactic acid fermentation on tofu whey has been studied less extensively than yeast fermentation, as only changes in volatile compounds and isoflavones were studied (Fei et al., 2017; Y. Zhu et al., 2019). The results from both studies reported by Fei et al. (2017); Y. Zhu et al. (2019) were similar as unpleasant flavour and aroma were reduced via the fermentation and isoflavone glucosides were converted into isoflavones aglycones via increased β-glucosidase activity. However, key aspects of both fermentation methods remain unknown, particularly regarding their effects on the structure and stability of tofu whey. A reduction in protein content could significantly alter the structural integrity and stability of tofu whey.

2.8 Functional properties of tofu whey

2.8.1 Emulsifying properties

Emulsification is important when trying to valorize waste products like tofu whey. Emulsions are the fusion of two liquids when two immiscible liquids, such as oil and water, are mixed but tend to separate over time. Many food systems are emulsions, including sauces, flavoured sodas, and mayonnaise (Prakash et al., 1990; Sikora et al., 2008) and cosmetic products like creams (Miner, 2017). If tofu whey exhibits emulsifying abilities, it could provide functional value to various products and systems, making it a valuable ingredient.

Emulsifying properties can be defined by emulsifying activity and emulsifying stability. The emulsifying activity refers to the amount of oil that can be emulsified per unit of protein, while the emulsifying stability describes how well the emulsion resists separation over time (Aryee et al., 2018). There is a lack of information on the emulsifying properties of fresh tofu whey, likely due to its high moisture content and low total solids. However, studies on

concentrated tofu whey obtained through freeze-drying or rotary evaporation have suggested that tofu whey has emulsifying properties that enhance oil-in-water emulsions (Kasran et al., 2013; Matemu et al., 2009; Ossa et al., 2022; Palazolo et al., 2013).

A study by Ossa et al. (2022) highlighted that tofu whey concentrate obtained via rotary evaporation at 80^{o}C exhibited superior emulsifying properties compared to that obtained at 50^{o}C. The higher-temperature concentrate showed a smaller mean droplet diameter (D (3,2) and D(4,3)), lower flocculation degree, and higher adsorbed protein percentage, all of which contribute to improved emulsifying stability and activity. The enhancement in emulsifying properties is attributed to partially unfolded proteins and protein-polysaccharide conjugates formed from heating. These conjugates prevent excessive protein-protein interactions, allowing polysaccharides to remain attached to proteins, improving their amphiphilic balance and producing better emulsifying properties (Kasran et al., 2013). Similarly, Matemu et al. (2009) reported that tofu whey glycoproteins have up to three times higher emulsifying activity and stability than tofu whey proteins alone.

Furthermore, studies have shown that concentrated tofu whey improves the stability of frozen and thawed emulsions, as emulsions are generally susceptible to destabilization during freezing and thawing treatments (Ossa et al., 2022; Palazolo et al., 2013). The studies reported by Palazolo et al. (2013) showed that after 130 days of frozen storage, the oil-in-water emulsions containing tofu whey remained stable, whereas the emulsions added with sucrose destabilized within 30 days. This stability is attributed to glycoproteins in tofu whey, which can help retard ice recrystallization, therefore preventing excessive ice growth (Palazolo et al., 2013). Large ice crystals are known to damage and alter protein structures, which can cause partial coalescence and creaming or sedimentation (Fredrick et al., 2010).

It appears that glycoproteins are the main contributing factor to improving the emulsifying properties of concentrated tofu whey. However, further research is required to understand how glycoproteins interact with protein in tofu whey and how they enhance emulsification. Additionally, the role of glycoproteins in fresh tofu whey remains largely unexplored.

2.8.2 Foaming properties

Proteins from soybean and soy protein isolates have been found to have foaming properties (Hettiarachchy & Kalapathy, 1998; Martínez et al., 2009). Improving foaming properties has contributed to better bread loaf volume and texture (Kenny et al., 2000). In other food products, such as cakes and meringues, foaming properties improve texture and rheology, enhancing overall product quality (Foegeding et al., 2006). Since tofu whey contains proteins similar to those found in soybeans and soy protein isolates, although there are some differences in composition and concentration, it has the potential to provide foaming properties, adding functional value to various food systems.

Foaming properties can be categorized into two aspects: foaming capacity and foaming stability. Foaming capacity is the ability of a protein to generate foam upon whipping. The amount of foam created is typically measured by the overrun percentage. Conversely, foam stability is the (time) required when 50% of the foam volume is lost, commonly measured by collapse time (Raymundo et al., 1998). While limited information exists on the foaming properties of tofu whey, even less is known about the foaming properties of concentrated tofu whey.

A study by Ossa et al. (2020)) reported a higher overrun percentage for tofu whey concentrated at 50^{o}C compared to tofu whey concentrated at 65 and 80^{o}C across all pH levels. This was due to lower protein aggregation in the system, resulting in higher molecular flexibility proteins. The reduced heat exposure at 50^{o}C likely minimized protein denaturation, thereby preserving foaming properties.

As for foaming stability, tofu whey concentrated at 80^{o}C demonstrated more extended stability between pH 3 and 5 than tofu whey concentrated at 50^{o}C, likely due to increased protein aggregation and a higher polysaccharide-soluble fraction. Higher protein aggregation enhances compaction at the air-water interface, forming a viscoelastic film that prevents film thinning and foam collapse, improving foam stability (Martínez et al., 2009; Ossa et al., 2020).

Additionally, for all concentrated tofu whey samples, foaming capacity and stability were generally better at pH values close to the isoelectric point (4-5), which is because, at this pH,

proteins carry a net charge close to zero, promoting aggregation and the formation of viscoelastic films, which enhance foaming properties (Martínez et al., 2009; Ossa et al., 2020). Although limited data are available on the foaming properties of concentrated tofu whey, the existing results indicate its strong potential as a functional ingredient. Tofu whey has shown an overrun of 1467-1900% and a stability of 19.9-28.8 seconds at pH 4-5. Compared to the foaming capacity of similar systems like aquafaba derived from chickpeas, peas, and soybeans, tofu whey exhibits superior foaming capacity. Studies report that aquafaba typically achieves a foaming capacity of 200-700% (Mustafa et al., 2018; Nguyen et al., 2020; Shim et al., 2021). However, further investigation into the foaming properties of fresh (non-concentrate) tofu whey is also required, as concentration methods can be costly. Developing a more economical approach could improve the valorisation of tofu whey as a functional ingredient.

2.8.3 Antioxidant properties

Tofu whey exhibits antioxidant properties and can be added to food systems for functional effects. Compounds like flavonoids, phenolic content, isoflavones, saponins, and phytic acids have been identified in tofu whey and contribute to its antioxidant activity (Chua et al., 2018; Sun et al., 2022; Zhao et al., 2020). Antioxidants are bioactive compounds that benefit health by inhibiting oxidative damage to cells caused by free radicals (Noguchi et al., 2000). By inhibiting damage to cells, antioxidants are essential in preventing the risk of cardiovascular diseases and cancers (Gómez-Zorita et al., 2020; Masunaga et al., 2019; Pabich & Materska, 2019) as well as other chronic diseases (Zhang et al., 2015).

Recent studies on the antioxidant properties of tofu whey have shown radical scavenging activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, while ferric reducing antioxidant power (FRAP) had lower activity compared to the other two methods (Benedetti et al., 2015; Tu, Tang, et al., 2019; Zhao et al., 2020). This trend is consistent with the findings on soybean-derived antioxidants, which also show lower FRAP activity than DPPH and ABTS (Ghanem et al., 2020; Yang et al., 2014). These results suggest that the antioxidant compounds naturally found in soybeans and tofu whey have a lower ability to reduce Fe^{3+} to Fe^{2+}.

The differences in antioxidant activity between DPPH and FRAP are further exacerbated when tofu whey is added to bread formulations. A control bread without tofu whey (0%) showed higher FRAP activity (409.72 µmol/g) and lower DPPH activity (185.85 µmol/g). However, when 6% tofu whey was added, both DPPH and FRAP values increased significantly to 885.08 µmol/g and 860.37 µmol/g, respectively, with DPPH showing a more pronounced increase (Zhao et al., 2021). Despite these results, the underlying mechanisms explaining why FRAP shows lower scavenging ability compared to DPPH and ABTS remain unclear and require further research. Furthermore, while tofu whey has been demonstrated to possess antioxidant properties, it is still unknown whether its consumption in dietary amounts can significantly decrease the adverse effects of reactive oxygen species, which cause the risk of cardiovascular diseases and cancers. Further investigations are necessary to determine whether tofu whey contains sufficient bioactive compounds before being classified as a functional ingredient with antioxidant properties.

2.8.4 Antimicrobial properties

Tofu whey may possess antimicrobial properties. The functions that antimicrobial properties bring to food systems are preservation from food spoilage and longer shelf life (Jamali et al., 2021; Villalobos et al., 2016). Antimicrobial agents inhibit the growth of bacteria, prevent the formation of microbial colonies, and, in some cases, destroy microorganisms (Elmogahzy, 2019).

Currently, there is little evidence on whether tofu whey has antimicrobial properties. Research by Tu, Tang, et al. (2019) found that fresh tofu whey showed no antimicrobial activity against *Staphylococcus aureus, Escherichia coli, and Bacillus subtilis*. However, when tofu whey was fermented with kombucha, which introduced acetic acid, it exhibited significant antimicrobial effects. However, tofu whey contains bioactive compounds such as saponins, isoflavones, flavonoids, and phenolic acids, demonstrating antimicrobial activity. Saponins, especially soyasaponins, have been identified as potent antimicrobial agents in small dosages of 0.25 mg, showing effective inhibition zones of 13.3 mm against *Escherichia coli* and 26.7 mm against *Staphylococcus aureus* (Sun et al., 2022).

Additionally, flavonoids and phenolic acids have displayed the ability to inhibit microbial metabolism by destabilizing and permeating microbial membranes (Pina-Pérez & Pérez,

2018; Sun et al., 2022). Furthermore, the research by Sun et al. (2022) highlighted that tofu whey media plates showed no growth of yeast, mould, or gram-negative bacteria. Further consolidation of the information on the antimicrobial properties of tofu whey is required, although they contain known antimicrobial compounds. This is because there are conflicting findings regarding the antimicrobial effectiveness of tofu whey.

2.8.5 Anti-browning properties

Anti-browning properties help prevent chemical deterioration in food products, increasing shelf life. Tofu whey has been shown to inhibit the browning effect in mashed potatoes (Sun et al., 2022). However, whether inhibition is due to enzymatic or non-enzymatic mechanisms remains unclear, as the study only assessed browning after 14 days of storage.

Enzymatic browning is induced by polyphenol oxidase activity, which catalyses the oxidation of phenolic compounds, leading to pigment formation (Tiwari, 2018). In contrast, non-enzymatic browning results from chemical reactions, such as caramelization or the Maillard reaction, which involve the interaction of reducing sugars and amino acids (Manzocco et al., 2000).

Although specific research on the anti-browning properties of tofu whey is limited, certain chemical compounds found in tofu whey have demonstrated potential for both enzymatic and non-enzymatic browning inhibition. For enzymatic browning, antioxidants in soy protein isolate are incorporated into active packaging materials to inhibit enzymatic browning in fruits and vegetables (Ghidelli et al., 2014; Shon & Haque, 2007). Furthermore, antioxidants found in tofu whey, such as flavonoids and isoflavone, may inhibit non-enzymatic browning reactions (e.g. Maillard reactions). Flavonoids have been shown to inhibit acrylamide formation by interfering with the Amadori product and preventing Schiff base conversion (Y. Zhang et al., 2015). Isoflavones, known as anti-glycation agents, can inhibit the formation of advanced glycation end products in Maillard reactions (Silvan et al., 2014). However, research demonstrating tofu whey's ability to prevent Maillard reactions and inhibit polyphenol oxidase is required before validating its anti-browning properties.

2.9 Application for tofu whey

Tofu whey is a byproduct with valuable functional and nutritional properties. These properties have been explored in various applications (Table 2.7), where tofu whey is added to different formulations to increase shelf life and improve the textural aspect of food products (Sun et al., 2022; Zhao et al., 2021). Although tofu whey provides similar functionality as aquafaba, tofu whey has a problem due to its beany and grassy taste and smell, which are attributed to specific compounds like 1-hexanol (Chua et al., 2018; Tu, Tang, et al., 2019; Zhao et al., 2021). This sensory issue must be addressed before tofu whey can be widely accepted as a functional ingredient in food applications, such as emulsifiers and foaming agents in food products like bread, cakes, and meringues, where undesirable flavours could deter consumer acceptance.

In terms of nutritional properties, tofu whey contains bioactive compounds, such as isoflavones, polyphenols, GABA, and phytic acid (Chua et al., 2017, 2018; Tu, Tang, et al., 2019), which could provide the functional effect of increasing antioxidant activity to fight stress in the body and therefore provide a healthier body with less radical molecules causing damage to cells. They also provide a good nitrogen source with an adaptable environment as a growth media (Ounis et al., 2008; Wang et al., 2018) while being less expensive than other growth media as it is currently a waste product being thrown away with high biological and chemical oxygen demands, which cost much money to treat (Chua & Liu, 2019; Wang & Serventi, 2019).

Table 2.7: Current application of Tofu whey

Application	Condition of tofu whey	Method for application	Results	Highlight	Reference
Added Functional Ingredient					
Tofu whey added to Wheat flour pan bread	Tofu whey was freeze-dried at -80^{o}C and 1.199Pa, with the protein and fat content being 13.26g/100g and 2.47g/100g.	Ingredient: 188g of wheat flour, 166g of water, 6g of shortening, 12g of sugar, 3g of salt, 2g of nonfat dairy milk, 2g of dry yeast, Tofu whey added 0, 2, 4, 6% of the flour. Cooking: The dough was mixed at 3 minutes low and then at 10 minutes medium. Proofed for 180 minutes at 37^{o}C and 90% RH Cooked at 200^{o}C for 30 minutes and cooled at room temperate	Increase in tofu whey % showed: Increase in bread moisture content Increase in Specific volume Decrease in Baking loss Increase in antioxidant activity and isoflavones, especially aglycone Increase hardness and textural chewiness properties	2% added tofu whey showed the best acceptable, while 6% added tofu whey showed the worst. Increased tofu whey above 2% gave off beany flavors in the bread. However, it also increased the softness of the bread compared to controls, and a lower level of tofu whey was added. The appearance was lowest at 6% tofu whey due to low lightness and nonuniform pores. Bread making process helps the transformation of the glucosidic isoflavones to aglycones.	(Zhao et al., 2021)

Tofu whey added to mashed potatoes	Tofu whey was freeze-dried at 105^{o}C until its stable weight.	Ingredient: 20g of tofu whey or water, 100g of mashed potatoes Cooking: potatoes pieces were boiled for 10 minutes	Tofu whey : Lactobacilli (MRS) 8.12 x 10^{6}, Yeast (YPD) no growth, Mould (YM-11) no growth, Gram-negative (MacConkey) no growth. Mash Potatoes with Water: Lactobacilli (MRS) 2.44 x 10^{6}, Yeast (YPD) 1.48 x 10^{8}, Mould (YM-11) no growth, Gram negative (MacConkey) 5.6 x 10^{7} Mash Potatoes with tofu whey: Lactobacilli (MRS) 1.43 x 10^{8}, Yeast (YPD) 5.24 x 10^{4}, Mould (YM-11) no growth, Gram negative (MacConkey) 2.99 x 10^{5}	The image of the mashed potatoes after 14 days in refrigerated conditions showed the control (water) sample was browning while the tofu whey added sample was still yellow/white, highlighting some anti-browning effect. Tofu whey shows an increased growth for lactobacilli, while there is decreased growth for yeast and gram-negative and no growth for mould in mash potato compared to water. An increase in the promotion of probiotic bacteria like lactobacilli has been shown to inhibit foodborne pathogens contributing to human health.	(Sun et al., 2022)
Base ingredient					

Kombucha-fermentation tofu whey beverage	Tofu whey was autoclaved at 108^{o}C for 15 minutes	Kombucha Inoculum contained *Pichia* and *Dekkera* as the most dominant yeast, while *Acetobacter lactobacillus* was the key bacterium. The kombucha inoculum was made using 10% w/v of sucrose in water at 100^{o}C for 5 minutes. Followed by the addition of 1% black tea, which sat in the liquid for 15 minutes. A starter culture and one piece of cellulose were added and fermented for seven days at 28^{o}C. The kombucha inoculum was added to soy whey at a 1:10 ratio and fermented at 28^{o}C	pH started at 4.89 and dropped to 4.25 on day 1, was stable until day three and dropped on day 4 to 3.28, and remained stable between 3.24 to 3.30 until day 8 (end) The fermentation produced the most acetic acid after eight days at 5.77g/L compared to formic acid at 0.78g/L and citric acid at 1.072g/L Kombucha fermented tofu whey showed D-glucuronic acid formation at 2.33g/L on day 6 Fermentation of tofu whey showed signs of converting total isoflavone glucosides to the aglycone	The most accepted fermentation period of the beverage was at day six, as day 8 showed a pungent and intense sour smell which decreased acceptance. The fermentation removes some unpleasant odours, such as 1-hexanol, known for beany odour, and the addition of pleasant odours, such as 2,4-dimethul-benzaldehyde, which is known for having an orange scent. The fermentation also showed an increase in antioxidant and antimicrobial functionality	(Tu, Tang, et al., 2019)

Alcoholic tofu whey beverage using saccharomyces yeasts (4 strains: Merit, EC1118, R2, 71B)	Tofu whey was adjusted to a pH of 4.0 and a Brix value of 15 using malic acid and sucrose, followed by pasteurization at 60^{o}C for 20 minutes	Tofu whey has been used as a preculture for fermentation at 5% (v/v) of the yeast cultures for three days at 20^{o}C. Fermentation: 300ml of tofu whey was added with 1% (v/v) of the fermented tofu whey for ten days at 20^{o}C	All four yeast were able to metabolize the sugar into ethanol. pH was 3.75 for Merit, 3.85 for EC118, 3.81 for R2 and 3.91 for 71B. Fermentation of tofu whey showed signs of converting total isoflavone glucosides to the aglycone All proteins besides Cysteine and Methionine showed a decrease in protein content while those two increased.	Remove unpleasant odours such as 1-hexanol, known for having a beany odour, and add a new ester to change the aroma. Yeast wine shows that tofu whey can make an alcoholic beverage with naturally free isoflavones to provide health benefits. The method also shows minimal changes and addition to tofu whey for the production.	(Chua et al., 2017)
Media for growth					

Tofu whey is used as a medium for microalgae	Tofu whey was filtered to remove large solid matter. Tofu whey had BG-11 medium added to it, and a control sample without. The pH of Tofu whey was adjusted using 1M of NaOH while 10g/L glucose was added. Tofu whey was then autoclaved at 121^{o}C for 20 minutes and cooled.	Inoculum Culture: *C. pyrenoidosa* FACH-9 was added to BG-11 medium and incubated at 25^{o}C in an orbital shaker at 120 rpm with exposure to 35 µmol·m^-2·s^-1 cool white fluorescent lights under a 16/8 hr light and dark cycle for two weeks. Microalgal culture growth conditions: Autotrophic and Mixtrophic - performed under 25^{o}C at 150 rpm in an orbital shaker with 35 µmol·m^-2·s^-1 light intensity and continuous illuminations Heterotrophic: performed under 25^{o}C at 150 rpm in an orbital shaker with 35 µmol·m^-2·s^-1 light intensity and in a dark environment	The best condition for *C. pyrenoidosa* in tofu whey is to adjust the initial pH to 9, as raw tofu whey pH is too acidic for the algal to grow. The result shows that the highest raw tofu can give growth of 83.5 x 10^6 mL^-1. All the different culture modes (Heterotrophic, Autotrophic, and Mixtrophic) showed that tofu whey produced a high level of biomass concentration with better productivity for lipids and proteins compared to BG-11 medium	Tofu whey shows high efficiency in growing microalgals compared to BG-11, as tofu whey showed 4.76 to 1.97 times high productivity. A great alternative source of nutrients for microalgae as food waste is generally safe and edible, meaning the microalgae grown from it can be used as human and animal while safe.	(Wang et al., 2018)

Tofu whey was used as a medium for *Lactobacillus Plantarum* LB17	Filtration of tofu whey: Cheesecloth and centrifuged at 12000 pm followed by microfiltration with a membrane pore size of 1.2µm. Demineralization of tofu whey: It was done via electrodialysis at 10V on filtrated tofu whey for final conductivity of 0, 25, or 50%. Eletroacidification of tofu whey: Was also done via electrodialysis at 10 A on the demineralized filtrated tofu whey and was taken out at 4.8 pH to 4.4 pH	Culture preparation: Stock is made from 20mL of fresh MRS grown culture with 50mL of 20% skim milk and 50 mL of 20% glycerol solution followed by freezing at -70^{o}C. 1ml of the thawed stock is added to 99ml of sterile MRS and incubated at 30^{o}C for 20 hr Tofu whey broth: The treated tofu whey broth is supplemented with 0.5% (w/v) yeast extract and 2% (w/v) sucrose and sterilized at 110^{o}C for 1 minute holding time. Fermentation: The fermentation is done on the tofu whey broth and MRS, both with 1% *Lactobacillus plantarum* LB17 at 30^{o}C with a pH of 6	There was a linear relationship between ash content and conductivity. Eletroacidification showed that pH reduction could cause a slight decrease in ash content. While Demineralization shows levitated protein soluble at 25% compared to that of 0 and 50% MRS showed better growth for *Lactobacillus plantarum* LB17 at all conductivity points of tofu whey and that there is not much different growth value between the different conductivity points	Although MRS is better for growth than tofu whey by four times, tofu whey is cheaper and could be used as an alternative solution. Demineralization and electronification did not affect the growth of *Lactobacillus plantarum* LB17. The soluble protein and salts can be recovered from tofu whey using demineralization and can be used in tofu production to increase yield	(Ounis et al., 2008)

Chapter 3. Proximate composition and characteristics of tofu whey liquid

3.1 Abstract

Tofu whey is a liquid by-product from tofu making, it is also considered a waste product as well with high BOD and COD, which requires much money to treat this wastewater. A better alternative is to upcycle and reuse tofu whey as a functional food ingredient as it contains a high content of valuable nutrients. The study was done to better understand the chemical and physical components of tofu whey and its functionality. The chemical characteristic of TWL was 97.34 ± 0.23% moisture, 0.38 ± 0.05% Crude protein, 0.10 ± 0.06% total fat, 0.43 ± 0.05 total ash and 1.76 ± 0.23 carbohydrate, with the primary sugar being sucrose and proteins mainly being made up of broken subunits and side chains of glycinin and conglycinin. TWL also display a high amount of antioxidant activity and saponin in wastewater, which is most likely why it has anti-browning ability. The functionality of TWL was shown to have high foamability around pH 2.5-5.5 and even emulsified 20% oil. However, it displayed poor stability at high oil ranges but decent stability at low oil ranges of 5%.

3.2 Introduction

Tofu whey is a liquid removed as a by-product in the final step of the tofu manufacturing process after the tofu is pressed. When producing 1 kg of tofu, approximately 1.5 to 10 litres of tofu whey is made, which has a biological oxygen demand (BOD) of 4.1-6.8 grams per litre and a chemical oxygen demand (COD) of 7.5-12 grams per litre (Hongyang et al., 2011; Wang & Serventi, 2019). This indicates that tofu whey has relatively high BOD and COD, which have environmental impacts if disposed of without proper treatment. It was also estimated by Barber et al. (2018) that it costs USD 130 per cubic meter of treated effluent, roughly $0.195- 1.3 USD per kg of tofu produced. Additionally, it is known that approximately 65 tons of tofu is made daily throughout East Asia (Shurtleff & Aoyagi, 2000). This means that in East Asia alone, treating wastewater daily costs $12,675 – USD 84,500. Because the cost of treating this wastewater is high and a certain amount of nutrients is present due to the removal of tofu whey with excessive force during tofu manufacture

(Chua & Liu, 2019), it would be better to upcycle and reuse it like aquafaba and make it into functional food ingredients through condensation, filtration and/or dehydration processes. Aquafaba is wastewater produced from chickpeas and has shown promising success in its use as an emulsifying and foaming agent in cakes and mayonnaise (Buhl et al., 2019; He, Purdy, et al., 2021; Mustafa & Reaney, 2020).

Upcycling tofu whey is not new. The literature shows its nutritional value, and it has been used as a coagulant, prebiotic, peptide producer, citric acid producer, and fungi cultivator. (Chua & Liu, 2019). Recently, as research on its use as a functional food material has increased, it has begun to attract more attention in the food application business. However, due to the lack of research in this area, there is a lack of basic information about the properties and functionality of tofu whey as a food ingredient, so more research is necessary to obtain in-depth scientific knowledge about its chemical composition and techno-functional properties. This will also lead to a way to reduce wastewater from the tofu industry that contributes and provides value to the tofu whey, thereby increasing the food industry's sustainability.

The objectives of this study were to determine the quantitative chemical composition of tofu whey by approximate analysis, antioxidant activity, antimicrobial activity, saponin content, and total phenolic content and also to conduct additional qualitative analyses such as SDS-PAGE to identify proteins and GC and ICP-MS for sugar and trace mineral profiles, respectively. In addition, the functionality of tofu whey was studied by examining its anti-browning, foaming and emulsifying properties. This study aims to provide insight into the characteristics and functionality of tofu whey as a functional food ingredient.

3.3 Materials and Methods

3.3.1 Materials

Fresh tofu whey liquid (TWL) was obtained from Tonzu (Auckland, New Zealand) that was produce when making firm tofu with nigari salt. After being obtained it was carefully decanted and filtered through a 200-micron sieve to remove broken tofu curds and sediments, and then it was immediately frozen until use. Frozen TWL was thawed at 20°C the night before use. All chemicals, including hydrochloric acid, sodium hydroxide, ethanol

(95%), methanol (99%), 2 N Folin-Ciocalteu reagent, gallic acid, sodium bicarbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2' -azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, sulfuric acid 96%, vanillin, soyasaponin Bb, diethyl ether, petroleum ether, citric acid, ascorbic acid, sodium bisulphite were purchased from Sigma-Aldrich (St. Louis, Missouri, USA) or Thermofisher (Waltham, Massachusetts, USA). All the chemicals used were of analytical grade. The water used in experiments was reverse osmosis water. Apples and pears were purchased at a local supermarket for enzymatic browning experiments.

3.3.2 Proximate analysis of TWL

The proximate analysis of TWL samples was performed at the Nutrition Lab, Massey University in Palmerston North, for its proximate composition. Before conducting the proximate analysis, the TWL samples were freeze-dried to remove their water content. The freeze-dried powders were then used for the proximate analysis. The methods used were standard analytical techniques used in food analysis. Moisture content was analysed using the air oven method (AOAC 925.10, 930.15), ash content by dry ashing at 550°C (AOAC 942.05), crude protein using the Dumas method (AOAC 968.06) with nitrogen to a protein conversion factor of 6.25, crude fat via the Mojonnier method (AOAC 922.06), and total dietary fibre (TDF), soluble dietary fibre (SDF), and insoluble dietary fibre (IDF) using the Megazymes kit (AOAC 991.43). Total carbohydrate content was calculated by subtracting the sum of protein, fat, moisture, ash and TDF from 100%. Additionally, minerals were analysed using Inductively coupled plasma-mass spectrometer (ICP-MS), and the sugar profile was determined via gas chromatography (GC).

3.3.3 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was conducted under reducing and non-reducing conditions to characterise the protein profile of TWL according to the method (Laemmli, 1970) With some modifications. For reducing condition, 20 µL of TWL was mixed with 6 µL of 4x Laemmli sample buffer and 0.8 µL of β-mercaptoethanol (reducing agent) at room temperature. The sample was then boiled for 10 minutes. In the non-reducing condition, the reducing agent, β-

mercaptoethanol, was replaced with Milli-Q water and other components were kept the same. A 10 µL of each sample was then loaded onto a Mini-Protean TGX Precast gel (Bio-Rad, USA) consisting of a 4% stacking gel and a 15% resolving gel. The electrophoresis was conducted at a constant 150 V using a PowerPac Basic (Bio-Rad, USA) in a running buffer prepared by diluting 10x Tris/Glycine/SDS solution. After electrophoresis, the gel was gently removed and stained with Coomassie Brilliant Blue R-250 solution to visualise the protein bands. After staining, the stained gel was stained with a destaining solution containing 10% isopropanol/glacial acetic acid under gentle shaking. The destaining solution was changed every 3 hours 3-4 times until the gel had a transparent background. Precision Plus ProteinTM Standards with a molecular range of 10-250 kDa were used as a protein molecular weight marker as a reference for identifying protein bands to determine their *approximate* molecular weights. Gels were imaged using a ChemiDoc XRS imaging system (Bio-Rad, USA).

3.3.4 Microbiological analysis of TWL

The growth of yeast and mould, growth of Lactic acid bacteria (LAB), growth of total coliforms, growth of faecal coliforms, and the growth of aerobic bacteria were applied to the retrieved tofu whey liquid samples. Ten-fold serial dilutions were used for the enumeration and propagation of microorganisms. The growth of yeast and mould was performed on Yeast Extract Glucose Chloramphenicol (YGC) agar (Merck, Germany). The growth of LAB was performed on de Man, Rogosa and Sharpe (MRS) agar (Oxoid, UK). The examination of total coliforms and faecal coliforms was applied using Violet Red Bile Glucose Agar (VRBGA) (Oxoid, UK) and Violet Red Bile Agar (VRBA) (Oxoid, UK), respectively. The aerobic bacteria were enumerated using Standard Plate Count (SPC) agar (Oxoid, UK). The incubation conditions of YGC, MRS, VRBGA, VRBA and SPC were summarised in Table 3.1.

Table 3.1: Incubation conditions of YGC, MRS, VRBGA, VRBA and SPC agar.

Type of agar	Incubation temperature (°C)	Incubation time (h)	Incubation environment
YGC	25	120 ± 2	Aerobic
MRS	37	48 ± 2	Anaerobic
VRBGA	35	48 ± 2	Aerobic
VRBA	35	48 ± 2	Aerobic
SPC	30	72 ± 2	Aerobic

3.3.5 Tofu whey extract preparation

Tofu whey extract was prepared based on a method described by Zhao et al. (2020) With slight modifications. Briefly, 10 mL of TWL was mixed with 10 mL of methanol, and the mixture was shaken on a standing shaker set at 50 rpm and at room temperature for 1 hour. After shaking, the mixture was centrifuged at 3000 × g at 4oC for 15 minutes. The supernatant was removed from the centrifuged mixture and used to determine the total phenolic content and antioxidant activity.

3.3.6 Total phenolic content of TWL

Total phenolic content was determined using the Folin-Ciocalteu assay method, as described by Huang et al. (2018). To prepare samples, 0.1 mL of tofu whey extract was added to a test tube, followed by 2.5 mL of 0.2 N Folin-Ciocalteu reagent, and within 7 minutes, 2.0 mL of 7.5% sodium carbonate solution was added to the test tube. The sample was mixed well and incubated for 2 hours at room temperature and in the dark. After incubation, the absorbance of samples was measured at 760 nm using a spectrophotometer (Shimadzu UV-1700 PharmaSpec UV-Vis Spectrophotometer) against a blank prepared by making a mixture with water instead of the sample. A calibration curve was created using gallic acid as a standard, and the results were expressed in µg/mL of gallic acid equivalent (GAE).

3.3.7 Antioxidant activities of TWL

Antioxidant activities of TWL were measured using the two standard methods, such as DPPH radical scavenging and ABTS radical scavenging assays, by comparing the ability of TWL to scavenge the DPPH free radicals and neutralise the ABTS radical cations to that of Trolox as a standard antioxidant compound.

3.3.7.1 DPPH assay

DPPH(2,2-diphenyl-1-picrylhydrazyl) assay was conducted to determine antioxidant activity in tofu whey using the method described by Fei et al. (2017) with slight modifications. A 0.15 mmol/L methanolic solution of DPPH. The solution was prepared using 99% methanol and then placed in the dark to react for 2 hours. Then, 0.1 mL of tofu whey extract was mixed with 3.9 mL of 0.15 mmol/L of DPPH solution. The sample-DPPH mixture was incubated at room temperature to react for 30 minutes in the dark. After the incubation, the absorbance of samples was measured at 517 nm using a spectrophotometer (Shimadzu UV-1700 PharmaSpec UV-Vis Spectrophotometer). A control sample was prepared using distilled water instead of the tofu whey extract, and methanol was used as a blank to correct for any background absorbance. A calibration curve was created using Trolox as a standard antioxidant compound, and the results were expressed as Trolox equivalent antioxidant capacity (TEAC) in units of µmol TE/100 mL sample.

3.3.7.2 ABTS assay

ABTS assay was also performed to determine the antioxidant activity of tofu whey. This method was used as described by Benedetti et al. (2015) with slight modifications. 7 mM of ABTS (2,2'-casino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) solution was mixed with 2.45 mM of potassium persulfate to generate ABTS radical cations. The mixture was then allowed to react in the dark for at least 16 hours before use. The working solution of ABTS was prepared by diluting with ethanol to an absorbance of 0.70 ± 0.03 at 734 nm before mixing with tofu whey extract. A 30 µL of tofu whey extract was mixed with 3 mL of the prepared ABTS working solution and incubated in the dark for reaction for 6 minutes. After the incubation, the absorbance of samples was measured at 734 nm using a spectrophotometer against ethanol as a blank. A calibration curve was created using Trolox as a standard antioxidant compound, and the results were expressed as Trolox equivalent antioxidant capacity (TEAC) in units of µmol TE/100 mL sample.

3.3.8 Analysis of saponin content in TWL

The total saponin content of tofu whey was measured using the vanillin-sulphuric acid assay as described by V. Le et al. (2018) With slight modifications. TWL was first diluted with

water and centrifuged at *3000 ×* g at 4℃ for 15 minutes to obtain tofu whey supernatant. Tofu whey foam was prepared by homogenising the tofu whey supernatant at 8000 rpm for 1 minute using a Silverson mixer (*Silverson, L4T, England*) and was transferred to a measuring cylinder where it was left to stand. After standing for 1 hour, 10 ml of foam at the top was collected with a spoon and mixed with 1 mL of distilled water. A 0.25 mL of the tofu whey/ foam supernatant was mixed with 0.25 mL of 8% (w/v) of vanillin in ethanol and 2.50 mL of 72% (v/v) sulphuric acid in water. The mixture was then transferred to a 60°C shaking water bath and left there for 15 minutes, followed by 5 minutes of cooling in an ice bath. The absorbance of the sample was measured at 560 nm using a spectrophotometer. A blank was prepared by making it with water instead of the sample. A calibration curve was created using soya-saponin I (95% purity) as a standard. Total saponin content was expressed as mg/ml.

3.3.9 Anti-browning effect of TWL on fruit juices

Fresh apples and pears sourced from a local supermarket served as specimens in evaluating the efficacy of TWL as a browning inhibitor in fresh fruit juices. The study's findings were juxtaposed with those of other agents. In this trial, the fruits underwent peeling, coring, and slicing into chunks. These prepared fruit chunks were then juiced using an Alter slow whole juicer model number BCAT (Biochef, BCWA400, Australia) with a coarse filter. The resulting liquid fruit juice was combined with five different agents (water, tofu whey, 0.2% ascorbic acid, 0.2% citric acid, and 0.2% sodium bisulfate) at varying ratios of agent to fruit juice (1:3, 1:1, and 3:1) (see Table 3.2). These agent-fruit juice mixtures were left at room temperature for 2 hours, after which the colour of the mixtures was assessed. This experiment aimed to ascertain whether tofu whey effectively inhibited or minimised browning in fresh fruit juice samples compared to specific chemical agents (e.g., ascorbic acid and sodium bisulfate) acknowledged for their anti-browning properties.

Table 3.2: The ratios of reagent to fruit juice sample

Samples	Ratios		
Reagent: Fruit Juice	**1:3**	**1:1**	**3:1**
Water: Fruit Juice	2.5mL:7.5mL	5mL:5mL	7.5mL:2.5mL
Tofu whey: Fruit Juice	2.5mL:7.5mL	5mL:5mL	7.5mL:2.5mL
0.2% Ascorbic acid: Fruit Juice	2.5mL:7.5mL	5mL:5mL	7.5mL:2.5mL
0.2% Citric acid: Fruit Juice	2.5mL:7.5mL	5mL:5mL	7.5mL:2.5mL
0.2% Sodium Bisulphite: Fruit Juice	2.5mL:7.5mL	5mL:5mL	7.5mL:2.5mL

The apple and pear juice samples mixed with different reagents were measured for their L*, a*, b*, C*, and h* colours via transmittance using a Konica Minolta Spectrophotometer CM-5. Centrifugation was done to remove extra pulp in the samples to make them as transparent as possible. Before measurement, the samples were inverted twice after the 2-hour incubation and then centrifuged at 3500 x g at 20^{o}C for 15 minutes. The browning index was calculated using Equations 1 and 2 (Ruangchakpet & Sajjaanantakul, 2007).

$$Browning\ index = \frac{[100\ (x-0.31)]}{0.17} \qquad \text{(Equation 3.1)}$$

$$x = \frac{(a^{*}+1.75L^{*})}{(5.645L^{*}+a^{*}-0.3012b^{*})} \qquad \text{(Equation 3.2)}$$

3.3.10 Foamability and foam stability of TWL

The foaming properties of TWL at different pH levels were investigated. The pH of the original TWL was 5.72 ± 0.13 and adjusted to different pHs (2.5, 4, 5.5, 7 and 9) using HCl and NaOH. The Waring blender method, as suggested by Li et al. (2022) It was used with slight modifications to produce foams. A 200 ml of TWL at each pH level was whipped using a Silverson stand mixer at 8000 rpm for 1 minute. Afterwards, the content was transferred into a 500 ml measuring cylinder, where foam properties such as capacity, stability, and drainage were observed over a period of 3 hours at regular intervals: 0, 10, 20, 30 minutes, 1 hour, 2 hours and 3 hours after foaming. These parameters were measured to characterise the volume of foam generated, the rate of foam collapse, and the volume of liquid drained from the foam over time. The foam capacity, stability and drainage were calculated using Equations 3-5 (Henao Ossa et al., 2020; Lomakina & Mikova, 2006; Zeng et al., 2013).

$$Foam\ capacity\ (\%) = \frac{(FL_1\ (mL) - V_1(mL))}{V_1(mL)} \times 100 \qquad \text{(Equation 3.3)}$$

$$Foam\ stability\ (\%) = \frac{(FL_2\ (mL) - LL_2(mL))}{FL_1(mL)} \times 100 \qquad \text{(Equation 3.4)}$$

$$Foam\ drainage\ (\%) = 100 - (\frac{V_1(mL) - LL_2(mL))}{V_1(mL) - LL_1(mL)} \times 100) \qquad \text{(Equation 3.5)}$$

Where V_1 is the initial volume of tofu whey used (mL), FL_1 is the initial height of foam produced (white colour) in a measuring cylinder at time 0 (mL), FL_2 is the height of foam produced (white colour) in a measuring cylinder (mL), LL_1 is the initial height of liquid in the measuring cylinder at time 0 (mL), and LL_2 is the height of liquid in measuring cylinder (mL).

3.3.11 Preparation of emulsions using TWL

Emulsions were prepared using TWL as an emulsifier solution at 5, 10, and 20% oil concentrations. This was to investigate the ability of TWL as an emulsifier to form and stabilise emulsions at different oil concentrations. In this experiment, Sodium Azide was initially added to the TWL solution to make a 0.02% (w/w) Sodium Azide solution as a preservative to inhibit microbial growth, thus preserving the emulsions during storage. Table 3.3 shows the formulation of emulsions in terms of the amounts of oil and TWL used.

Table 3.3: The oil concentration of the TWL emulsions

Oil concentration (%)	TWL (g)	Oil (g)	Total (g)
5	285	15	300
10	270	30	300
20	240	60	300

Canola Oil was added to TWL at ambient temperature (20-25°C). The mixture of oil and TWL was pre-homogenized using a Silverson mixer (Silverson, L4T, England) at 6000 rpm for 2 minutes to form a coarse emulsion. The coarse emulsion was then homogenised four times using a two-stage high-pressure homogeniser (SPX Flow Technology, APV-2000, Australia) at 500 bar for the first stage and 50 bar for the second stage. Immediately after preparation, the tofu whey emulsions were used for analysis and stored at 4°C to determine the stability of emulsions over seven days.

3.3.12 Analysis of emulsions

3.3.12.1 Creaming

10ml of the emulsions was stored in a flat bottom tube (10ml).

The creaming index (%) was measured over days 0, 1, 4, and 7 during storage and calculated using Equation 6. This was to determine the emulsion's stability over time against creaming or phase separation.

$$Creaming\ index\ (\%) = (\frac{H_S}{H_T} \times 100\%) \qquad \text{(Equation 3.6)}$$

Where H_s is the height of the serum phase (cm) and H_T is the total height of liquid in the test tube (cm).

3.3.12.2 Particle size and size distribution

The particle size and distribution of tofu whey emulsions containing different oil concentrations were measured using a Mastersizer 3000 (Malvern Instrument Inc., Westborough, UK), a static light scattering technique. The refractive indices of 1.465 and 1.33 were used for the emulsions' dispersion phase (canola oil) and continuous phase (tofu whey). The analyses were conducted on freshly made tofu whey emulsions and those stored at 4°C over 7 days to determine if any changes in particle size or distribution were made to indicate their stability.

3.3.13 Statistical analysis

All experiments and measurements were performed in triplicate, and the data obtained were expressed as means ± standard deviations (SD). Minitab Software (version 21.1.0.0, Pennsylvania, USA) was used for data analysis through one-way analysis of variance (ANOVA), and Fisher's Least Significance Difference (LSD) test was used to identify differences between the results.

3.4 Results and Discussion

3.4.1 Chemical composition of TWL

Proximate analysis of TWL was conducted to quantitatively determine its chemical composition to evaluate the value of tofu whey, thereby valorising it. The results are shown in Table 3.4. The content of protein, fat, carbohydrate, dietary fibre, ash, and moisture was 0.38 ± 0.05, 0.10 ± 0.06, 1.76 ± 0.23, 0.10 ± ND, 0.43 ± 0.05 and 97.34 ± 0.23 g/100 g, respectively. This was in agreement with other studies shown in Table 3.4 in the literature review section (Benedetti et al., 2016; Li et al., 2017; Ossa et al., 2020; Tu, Azi, et al., 2019; Tu, Tang, et al., 2019; Zhang et al., 2012) although the fat content of 0.1g/100 g was lower. Differences can also arise from variations in the ingredients (e.g. soybean variety, coagulating salt type and concentration, etc.) and methods (e.g. heat treatment temperature and time, pressure applied to remove tofu whey, etc.) used in tofu making as well as the treatment (e.g. filtration, centrifugation, etc.) of fresh tofu whey after collection and before analysis.

Table 3.4: The chemical composition of TWL was analysed using proximate analysis, with minerals contained via ICP-MS and sugar contained via GC with standard deviation (n= 6).

Component	
Moisture (g/100g)	97.34 ± 0.23
Total Solids (g/100g)	2.66 ± 0.23
Crude protein (g/100g)	0.38 ± 0.05
Fat (g/100g)	0.10 ± 0.06
Total Carbohydrate (g/100g)	1.76 ± 0.23
Total Sugar (g/100g)	0.55 ± 0.06
Glucose (g/100g)	<0.1 ± ND
Fructose (g/100g)	<0.1 ± ND
Sucrose (g/100g)	0.48 ± 0.13
Galactose (g/100g)	<0.1 ± ND
Total Dietary Fibre (g/100g)	0.10 ± ND
Insoluble Dietary Fibre (g/100g)	ND
Soluble Dietary Fibre (g/100g)	0.10 ± ND
Ash (g/100g)	0.43 ± 0.05
Calcium (mg/100g)	3.25 ± 3.20
Magnesium (g/100g)	0.04 ± 0.02
Potassium (g/100g)	0.16 ± 0.02
Sodium (g/100g)	0.03 ± 0.02
Phosphorus (g/100g)	0.01 ± 0.01
Sulphur (mg/100g)	0.01 ± 0.01
Iron (mg/100g)	0.10 ± 0.01
Copper (mg/100g)	0.08 ± 0.05
Manganese (mg/100g)	0.01 ± 0.01
Zinc (mg/100g)	0.02 ± 0.01
Chloride (g/100g)	0.12 ± 0.05

Additionally, when tofu whey was compared to other wastewater like aquafaba (Fuentes Choya et al., 2023), its protein and carbohydrate contents were four times lower than those of aquafaba. This means that tofu whey may not be useful as a functional ingredient unless the fresh tofu whey liquid is concentrated to increase the concentration of protein and carbohydrates, thus effectively being used in food applications.

As for the results of analysing simple sugars in tofu whey, sucrose was the only sugar contained in large quantities at 0.48 ± 0.13 g/100 g. This value was consistent with data from other studies where sucrose content was found between 0.16g/100g and 1.13g/100 (Champagne et al., 2003; Fei et al., 2017; Sriniworn et al., 2015). The data for dietary fibre showed that tofu whey contained only a small amount of soluble dietary fibre of 0.10 ± ND g

/100 g, while insoluble dietary fibre was not detected in the test. Both types of dietary fibre have different benefits for humans. Soluble dietary fibre improves digestion and helps lower blood sugar levels, while insoluble dietary fibre promotes the movement of substances through the digestive system and increases stool volume (Dhingra et al., 2012).

The analysis of minerals in tofu whey showed that potassium (P), chloride (Cl), magnesium (Mg) and sodium (Na) were found to contain the highest amounts of minerals being as 0.16 ± 0.02, 0.12 ± 0.05, 0.04 ± 0.02 and 0.03 ± 0.02 g/100 g, respectively, while other minerals such as iron (Fe), copper (Cu) and zinc (Zn) were found to be present in lower amounts. According to a study of tofu whey by Yang et al. (2016), the magnesium, calcium and chloride contents of tofu whey were similar (0.042g/100g of magnesium, 0.048g/100g of calcium and 0.06g/100g of chloride). Differences could be due to various factors, including the type of coagulant used to make the tofu. In the study by Yang et al. (2016), tofu was made using two coagulants, gypsum and bittern salt, comprised of calcium sulfate and magnesium chloride, respectively. On the other hand, the tofu whey samples collected in this study used a different coagulant called 'Nigari'. It is made from seawater and brine (salt water) and contains a large amount of magnesium chloride, sodium chloride and potassium chloride, which is why the analysed calcium was significantly lower at 0.00325g/100g compared to 0.048g/100g of calcium in Yang et al. (2016). These minerals are crucial for human health as they not only have functional roles, acting as catalysts in enzyme systems and participating in oxidation-reduction reactions but also help the function and movement of muscles and cells in the body (Council, 1989)Therefore, the minerals contained in tofu whey can provide new value to tofu whey as wastewater. Further, future research for quantitative analysis of tofu whey would be to investigate the vitamins contained in tofu whey, as they also provide health benefits, but not much research has been done on them.

3.4.2 Protein profile of TWL by SDS-PAGE

The protein profile of TWL from two different batches (T1 and T2) was characterised by both reducing and non-reducing SDS-PAGE (Figure 3.1). The results show the TWL protein profile consisting of 7S (β-conglycinin subunit α and α') approximately around 83-57 kDa, 7S (γ-conglycinin) approximately around 64 kDa, 7S (β-conglycinin subunit β and β') approximately around 53-42 kDa, 11S (glycinin acid polypeptide chain) approximately

around 40-32 kDa, 11S (glycinin essential polypeptide chain) approximately around 25-17 kDa, and other low molecular weight proteins below 16 kDa (Kao et al., 2003; Stanojević et al., 2023). Most of the low molecular weight proteins in TWL could be a 2S albumin storage protein (18-21kD), as suggested by Ebisawa et al. (2013); Lin et al. (2006)). The predominant ones were the β-conglycinin subunit β and β', the glycinin acid polypeptide chain, the essential polypeptide chain, and the low molecular weight protein of 15 kDa. Kao et al. (2003) suggested that the high amount of low molecular weight proteins in tofu whey was due to the coagulant used in manufacturing. During tofu production, it appears that tofu whey contains fewer low molecular-weight proteins when more networks are formed in the tofu, and lower molecular-weight proteins are present when there are fewer networks. This could also mean that different types of tofu production would have different effects on the tofu whey as firm tofu, which requires force to push out water from the tofu, could disrupt networks, therefore leaking out low molecular weight proteins, while soft tofu, which does not push out might have more low molecular weight proteins as the network is not disrupted. This might have a negligible effect on the functionality of tofu whey, which opens the way for future research on how different types of tofu could affect its properties and functionality.

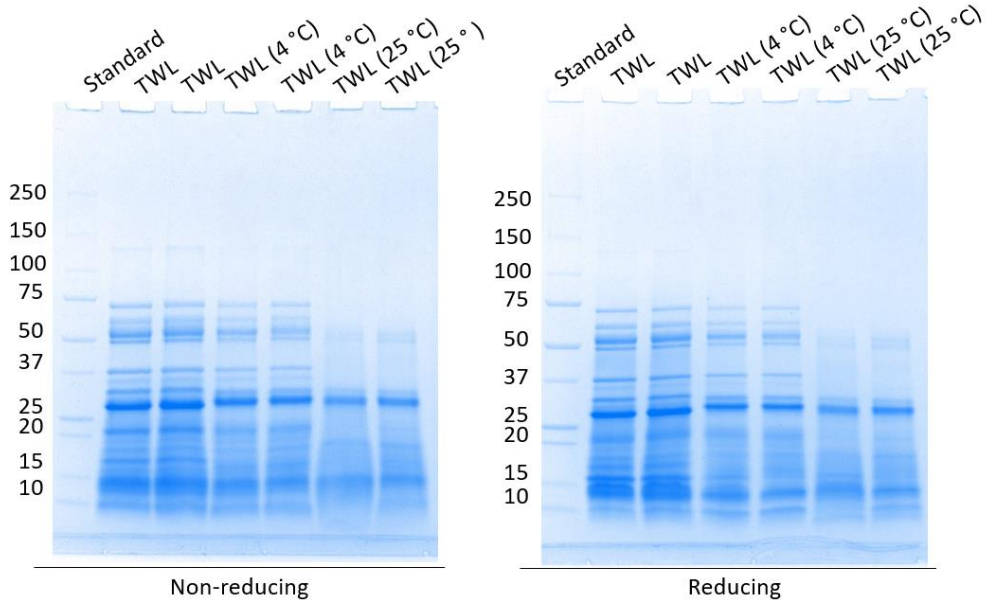


Figure 3.1: SDS-PAGE profile of TWL under non-reducing and reducing conditions.

The SDS-PAGE analysis of the protein profile of tofu whey also suggests that storage at different temperatures can lead to protein degradation. Tofu whey stored at 4^{o}C displayed all protein bands as indicated in previous studies by Kao et al. (2003) Stanojević et al. (2023), while tofu whey stored at 25^{o}C had lower amounts of β-conglycinin subunit β and β', glycinin acid polypeptide chain, glycinin essential polypeptide chain and the low molecular weight protein, and the bands corresponding to the higher molecular weight proteins such as β-conglycinin subunit α and α' and γ-conglycinin disappeared. Changes in the protein profiles between the two temperatures may be due to microbial growth. As lactic acid microbial growth occurs, bacteria produce proteolytic enzymes, which cause protein degradation. A study by Ren & Li (2022) indicates that lactic acid bacteria (LAB) predominantly initiate the breakdown of α and α' subunits, likely due to their abundance of hydrophilic areas. This implies that tofu whey might be a favourable medium for LAB proliferation. However, further analysis of microbial growth in tofu whey can help gain additional insight and explain the proteolytic enzymatic breakdown of protein in TWL stored at 25^{o}C.

3.4.3 Phenolic content and antioxidant activity of TWL

A quantitative antioxidant activity, phenolic, and saponin content analysis was conducted on TWL. The results are presented in Table 3.5. The DPPH antioxidant activity in TWL was 121.41 ± 14.52 µmol TE/100 mL, which exhibited twice the activity compared to the 66.11 ± 1.75 µmol TE/100 mL reported by Zhao et al. (2020). Similarly, the ABTS antioxidant activity in TWL was 145.96 ± 7.1 8 µmol TE/100 mL, also displaying twice the activity compared to the 75 ± 4.00 µmol TE/100 mL reported by Benedetti et al. (2015)The difference in antioxidant activity could be due to several factors (Ling et al., 2022), including the soybean cultivar, cooking temperature or heat, which can degrade antioxidants, and storage conditions that may impact antioxidant levels.

Additionally, the results from Fei et al. (2017) Support a similar hypothesis regarding the antioxidant system in tofu whey. Their study suggests that tofu whey possesses a predominantly hydrophobic antioxidant system, as evidenced by the comparable ABTS and DPPH activities. This observation is because of the nature of the ABTS and DPPH assays, where the former is known to assess both hydrophobic and hydrophilic antioxidant systems,

while the latter is more inclined toward evaluating hydrophobic systems. (Floegel et al., 2011)Consequently, a greater disparity between ATBS and DPPH activities indicates a higher proportion of hydrophilic antioxidants, whereas a smaller difference suggests a prevalence of hydrophobic antioxidants.

Table 3.5: Antioxidant activities, total phenolic and total saponin contents in TWL with standard deviation (n= 6).

	Total Average	Wet basis	Dry basis
Antioxidant	**(µmol TE/100 mL)**	**(µmol TE/g)**	**(µmol TE/g)**
DPPH	121.41	1.20	44.92
	±14.52	±0.14	±5.37
ABTS	145.96	1.44	52.56
	±7.18	±0.07	±2.59
Phenols	**(mg/mL)**	**(mg/g)**	**(mg/g)**
Total phenolic content	0.24	0.24	8.81
	±0.07	±0.07	±2.43
Saponins	**(mg/mL)**	**(mg/g)**	**(mg/g)**
Total saponin content	0.67	0.66	23.95
	±0.05	±0.05	±1.74
Total saponin content in the foam	0.21	0.21	3.98
	±0.06	±0.05	±1.19

The total phenolic content in TWL was measured to be 24.00 ± 7.00 mg/100 mL, which was approximately three times lower than the 60.87 ± 0.09 mg/100 mL reported by Zhao et al. (2020) In contrast, the total saponin content in TWL was 0.24 ± 0.02 g/100 g, similar to the 0.2 g/100g reported by W. Yang et al. (2016) The difference in phenolic content could be due to differences in the amount of regent used, as Zhao utilised only 800 µL of 0.2N Folin-Ciocalteu reagent compared to 2.5 mL, which is three times as much.

The total saponin content in TWL is relatively high at 0.67 ± 0.05 mg/ml compared to other literature values for TWL wastewater, which typically contains around 0.2 g/100g (W. Yang et al., 2016), but was more in line when compared to soybeans, which typically contain between 0.62 to 1.86 g/100 g of saponins depending on the cultivar (Berhow et al., 2020; Lee et al., 2011). Furthermore, saponins offer various health benefits, such as anti-cancer activity in the body, and the lower amounts found in tofu whey may not lead to anti-nutrient effects, as stated in the literature review section 2.8.4.

Further studies can be done on quantitative analysis of total phenolic and saponin content to gain deeper insights into what chemical compounds drive antioxidant activity and determine whether the saponins in tofu whey are the same ones responsible for providing health benefits.

3.4.4 Microbial growth of TWL

Microbial growth on TWL was studied to understand the microorganisms that can grow in TWL and their effects on the pH of TWL. The microbial growth of TWL is shown in Figure 3.2. On collection (Day 0), yeast and mould (A), coliforms, and E. coli were absent, while LAB (D) (7.4×10^3) and SPC bacteria (C) (9.6×10^3) were present in the tofu whey. These findings agree with studies conducted by Sun et al. (2022), which similarly reported the absence of yeast and mould in tofu whey. However, they observed a *Lactobacilli* cell count of 8.12×10^6, twofold compared to our study's 7.4 x 103 count. Another study reported by Dai et al. (2023) on soy whey showed that naturally fermented soy whey at 25^{o}C contained Lactobacillus as the dominant LAB and Firmicutes as the predominant SPC bacteria. This supports our observation of a high count of LAB and SPC bacteria after two days of storage.

Furthermore, our findings agree with the theory proposed by Ponomarova et al. (2017) that yeast can grow symbiotically with LAB. Figure 3.2 shows that yeast and mould can proliferate alongside LAB over time at 20^{o}C. However, once the nutrient source is depleted, both microbial populations begin declining. Conversely, at 4^{o}C, yeast and mould exhibit more robust growth compared to LAB, which appears to survive without significant proliferation. The growth curve of yeast and mould is as expected since it requires an incubation period before observable cell count increases, given that the yeast and mould spore need time to colonise before active cell growth occurs. (Tournas et al., 2001)The results align with changes in pH (B) over time, as LAB produces lactic acid, which decreases pH. In contrast, yeast and moulds can metabolise natural acids, increasing pH (Demain & Martens, 2017; Singhvi et al., 2018). Both pH changes coincide with the exponential growth phase of each respective microorganism, suggesting that these shifts in pH may be attributed to their metabolic activities during active growth phases.

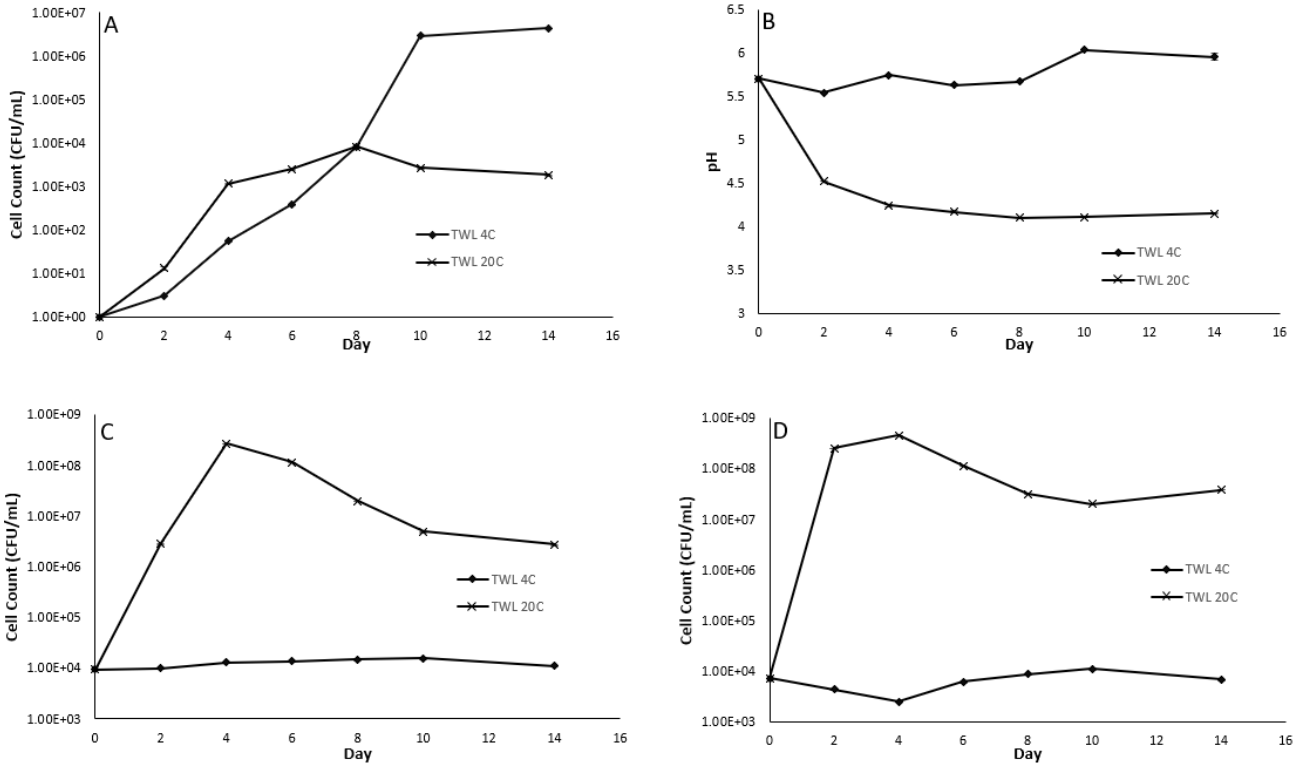


Figure 3.2: Microbial growth of yeast and mould (A), pH change (B), standard plate count (SPC) (C) and lactic acid bacteria (LAB) (D) on TWL stored at 20^{o}C and 4^{o}C.

Although the data shows potential LAB, yeast, and mould growth in tofu whey, the generalizability of these findings is limited due to the study's focus solely on microbes naturally present in tofu whey. Consequently, there remains a lack of insight into the growth patterns of specific microbes, necessitating future study in this area. Nevertheless, the observed growth of these microbes is a good sign of the use of tofu whey as a medium for the proliferation of LAB, yeast, and mould. This implies that wastewater streams like tofu whey possess significant potential as media for the microbial production of bioactive natural compounds, even without undergoing concentration to enhance sugar and protein content. The new business of extracting bioactive compounds from microbial sources is proving to be highly profitable (Demain & Martens, 2017; Pham et al., 2019), and the potential utilisation of wastewater such as tofu whey as a growth medium could further enhance the revenue generation within this sector.

3.4.5 Anti-browning effect of TWL

The anti-browning effect of TWL was analysed because studies done by Sun et al. (2022) TWL infused into mashed potatoes showed insignificantly less browning than mashed potatoes without TWL after 14 days. However, the paper did not study the effect of anti-browning in TWL; further research in this area was required. The colours of apple and pear juice samples were analysed at different ratios to investigate the potential effect of tofu whey as an anti-browning agent. The results of the colour measurement are expressed in L*, a*, and b* values. L* value represents lightness and darkness. The positive values for a* represent redness and yellowness, while the negative values for a*and b* represent greenness and blueness, respectively. Tables 3.6 and 3.7 show the results of apple juice and pear juice, respectively (Figure 33 in the appendix shows photos of the samples).

The results for both apple and pear samples show that 0.2% ascorbic acid and sodium bisulphite were significantly more effective than other reagents in inhibiting enzymatic browning visually ($p < 0.05$). However, concerning the anti-browning effect of tofu whey, apple samples at three different ratios exhibited lower a* values and at the 1:1 and 1:3 ratios for b*colour values, which was a significant difference compared to water and 0.2% citric acid ($p <0.05$). Meanwhile, a significant difference was observed only at the 1:3 ratio in the pear sample ($p<0.05$).

The results for 0.2% ascorbic acid and sodium bisulphite were in line with expectations, as both are recognised as effective anti-browning agents. Ascorbic acid reduces the oxidising substrate, which is thus an indirect inhibition of polyphenol oxidase (PPO) activity (Moon et al., 2020), an effect previously demonstrated at 1% concentration by Rojas‐Graü et al. (2008). Sodium bisulphite, on the other hand, inhibits PPO by lowering the pH (effective at 0.5 g L^{-1}) (Lu et al., 2007) and acts as a reducing agent. It reacts with intermediate ortho-quinones, converting them back to their colourless form, thus stopping melanin formation and reducing browning (Embs & Markakis, 1965; Sapers, 1993). In contrast, the 0.2% citric acid results suggest that it is not an optimal anti-browning agent. This contradicts the claim made by Suttirak and Manurakchinakorn (2010) and Tortoe et al. (2007) that suggest citric acid is an excellent anti-browning agent, as 1% and 0.5mM of citric acid showed decent results in reducing browning in cut fruit and vegetable. However, based on the findings of a

similar study (Tsouvaltzis & Brecht, 2017), the discrepancy may stem from the inadequate use of citric acid, as > 0.5% citric acid was required to inhibit browning effectively. Since 0.2% citric acid was used in this study, it is plausible that this concentration was not enough to chelate all ions to stop polyphenol oxidase activity, as studies by Altunkaya and Gökmen (2008) agree with the result, as 0.05% citric acid was not as effective in reducing PPO activity in lettuce at pH 4-9. Similarly, the study reported by Sun et al. (2022) showed that tofu whey infused into mashed potatoes exhibited anti-browning effects, which was different from our results, suggesting a similar theory for tofu whey. It is conceivable that a higher concentration of tofu whey might be required to stop browning in apple and pear juice effectively. These results build on existing evidence that different mechanisms to inhibit PPO in enzymatic browning reactions have different limits and that reducing agents such as ascorbic acid and sodium bisulphite inhibit PPO better compared to citric acid which is primarily recognised as a chelator in anti-browning applications (Ali et al., 2015; Suttirak & Manurakchinakorn, 2010). Although the mechanisms underlying tofu whey's anti-browning effects are still not clearly understood, based on results from the data, tofu whey could have a similar mechanism to citric acid as an anti-browning agent, as tofu whey is known for having phytic acid, which is known to be a potent chelator as mentioned in the literature reviews section 2.8.2. However, beyond simple visual analysis, comprehensive studies are needed to confirm the potential for PPO inhibition of tofu whey.

Table 3.6: The colour parameter for different reagents at three different ratios of apple juice after 2 hours

APPLE JUICE	L*(D65)	A*(D65)	B*(D65)	C*(D65)	H(D65)
CONTROL 100% JUICE	73.76	7.64	28.95	29.94	75.22
	±1.46	±0.43	±0.50	±0.55	±0.70
H_2O: JUICE 3:1	91.83	1.19	17.84	17.88	86.19
	$\pm0.72^{B}$	$\pm0.17^{A}$	$\pm1.10^{A}$	$\pm1.11^{A}$	$\pm0.31^{B}$
TOFU WHEY: JUICE 3:1	88.05	-0.07	18.37	18.38	90.3
	$\pm2.91^{C}$	$\pm0.56^{B}$	$\pm1.68^{A}$	$\pm1.68^{A}$	$\pm1.72^{A}$
ASCORBIC ACID: JUICE 3:1	96.12	0.31	4.57	4.58	86.17
	$\pm0.48^{A}$	$\pm0.04^{B}$	$\pm0.51^{C}$	$\pm0.51^{C}$	$\pm0.61^{B}$
CITRIC ACID: JUICE 3:1	92.88	1.1	13.31	13.36	85.26
	$\pm0.39^{B}$	$\pm0.03^{A}$	$\pm1.19^{B}$	$\pm1.19^{B}$	$\pm0.36^{B}$
SODIUM BISULPHITE: JUICE 3:1	96.15	0.31	4.14	4.15	85.82
	$\pm0.66^{A}$	$\pm0.06^{B}$	$\pm0.33^{C}$	$\pm0.34^{C}$	$\pm0.63^{B}$
H_2O: JUICE 1:1	82.99	4.87	28.68	29.1	80.41
	$\pm2.47^{B}$	$\pm0.96^{A}$	$\pm1.21^{A}$	$\pm1.35^{A}$	$\pm1.49^{C}$
TOFU WHEY: JUICE 1:1	84.9	1.65	19.58	19.69	85.65
	$\pm4.95^{B}$	$\pm1.50^{B}$	$\pm2.90^{B}$	$\pm3.01^{B}$	$\pm3.69^{A}$
ASCORBIC ACID: JUICE 1:1	92.89	0.72	8.24	8.27	84.98
	$\pm0.70^{A}$	$\pm0.10^{B}$	$\pm0.88^{C}$	$\pm0.89^{C}$	$\pm0.16^{AB}$
CITRIC ACID: JUICE 1:1	85.74	3.83	26.01	26.29	81.65
	$\pm1.55^{B}$	$\pm0.59^{A}$	$\pm0.96^{A}$	$\pm1.03^{A}$	$\pm0.97^{BC}$
SODIUM BISULPHITE: JUICE 1:1	94.21	0.57	6.35	6.38	84.97
	$\pm2.04^{A}$	$\pm0.25^{B}$	$\pm2.19^{C}$	$\pm2.20^{C}$	$\pm0.50^{AB}$
H_2O: JUICE 1:3	81.89	5.4	29.1	29.6	79.57
	$\pm2.51^{B}$	$\pm1.16^{A}$	$\pm1.40^{A}$	$\pm1.59^{A}$	$\pm1.73^{B}$
TOFU WHEY: JUICE 1:3	78.12	3.87	22.08	22.44	80.12
	$\pm5.39^{B}$	$\pm1.29^{B}$	$\pm1.10^{B}$	$\pm1.16^{B}$	$\pm3.19^{B}$
ASCORBIC ACID: JUICE 1:3	88.41	1.5	12.2	12.29	83.03
	$\pm1.11^{A}$	$\pm0.25^{C}$	$\pm0.85^{C}$	$\pm0.87^{C}$	$\pm0.70^{B}$
CITRIC ACID: JUICE 1:3	80.59	6.18	30.49	31.11	78.55
	$\pm1.56^{B}$	$\pm0.41^{A}$	$\pm0.17^{A}$	$\pm0.17^{A}$	$\pm0.74^{A}$
SODIUM BISULPHITE: JUICE 1:3	90.45	1.11	9.84	9.9	83.91
	$\pm0.51^{A}$	$\pm0.58^{C}$	$\pm3.10^{D}$	$\pm3.14^{D}$	$\pm1.49^{A}$

$^{a-d}$ The same letter within the column indicates no significant differences between samples (n=6) ($p < 0.05$).

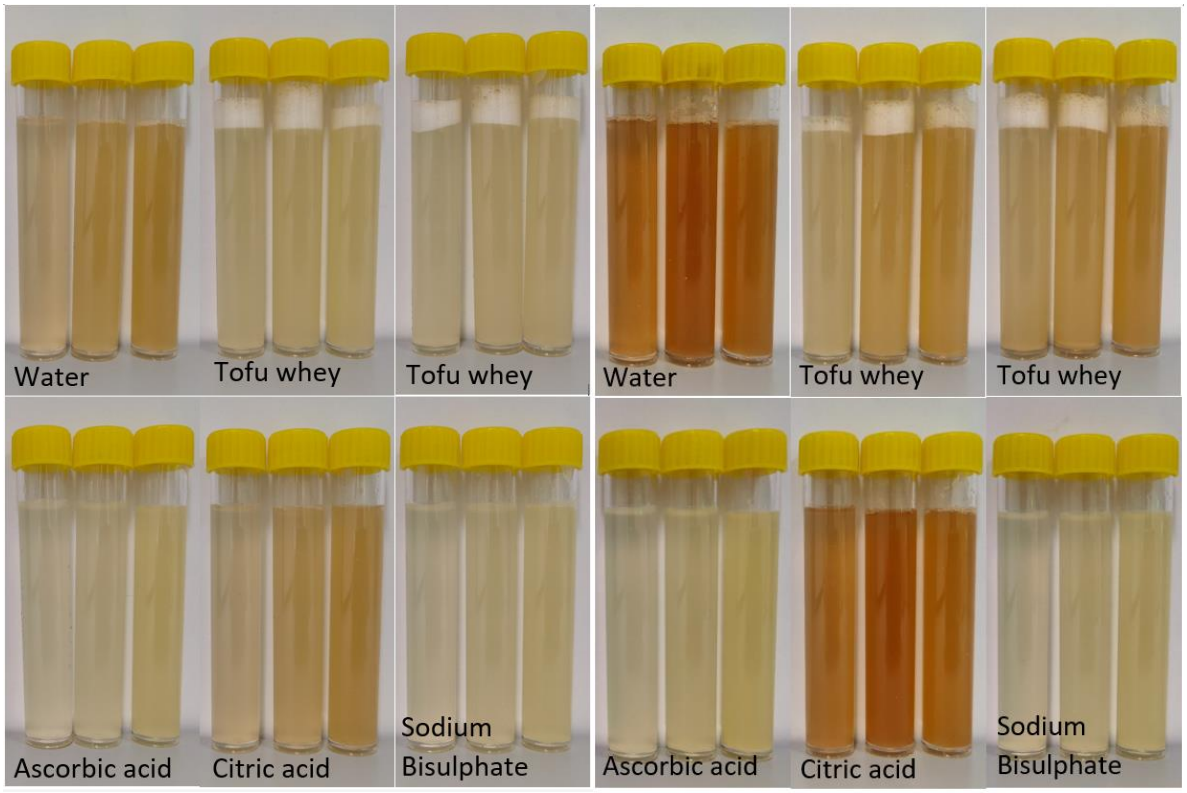


Figure 3.3 Picture of apple juice sample (left to right 3:1, 1:1 and 1:3), begin and 2 hours after

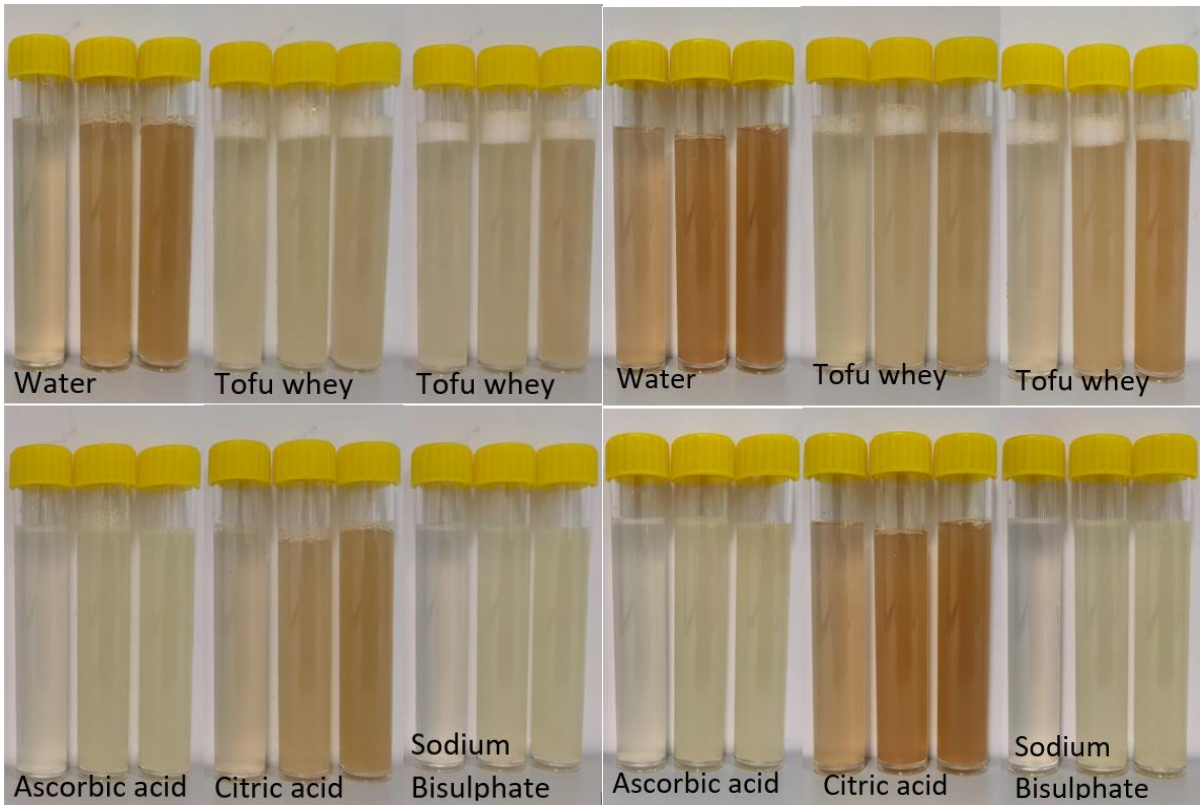


Figure 3.4 Picture of pear juice sample (left to right 3:1, 1:1 and 1:3), begin and 2 hours after.

Table 3.7: The colour parameter for different reagents at three different ratios of pear juice after 2 hours

PEAR JUICE	L*(D65)	A*(D65)	B*(D65)	C*(D65)	H(D65)
CONTROL 100% JUICE	76.71 ±3.07	5.79 ±0.72	24.72 ±0.21	25.4 ±0.30	76.55 ±1.88
H_2O: JUICE 3:1	93.1 ±0.72^{B}	0.86 ±0.15^{A}	9.84 ±0.31^{B}	9.88 ±0.32^{B}	86.05 ±0.44^{B}
TOFU WHEY: JUICE 3:1	93.1 ±0.69^{B}	-1.46 ±0.59^{C}	13.5 ±1.96^{A}	13.58 ±2.01^{A}	93.04 ±4.54^{A}
ASCORBIC ACID: JUICE 3:1	95.42 ±0.36^{A}	0.22 ±0.05^{B}	5.12 ±0.68^{C}	5.13 ±0.68^{C}	86.68 ±0.21^{B}
CITRIC ACID: JUICE 3:1	92.64 ±1.69^{B}	0.86 ±0.27^{A}	9.29 ±1.18^{B}	9.33 ±1.20^{B}	85.18 ±0.41^{B}
SODIUM BISULPHITE: JUICE 3:1	95.88 ±1.07^{A}	0.22 ±0.08^{B}	3.98 ±0.65^{C}	3.99 ±0.66^{C}	86.31 ±1.11^{B}
H_2O: JUICE 1:1	85.99 ±3.34^{B}	2.75 ±1.03^{A}	18.17 ±2.74^{A}	18.38 ±2.87^{A}	81.56 ±1.92^{B}
TOFU WHEY: JUICE 1:1	88.03 ±1.38^{B}	1.06 ±0.81^{B}	16.67 ±0.80^{AB}	16.72 ±0.82^{AB}	85.32 ±3.54^{A}
ASCORBIC ACID: JUICE 1:1	91.54 ±1.42^{A}	0.63 ±0.14^{B}	8.74 ±1.25^{C}	8.76 ±1.26^{C}	85.50 ±0.74^{A}
CITRIC ACID: JUICE 1:1	88.53 ±1.90^{B}	1.92 ±0.47^{A}	14.83 ±1.61^{B}	14.96 ±1.65^{B}	82.19 ±1.59^{AB}
SODIUM BISULPHITE: JUICE 1:1	93.02 ±1.35^{A}	0.48 ±0.15^{B}	7.00 ±1.06^{D}	7.02 ±1.07^{C}	85.60 ±1.24^{A}
H_2O: JUICE 1:3	83.77 ±2.18^{B}	3.45 ±0.69^{A}	20.54 ±2.12^{A}	20.83 ±2.19^{A}	80.23 ±1.49^{B}
TOFU WHEY: JUICE 1:3	82.35 ±2.14^{B}	3.54 ±0.65^{A}	20.44 ±1.14^{A}	20.74 ±1.23^{A}	79.28 ±2.29^{B}
ASCORBIC ACID: JUICE 1:3	87.97 ±2.28^{A}	1.10 ±0.34^{B}	11.68 ±1.59^{B}	11.73 ±1.62^{B}	84.00 ±1.76^{A}
CITRIC ACID: JUICE 1:3	81.82 ±3.18^{B}	3.80 ±0.80^{A}	21.01 ±1.01^{A}	21.35 ±1.13^{A}	79.56 ±1.90^{B}
SODIUM BISULPHITE: JUICE 1:3	89.67 ±1.66^{A}	0.82 ±0.23^{B}	9.71 ±1.26^{C}	9.75 ±1.28^{B}	84.11 ±1.68^{A}

$^{a-d}$ The same letter within the column indicates no significant differences between samples (n=6) ($p < 0.05$).

Figure 3.5 shows the browning index, calculated from the L*, a*, and b* values from Tables 3.6 and 3.7, aiming to understand better the browning occurring in the different samples. Figures 3.3 and 3.4 show a visual understanding of the change in browning over time with

various reagents. A more accurate understanding of the browning phenomenon was identified through the browning index. It appears that ascorbic acid and sodium bisulphite were noticeably more effective, as the 1:3 ratio for these two reagents had a lower browning index than the 3:1 ratio for citric acid, tofu whey and water. This indicates that ascorbic acid and sodium bisulphite at 0.2% concentration exhibited superiorly in reducing enzymatic browning compared to the other reagents.

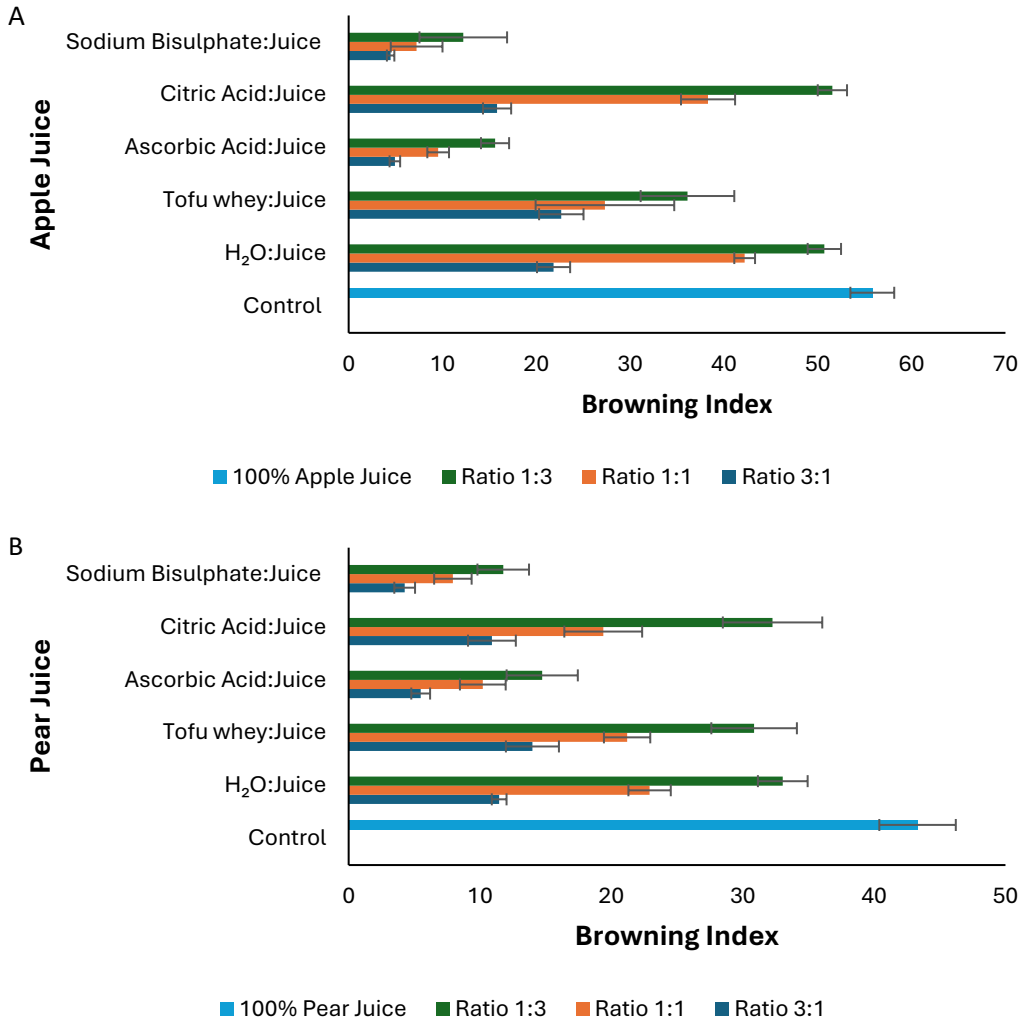


Figure 3.5: The browning index value for apple juice (A) and pear juice (B) at 3 different ratios, 1:3, 1:1 and 3:1 of reagent: juice with standard deviation (n=6) ($p < 0.05$).

Additionally, an interesting trend was observed in tofu whey for both juice samples. At lower juice ratios (3:1), tofu whey appeared less effective in inhibiting the browning reaction than citric acid and water. However, at higher juice ratios (1:1 and 1:3), tofu whey demonstrated superior efficacy in inhibiting the browning reaction, resulting in a reduced browning index compared to the other two reagents. However, the data suggest this.

Figures 3.3, 3.4, 6.1 and 6.2 in the appendix reveal that tofu appeared less brown than water and citric acid in both juice samples. This finding complies with research done by Sommano et al. (2011), which suggests that polyphenol oxidase activity may not correlate with the browning index but exhibits a stronger correlation with the* value and hue angle. This correlation implies that tofu whey might be a better anti-browning agent than what is visually observed. However, due to the lack of data on PPO activity in these samples, the current results cannot confirm the effect of tofu whey on PPO activity. Nonetheless, based on the present findings, it can be stated that tofu whey visually reduces the browning colour observed in apple and pear juice, resulting from enzymatic reactions that are not as strong as ascorbic acid and sodium bisulphite. Therefore, tofu whey can potentially be a browning inhibitor in fruit juices. However, three key avenues for future research are required to enhance the understanding of tofu whey's anti-browning agent. Firstly, investigation into tofu whey's capacity to reduce PPO activity, and secondly, exploration of the mechanisms or substances in tofu whey which causes a reduction in PPO activity. The final avenue for research is to conduct research on the sensory properties of tofu whey when incorporated into fruit juices, as it known to have a beany flavour which may negatively affect the overall sensory property. This could limit its viability as an anti-browning agent in the juice industry.

3.4.6 Effect of pH on foamability of TWL

The effect of pH on the foamability of TWL was investigated to determine its behaviour as a foaming agent at different pH levels. The original pH of TWL was around 5.8, which was adjusted to pH 2.5, 4, 5.5, 7 and 9. The results in Figure 3.6 show that foam capacity was significantly affected by pH ($p < 0.05$) as pHs 2.5, 4, and 5.5 had greater foaming capacity (85.75 ± 1.77%, 90.25 ± 5.30%, 88.75 ±7.38%) than pHs 7 and 9 (63.75 ± 5.30% and 62.5± 5.30%). The results also indicated that between the two groups of pHs 2.5, 4, 5.5 and 7, 9, there was no significant difference between foam capacity and each other. The reason for better foam capacity at low pHs is due to reduced particle charge, which reduces repulsion among proteins. Meanwhile, at higher pHs, the particle charges are higher, which increases repulsion and causes a reduction in foam capacity. (Henao Ossa et al., 2020). These results had similar trends to studies done by Henao Ossa et al. (2020), on tofu whey concentrates where pH 4 showed the best foam capacity as it is closest to the isoelectric point (pH 4.5) of soy protein, and as pHs increase or decrease, the foam capacity started to decrease as the

particle charges began to grow, resulting in increased repulsion among proteins. Furthermore, it seems like the main components for foaming in TWL are a combination of 7s (β-Conglycinin) and soy protein selective hydrolysate, which are both found in TWL based on Figure 3.1 and the fact that hydrolysate is soy protein, which is hydrolysis and can be done under heating condition, during tofu manufacturing. Studies done by Wang et al. (2021) Show similar foam capacity trend for 7s and soy protein selective hydrolysate, which lack electrostatic charges at pH 4, which is a similar theory that is presented by Henao Ossa et al. (2020).

For attributes such as foam stability and drainage, the varying pH level of TWL is a significant factor over time. The pH 4 foam was significantly more stable and had less liquid drainage at the beginning of the measurement compared with the other pHs ($p < 0.05$). On the other hand, the pH 9 sample had significantly lower stability and more liquid drainage at the start ($p < 0.05$). However, as time increased near the end, only pH 9 foam had significantly lower stability and more liquid drainage, while pH 4 was no longer much better than pH 2.5 and 5.5 in foam stability and fluid drainage. The results for foamability (Foam capacity, stability and liquid drainage) align with a study by Dachmann et al. (2020) and Zeng et al. (2013) on plant protein and soy protein hydrolysate, as at the isoelectric point, it seems that the foam has a lower surface tension, quicker adsorption, and better viscoelastic properties. This culminates in enhancing network formation at the air/water interface, resulting in better foam stability.

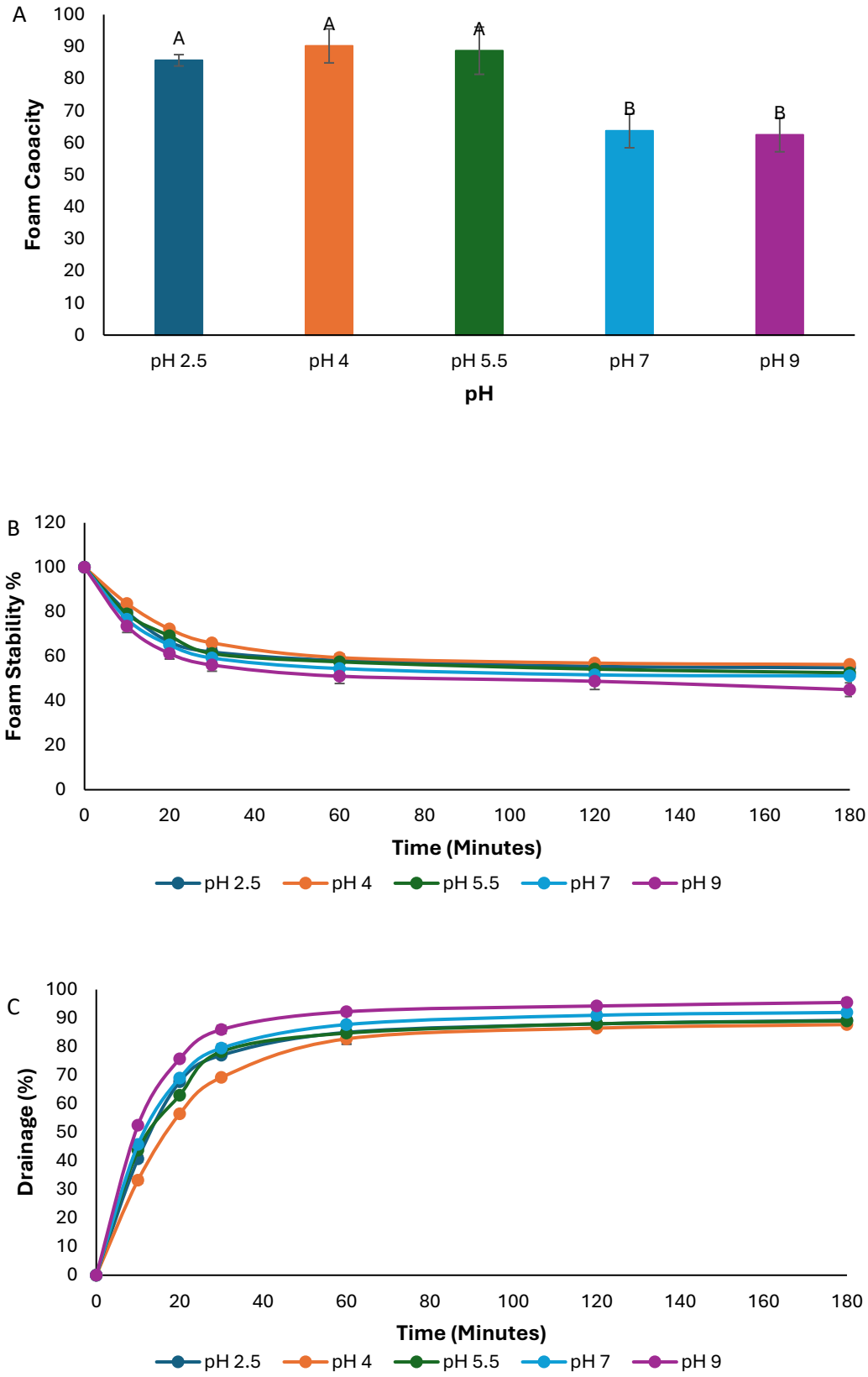


Figure 3.6 Effect of pH on foam capacity (A), foam stability (B) and liquid drainage (C) in TWL (the capital letter on the graph is a significant difference between pH for foaming capacity as determined by Fisher's test) (n=6) ($p < 0.05$).

Additionally, a study by Zhu et al. (2020) showed that soy saponins and 7S or 11S proteins can interact via weak bonds, which changes the protein conformational state, leading to synergistic dynamic surface properties that reduce surface tension. The elasticity of the air/water surface improves, resulting in enhanced foamability. Because tofu whey contains both components, the interactions between soy saponins and 7S or 11S soy proteins can also occur in tofu whey. This suggests that tofu whey with moderate saponin content is a suitable foaming agent. The results in Table 3.5 indicate that there was 0.21 ± 0.06 mg/mL of saponin in the tofu whey foam. Furthermore, comparing tofu whey with other plant and egg proteins (Ding et al., 2022; Ivanova et al., 2018), it seems that tofu whey at lower pH exhibits the same foaming capacity and stability, while at the isoelectric point, the foamability was better. However, to better understand the functionality of tofu whey as a foaming agent, further studies should be done to determine the interaction between saponins, 7s and hydrolysates to see their interaction and how their interaction affects foaming properties in TWL.

3.4.7 Particle Size and particle distribution of TWL emulsion at different oil concentrations

The particle size and particle distribution of emulsion oil droplets are essential parameters for understanding the properties and stability of an emulsion. Various factors, including the type and concentration of emulsifier and the concentration of oil, can significantly influence these parameters. In this study, TWL was investigated to determine its potential use as an emulsifier solution. The emulsion samples were prepared at three different oil concentrations (5, 10, and 20%) using a two-stage high-pressure homogeniser.

The results of the mean particle size of emulsions analysed after preparation (0 days), expressed as D (3,2) and D (4,3), are shown in Table 3.8. During storage for 7 days at 4^{o}C, the change in particle size of the emulsions was also measured. The results are shown in Figure 3.5.

Table 3.8: The particle size D(3,2) and D(4,3) of TWL emulsion at different concentration on day 0

Oil concentration	D(3,2) (µm)	D(4,3) (µm)
5%	2.89±0.13^{C}	6.00±0.96^{B}
10%	3.31±0.15^{B}	6.25±1.01^{B}
20%	4.21±0.19^{A}	8.36±1.76^{A}

$^{a-c}$ The same letter within the column indicates no significant differences between samples (n= 6) ($p < 0.05$).

The particle size changes are measured with D(4,3), which measures the bulk or coarse particles in the emulsion, while D(3,2) measures the fine particles in the emulsions. The particle size of TWL emulsion with different oil concentrations shows that as oil increases, so does the particle size for D(3,2) (B) significantly at all oil concentration levels, while for D(4,3) (A), the particle size changes are only significant when 20% oil concentration is added and there not much difference when comparing 5% and 10% oil concentration as seen in Table 3.8. The particle size of TWL emulsion compared to Tween 80 and sodium caseinate emulsion was significantly larger. The studies were done by Calligaris et al. (2016) on 3% w/w tween 80 and 15% w/w oil emulsion at 50 MPa and 5 MPa using a two-stage high-pressure homogenisation showed a D(3,2) value of 0.29 µm and studies by Perrechil and Cunha (2010) On 6% w/w sodium caseinate and 30% w/w oil emulsion at 50 MPa using a two-stage high-pressure homogenisation showed a D(3,2) value of 0.41 µm. However, the particle size of the TWL emulsion was much more comparable to that of the whey protein emulsion, but the TWL emulsions were still relatively more extensive than those of the whey protein emulsions. A study by Kuhn and Cunha (2012) On 3% w/w whey protein and 30% w/w oil emulsion at 20 MPa and 5 MPa using a two-stage high-pressure homogenisation passed through 4 times, showed a D(4,3) value of 1.14 µm. The reason for this could be due to the fact the TWL has only 0.38g/100g of protein, which means that in the emulsion, there is only about 0.3% w/w of TWL protein, which is why large particle sizes are displayed compared to these alternative emulsifiers.

As seen in Figure 3.7, the D(4,3) (A) particle size results only indicate a significant difference (change) during storage of the emulsions at 10% oil concentrations on day 7, and similarly for the D(3,2) (B) particle size for 20% oil concentration on day 7.

The particle size distribution of emulsions with different oil concentrations, measured at day 0 (A) and day 7 (B), is shown in Figure 3.8. The results also show no significant change in the particle size distribution between day 0 and day 7 at a given oil concentration. This indicates that during storage up to 7 days, all emulsion samples containing 5, 10 and 20% oil concentrations remained stable without noticeable change in oil-containing emulsions ($P < 0.05$). Interestingly, on day 1, the distribution, however, showed that the particle size distribution of emulsions was measured to be multimodal in all cases, with a prominent peak between 1-10 µm, a small peak between 0.2-1 µm and a minor peak in the large particle size region (>30 µm).

The results showing an increase in droplet size as oil concentration increased were as expected since increasing oil while keeping protein the same would decrease the oil-to-protein ratio, assuming the protein acts as an emulsifier. In other words, as protein proportions decrease compared to the oil proportions, a larger droplet size is generated to stabilise the emulsion. (Linke et al., 2020). Further, when comparing tofu whey emulsions to soy protein isolate emulsions from a study carried out by (Zhang et al., 2023), on soy protein isolate emulsion (5, 10, 15, 20 and 25 mg/mL SPI) using microfluidic device at 140 mbar for continuous phase and 80 mbar at dispersed phase seems to have a high droplet size even with a high protein-to-oil ratio. This could be because tofu whey emulsions are stabilised by proteins and saponins present in tofu whey, as determined in this study. A study reported by Zhu, Xu, et al. (2020) Suggest that using soyasaponins with 11S protein seems to produce emulsions with smaller droplet size and more extended stability because it is speculated that when 11S protein interacts with soya-saponin, the 11S protein structure gets partially denatured, which allows for more protein adsorption at the interface, resulting in lower interfacial tensions which significantly improve emulsion stability and reduce droplet size. Although the mechanism for how tofu whey proteins or saponins are adsorbed on the surface of oil droplets to form emulsion oil droplets is unclear, this study demonstrates that the proteins and saponins in tofu whey have excellent emulsifying ability and can be used in emulsion applications as functional food ingredients.

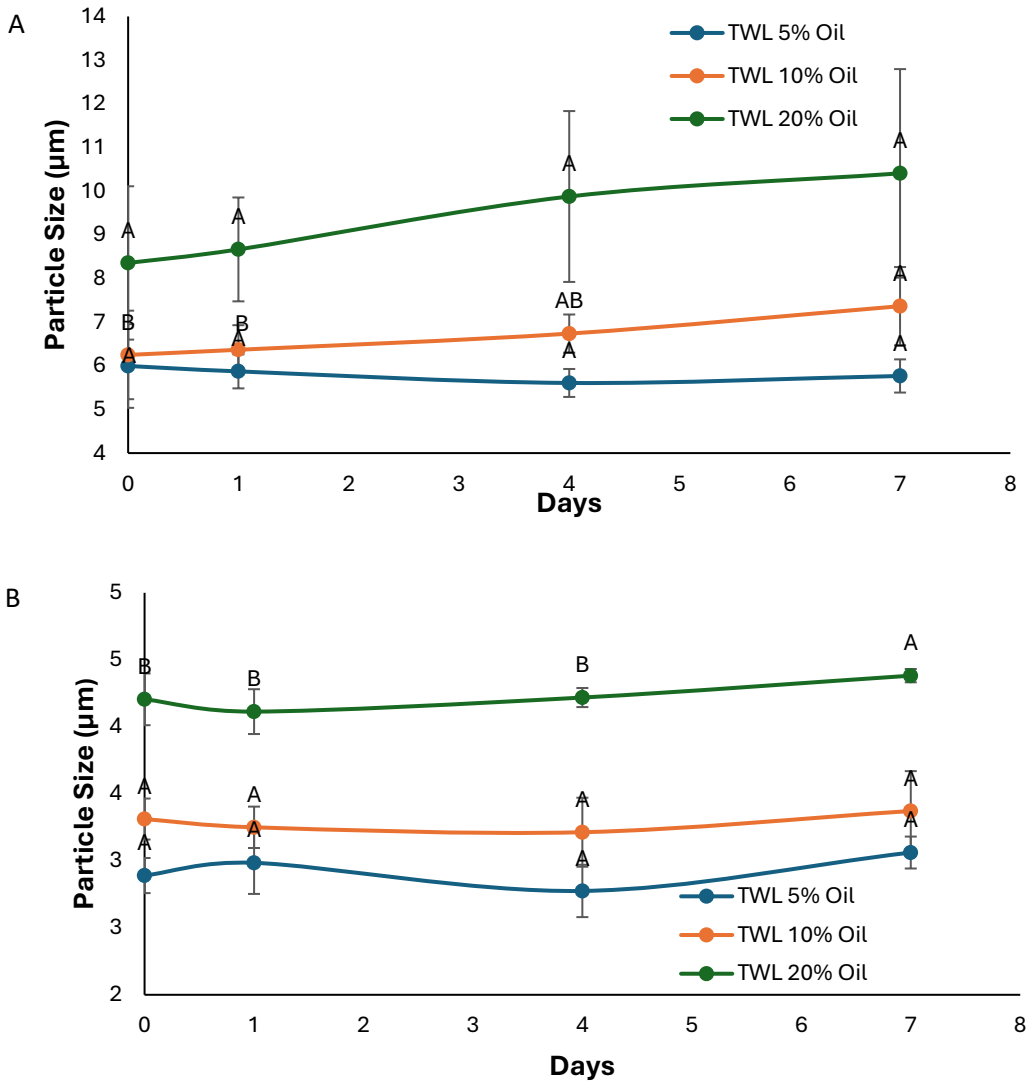


Figure 3.7 The change in particle size D(4,3) (A) and D(3,2) (B) of TWL emulsions at different oil concentrations (5, 10 and 20%) measured during storage for 7 days at 4°C (The capital letter symbol on the graph displays the significant difference between sample during storage as determined by the Fisher's test) with standard deviation (n=6) ($p < 0.05$).

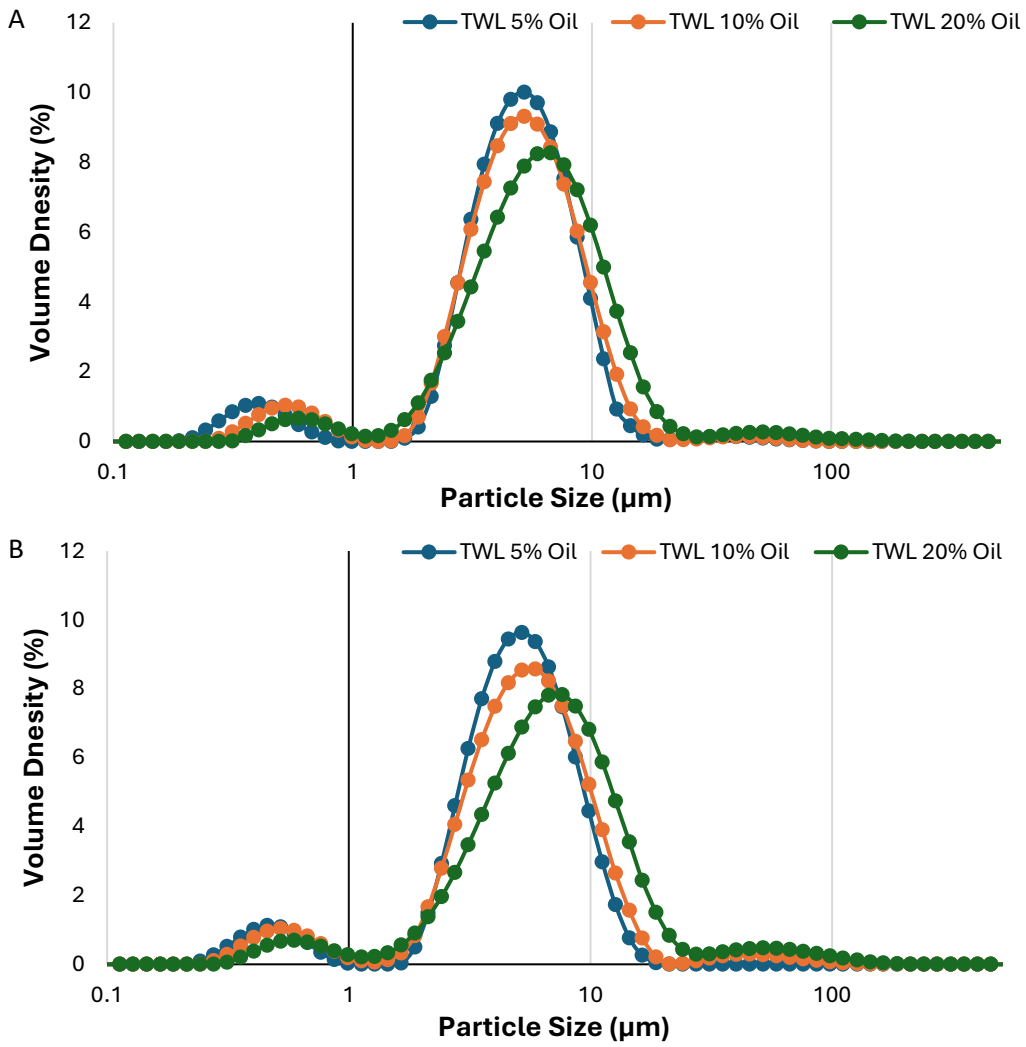


Figure 3.8: Change in particle distribution of TWL emulsions at different oil concentrations from day 0 (A) to day 7 (B) storage at 4^{o}C.

3.4.8 Stability of TWL emulsions at different oil concentrations

The creaming index is used to define the stability of an emulsion, where an emulsion with a lower creaming index generally exhibits good stability (Hong et al., 2018). As described above, in addition to analysing particle size changes during emulsion storage, determining the creaming index of tofu and why emulsions can also help identify whether TWL is a good emulsifier. The results in Figure 3.9 show that emulsion samples containing lower oil concentrations, i.e. 5% oil concentration, have significantly better stability over time compared to 10% and 20% oil-containing emulsions ($P < 0.05$), as on day 1, it seems that there is no significant between the different oil concentration.

Regarding creaming over storage, 5% oil concentration emulsion showed no significant changes to creaming over 7 days of storage, while 10% and 20% oil-containing emulsions showed significant changes in the cream layer on day 7 and day 4, respectively. The data was expected since it was in line with droplet size distribution, as seen in Figures 3.5 and 3.6, as the droplet size of the emulsion increases over time or as more oil is added to the emulsion. The stability of the emulsion decreased, and this caused more creaming, as shown by a rise in the creaming index as oil concentration increased. This is due to Stokes' law, which relates to the three main factors" particle size, the difference in density between the oil and water phases, and the viscosity of the continuous phase" that control the creaming rate (Robins & Hibberd, 1998). This implies that tofu whey should be used as an emulsifier solution at low oil concentrations to have smaller oil droplets and good emulsion stability and that stability is relatively low at high oil levels. However, increasing tofu whey concentration might be able to retard creaming at higher oil concentrations and should be investigated for future tofu whey application as a functional food ingredient.

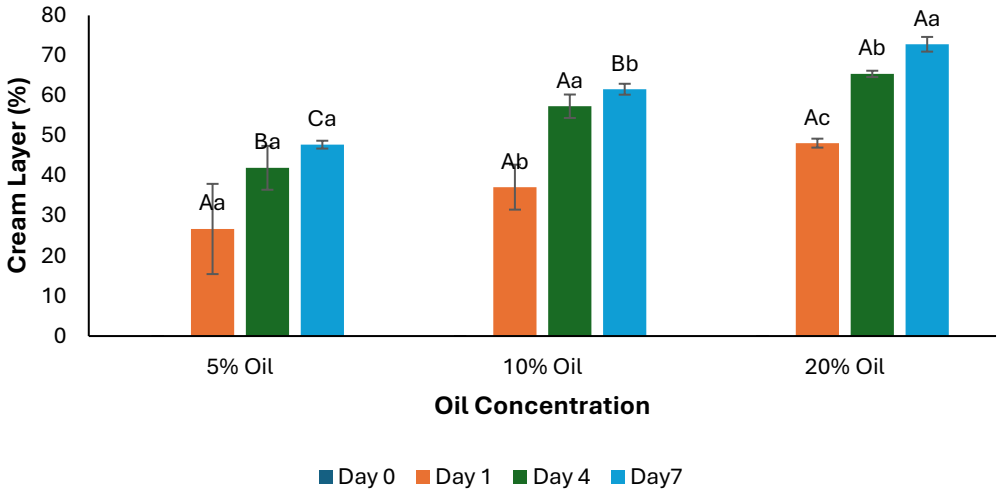


Figure 3.9: Creaming index of TWL emulsions containing different oil concentrations (5, 10 and 20% oil) analysed during storage over 7 days at 4^{o}C. (The capital letter shows a significant difference between oil concentrations while the lowercase letter shows a considerable difference between days between storage as determined by Fisher's test) with standard deviation (n=6) ($p < 0.05$).

3.5 Conclusions

The chemical composition of TWL was 97.34 ± 0.23% moisture, 0.38 ± 0.05% crude protein, 0.10 ± 0.06% fat, 0.43 ± 0.05% ash, 1.76 ± 0.23% carbohydrate, and 0.10 ± ND% dietary

fibre. The main sugar compound found in tofu whey was sucrose (0.48 ± 0.13%), while magnesium, potassium, sodium, and chloride were the main mineral constituents. The results of the SDS-PAGE indicate that the main protein components in tofu whey appear to be β-conglycinin subunit β and β', glycinin acid polypeptide chain, essential polypeptide chains, and low molecular weight protein of 15 kDa. Tofu whey's sugar and protein content is considered a good source for microbial growth. Additionally, tofu whey exhibited adequate antioxidant activity, with the phenolic and saponin content found in tofu whey.

Tofu whey was also analysed for its potential effect as an anti-browning agent using fruit juice samples as a mediator for an enzymatic browning reaction. The results showed that tofu whey is not comparable to ascorbic acid and sodium bisulphite but is superior to a low concentration of citric acid at stopping polyphenol oxidase activity. Furthermore, compared to anti-browning agents, tofu whey exhibited better potential as an emulsifying and foaming agent. It showed high foam capacity and stability near pH 2.5- 5.5, considered the natural pH of most foods. In the case of emulsions, tofu whey was shown to have good emulsifying properties, although the higher the oil concentration, the lower the stability. Tofu whey is a promising functional food ingredient with these functionalities, so further research on its concentration needs to be carried out. This was studied in the following chapter (Chapter 4).

Chapter 4. Preparation and Characterization of Tofu Whey Powder from Tofu Whey Liquid

4.1 Abstract

Upcycling tofu whey from liquid as a by-product of making tofu is insufficient way to valorise it. It must be dried into a powder like other by-products on the market, like aquafaba and legume powder. To maximise its use as a functional food ingredient, it can be used in products like mayonnaise, yoghurt, and bread, as shown by legume powders. However, unlike legume powder, the downside of a clean-label product would now show a soy label. To better understand tofu whey as a dried powder, a chemical and physical analysis was done on the components of tofu whey, as well as further investigation into its functionality. The chemical characteristic of TWP was 5.23 ± 1.04% moisture, 15.08 ± 0.35% Crude protein, 1.54 ± 0.29% total fat, 15.09 ± 1.74% total ash and 62.88 ± 2.27% carbohydrate, with the main salts being Copper, Chloride, Iron, Magnesium and Potassium. TWP showed high antioxidant activity and saponins, which have shown emulsification. The functionality of TWP displays that foamability was better at an average pH of 4 with higher foam capacity and stability, while emulsification showed better stability at higher and lower pH due to better zeta potential at higher pH, forming more potent negative and positive electron clouds. While at high concentrations of NaCl (500mM) due to the hydrophilic properties of soy protein molecule when interaction with NaCl, it forms double Na^{+}-water layers which cause electrostatic force cloud around the particle making it more stable.

4.2 Introduction

Tofu whey is a nutrient-rich wastewater that is high in COD and BOD(Sun et al., 2022). High COD and BOD require high costs for waste treatment. Instead, upcycling and reusing tofu whey as a functional food ingredient can be another option over its disposable via wastewater treatment. This can cause a waste reduction and can lead to a more environmentally sustainable way to use up the tofu whey wastewater, as some methods of treating wastewater are not sustainable and eco-friendly (Bolisetty et al., 2019). As such, upcycling tofu whey wastewater is quite like the upcycling of legume wastewater. However,

legume wastewater has the same problem as tofu whey, as it is a high-water content ingredient, meaning they must be dried into a powder to enhance their usability and economic value.

The two most common methods for powder conversion of legume wastewater are freeze-drying and spray-drying. Both these methods seem to be effective in powdering the legume wastewater but have shown significant effects on the composition during the conversion, as studies by W. Chen et al. (2021) result in high fibre content for spray drying and lower soluble carbohydrates and mineral content after freeze drying as well as spray drying showing less protein content, particle size and colouration, while the opposite was true for freeze drying. Furthermore, spray-drying seems to lead to functionality changes in these proteins due to aggregation of globulins, causing higher surface hydrophobicity and better surface activity (Yang et al., 2022).

The application of these legume powders includes but is not limited to being a clean-label hydrocolloid with foaming and emulsifying abilities, as they can replace egg, dairy, and gluten hydrocolloids. In products such as mayonnaise, typically used eggs can be replaced with legume powder to make mayonnaise to stabilise foam and water in oil emulsion. At the same time, bread made with legume powder instead of gluten can display better load volume and soft texture as it provides stabilisation of air bubbles through saponins and fibre. Last, dairy-free yoghurt reduces syneresis through fibre-binding moisture and solids and better prebiotic activity via oligosaccharides (Serventi & McNeill, 2024). With tofu whey sharing similarities with legume wastewater, further studies on tofu whey, which is less known, are required to provide the food industries with other alternative clean-label ingredients that can provide similar applications. However, the downside of using tofu whey would be that these labels are getting replaced, but another soy label would be used instead.

The total solids content of tofu whey is very low, less than 2-3%, based on the data from the previous chapter. This makes tofu whey liquid less valuable as it is too diluted, but like aquafaba and legume wastewater, it can be processed into a dry powder for its better utilisation and application in foods (Ossa et al., 2022; Zhang et al., 2012). In order to give more value to tofu whey, it needs to be concentrated by removing water or dried into a tofu

whey powder (TWP) through drying techniques. However, there is a lack of research on TWP. This chapter aimed to convert a liquid tofu whey into a freeze-dried powder for its use as a functional food ingredient. This study was to provide knowledge on the functionality of TWP and its characteristics for the tofu industry as it tries to reduce waste. This will help save costs when treating wastewater and provide the tofu industry with a more sustainable and environmentally friendly disposal method.

The study was to determine the chemical composition of TWP with proximate analysis and measure mineral profiles, saponin content, total phenolic content, and antioxidant activity. Furthermore, the effects of concentration of tofu whey and pH were investigated for foaming, while the effects of concentration, pH and salts on the emulsifying properties of TWP were also determined.

4.3 Materials and Methods

4.3.1 Materials

Fresh tofu whey liquid (TWL) was obtained from Tonzu (Auckland, New Zealand). All chemicals described in Section 2.2.1 were also used in this chapter.

4.3.2 Preparation of freeze-dried tofu whey powder

Fresh TWL was collected in different batches and filtered using a 200-micron filter bag before being frozen. The frozen tofu whey was then taken to the Food Bowl (Auckland, New Zealand), where it was freeze-dried using a freeze dryer (Cuddon 80L Karen unit, New Zealand) with a capacity of up to 80 litres. The freeze-dried tofu whey was powderized using a Robot Coupe Blixer 60 (USA). The resulting tofu whey powder (TWP) was then vacuum sealed using a Multivac C200 (USA). The water activity (a_w) of TWP was around 0.37 at 25^{o}C. The chemical composition and physical properties of the freeze-dried samples were similar between different batches and, as such, were treated the same. Some frozen samples of tofu whey liquid were freeze-dried in the Food Technology Laboratory at Massey University (Auckland, New Zealand) using a Labconco freeze dryer No.7806030 unit (USA) with a maximum volume of 3 litres. The freeze-dried powder was vacuum sealed using an AudionVac VMS103 (Amsterdam, Netherlands).

4.3.3 Reconstitution of tofu whey powder

Freeze-dried TWP was reconstituted in water at different concentrations (3, 5, 6, 10 and 20% w/w) by adding TWP to distilled water containing 0.2% sodium azide. The mixture was gently agitated with a magnetic stir bar for 2 hours at ambient temperature to ensure complete dissolution and hydration. The reconstitution of tofu whey powder was used to measure total phenolic, antioxidant, and saponin activities, foam capacity and stability, emulsion preparation, stability, and zeta potential.

4.3.4 Proximate composition and mineral content of tofu whey powder

The proximate composition of TWP was determined using standard analytical methods to assess moisture, ash, fat, protein, dietary fibre, and carbohydrate content. The mineral content was assessed using Inductively Coupled Plasma – Mass Spectrometry.

4.3.4.1 Moisture content

Moisture content was determined using the air-oven method with slight modifications from AOAC Official Methods 925.10. Approximately 2 g of TWP was placed on an aluminium moisture dish. The tofu whey powder samples were placed in an oven at 105^{o}C. The samples were removed and weighed the following day. The moisture content was calculated using the following equation.

$$Moisture\ (\%) = \frac{(W_2 - W_3) \times 100}{(W_2 - W_1)} \qquad \text{(Equation 4.1)}$$

where W_1 is the weight of the moisture dish (g), W_2 is the weight of the moisture dish and sample before drying (g), and W_3 is the weight of the moisture dish and sample after drying) (g. The experiment was conducted in triplicate.

4.3.4.2 Ash content

Ash content was determined using the dry ashing method with slight modifications from AOAC Official Methods 942.05. A crucible was heated in a muffle furnace at 550^{o}C for 60 minutes and then cooled in a desiccator for 60 minutes. Approximately 2 g of TWP was added to the crucible and charred until black over a Bunsen burner. The tofu whey powder sample in the crucible was placed in the muffle furnace for 5 hours. After the sample was

ashed, the crucible was placed in a desiccator to cool down for 60 minutes before weighing. The ash content was calculated using the following equation.

$$Ash\ content\ (\%) = \frac{(W_2 - W_1)}{(W_3)} \times 100 \qquad \text{(Equation 4.2)}$$

where W_1 is the weight of the crucible (g), W_2 is the weight after ashing (g), and W_3 is the weight of the TWP before drying (g). The experiment was conducted in triplicate.

4.3.4.3 Fat content

Fat content was determined using the Mojonnier method (AACC 30-10) with slight modifications (Chemists & Horwitz, 1990; Nielsen, 2003). Approximately 2 g of TWP was added to a test tube and mixed with 2 mL of water, followed by 10 mL of 70% concentrated HCl. The test tube was placed in a boiling water bath at 60^{o}C for 40 minutes. Afterwards, the test tube's content was mixed with 10 mL of ethanol and poured into a Mojonnier flask. The test tube was then rinsed three times with 25 mL of diethyl ether, which was poured into the Mojonnier flask and shaken for 2 minutes. Next, 25 mL of petroleum ether was added to the Mojonnier flask and shaken for 2 minutes. The Mojonnier flask was left to stand for 30 minutes, and the organic solvent layer was decanted into an aluminium fat dish. The remaining mixture in the Mojonnier flask was prepared for a second fat extraction using the same methods as above, but only with 5 mL of ethanol and 15 mL of diethyl ether and petroleum ether. The aluminium fat dish was placed on a hot plate and heated to 100^{o}C until the liquid evaporated. The aluminium fat dish was then placed in a desiccator to cool before being weighed. The fat content was calculated using the following equation.

$$Fat\ content\ (\%) = \frac{(W_2 - W_1)}{(W_3)} \times 100 \qquad \text{(Equation 4.3)}$$

where W_1 is the weight of the empty fat dish (g), W_2 is the weight of the fat dish and fat (g), and W_3 is the weight of TWP (g). The experiment was conducted in triplicate.

4.3.4.4 Protein content

Protein content was determined using the semi-automated Kjeldahl method (Kjeldtec 1030 system, Sweden, Buchi K424 Digestion unit and Buchi Kjeldahl B-316 Distillation unit, Switzerland) with slight modifications from AOAC Official Methods 976.05 (Chemists &

Horwitz, 1990; Nielsen, 2003). Approximately 1 g f TWP was added to a digestion tube with 7 g of K_2So_4, 0.007 g of Se and 25 mL of concentrated H_2SO_4. The semi-automated digestion unit was heated to 420^{o}C for 6 hours and then cooled. Afterwards, 70 mL of water was added to the digestion tube and 70 mL of 40% NaOH. The mixture was distilled into 50 mL of 4% boric acid solution with 0.1% bromocresol green and methyl red. The distilled sample was titrated against 0.1M of HCl to a grey-mauve point. The protein content was calculated using the following equations.

$$\text{Nitrogen } (\%) = \frac{(\text{A} \times 0.1) \times 14 \times 100}{1000 \times \text{B}} \qquad \text{(Equation 4.4)}$$

$$\text{Protein } (\%) = \text{Nitrogen } (\%) \times 6.25 \qquad \text{(Equation 4.5)}$$

where A is the amount of HCl (mL) used, and B is the weight of TWP (g)

4.3.4.5 Total dietary fibre content

The total dietary fibre content of TWP samples was analysed enzymatically using the Megazymes assay kit K-INTDF in standard with AOAC Method 991.43 (Chemists & Horwitz, 1990) at the Nutrition Lab, Massey University in Palmerston North.

4.3.4.6 Carbohydrate content

Carbohydrate content was calculated using subtraction methods using the following equation.

$$\text{Carbohydrate content} = 100 - (\text{Crude Protein } \% + \text{Total Fat } \% + \text{Total Moisture } \% + \text{Total ash } \%) \qquad \text{(Equation 4.6)}$$

4.3.4.7 Mineral content

The content of minerals (Ca, Mg, P, Na, P, S, Fe, Cu, Mn, Zn) in TWP was assessed via Inductively Couple Plasma – Mass Spectrometry (ICP-MS). This was also performed in the Nutrition Lab at Massey University in Palmerston North. Tofu whey powder samples were digested using HNO_3 and HCl and then filtered according to APHA3030. The analysis was performed on a Perkin Elmer Sciex Elan DRCII ICP-MS (Canada). Chloride (Cl) was analysed via potentiometric titration with 2% nitric acid extraction, following AOAC Official Method 971.27 with slight modifications (Chemists & Horwitz, 1990).

4.3.5 Colour measurement

TWPs were analysed for colour measurement to determine CIE L*, a*, and b* values using a Konica Minolta Spectrophotometer CM-5. The CIE L*, a*, and b* values were then used to calculate the yellowing index using the following equation (Zhao et al., 2021).

$$Yellowing\ Index = \frac{142.86 \times b^*}{L^*} \qquad \text{(Equation 4.7)}$$

4.3.6 Scanning electron microscopy

TWP's morphological shape and structure were examined using scanning electron microscopy (SEM) (Hitachi TM-3030, Japan) operating at 15kV. The powder was placed gently on the surface of a carbon tape on the sample holder to stabilise it during the examination.

4.3.7 Preparation of TWP extract

The tofu whey concentrates of 5% TWP were prepared as described in Section 3.3.5 for TWL.

4.3.8 Antioxidant activities of TWP

4.3.8.1 DPPH assay

DPPH assay was conducted using 5% tofu whey concentrate as described in Section 3.3.7.1

4.3.8.2 ABTS assay

ABTS assay was performed using 5% tofu whey concentrate as described in Section 3.3.7.2

4.3.9 Total phenolic content

Total phenolic content was determined using 5% tofu whey concentrate as described in Section 3.3.6.

4.3.10 Determination of Saponin in TWP

Saponin content was determined using 5% tofu whey concentrate as described in Section 3.3.8.

4.3.11 Foamability and foam stability of TWP

The foaming properties of TWP were evaluated using 3%, 6% and 10% TWP. Additionally, the effect of pH on the foaming properties was measured using a 6% TWP solution at pH 3, 4, 5, and 6. The foaming process was conducted according to the method described in Section 3.3.10 for TWL.

4.3.12 Preparation and analysis of TWP emulsions

Emulsions containing 5, 10 and 20% canola oil were prepared with 5% and 20% TWP following the method described in Section 3.3.11 for TWL. After preparing the emulsions, a 5% concentrated tofu whey emulsion was used to determine the effects of pH and salt. The effect of pH was measured by adjusting the 5% tofu whey emulsion to pH 2, 3, 4, 5, 6, 7, 8 and 9. Simultaneously, the salt effect was measured by adding 100, 200, and 500 mM of NaCl and 10, 20, and 50 mM of $CaCl_2$. The prepared emulsions were then analysed for particle size, particle size distribution, and creaming index. Zeta potential and microstructure.

4.3.12.1 Creaming

The creaming index was measured using the method described in Section 3.3.12.1 for TWL.

4.3.12.2 Particle size and size distribution

The particle size and size distribution of emulsions were measured using the method described in Section 3.3.12.2 for TWL.

4.3.13 Zeta potential

To measure zeta potential, 5% tofu whey solution and 5% tofu whey emulsion containing 5% oil were prepared and dialysed using a Float-A-Lyzer 10 mL kit (Repligen, Massachusetts, USA). The Float-A-Lyzer tube was rinsed with distilled water and then cleaned with a 30% ethanol solution. The ethanol solution was added to the tube and left to stand for 10 minutes. After disposing of the ethanol solution, the Float-A-Lyzer was rinsed three times and then cleaned with distilled water. Distilled water was added to the tube, and the tube was left standing for 20 minutes. Following this, the Float-A-Lyzer was rinsed again three

times with distilled water. Then, 10 mL of the sample was poured into the Float-A-Lyzer tube and left standing in milli-Q water for 24 hours under a magnetic stirrer. The milli-Q water was changed three times during the 24 hours: once at the 4th hour, another at the 8th hour, and finally at the 12th hour.

The dialysed tofu whey solution and tofu whey emulsion were then measured for zeta potential. After dialysis, the 5% tofu whey sample was adjusted to different pH levels of 2, 3, 4, 5, 7, 8, and 9. After dialysis, the 5% tofu whey emulsion was adjusted to pH 3, 4, 5, 6, 7, 8, and 9, diluted with distilled water to a 1:25 ratio. The samples were measured using a Malvern Zetasizer Nanoseries NANO-ZS with a dip cell. For 5% tofu whey, a refractive index of 1.33 (water) for the dispersed phase and 1.52 (soy protein) for the continuous phase was used. For the 5% tofu whey emulsion, a refractive index of 1.465 was used for the dispersed phase (canola oil) and 1.33 for the continuous phase (tofu whey).

4.3.14 CLSM of tofu whey emulsions

Emulsion samples were analysed by confocal laser scanning microscopy (CLSM). A small sample was placed in a cavity slide and added with 50 µL each of 0.2 g/L Nile Red (oil droplet staining) and Fast Green (protein staining) before being covered with a coverslip. Imaging was captured by the Leica DM6000B SP5 confocal laser scanning microscope system with LAS AF software (version 2.7.3.9723, Leica Microsystems CMS GmbH, Germany). Images were acquired with an HCX PL APO CS 40x oil (N.A. 1.25) using the super-resolution mode. Nile Red and Fast Green were sequentially imaged through excitation at 488 nm (argon laser) and 633 nm (HeNe 633 laser), respectively. Emission collection was conducted at 498-569 nm and 643-787 nm, respectively.

4.3.15 Statistical analysis

All experiments and measurements were carried out in triplicate, and the data were expressed as means ± standard deviations (SD). Minitab Software (Version 21.1.0.0, Pennsylvania, USA) was used for one-way analysis of variance (ANOVA), and Fisher's Least Significance Difference (LSD) test was used to identify differences between the results.

4.4 Results and Discussion

4.4.1 Chemical Composition of TWP

Proximate analysis was conducted to quantitatively estimate the chemical composition of TWP, including moisture, crude protein, fat, ash, dietary fibre and carbohydrate. Understanding the chemical composition can help apply tofu whey wastewater as a functional food ingredient. The proximate analysis results for TWP are shown in Table 4.1. TWP's moisture content was observed to be 5.23 ± 1.04 g/100 g, which is expected as freeze drying does not remove 100% of water from food but only reduces it. Similarly, the protein, total ash, and carbohydrate content results in TWP were 15.08 ± 0.35 g/100 g, 15.09 ± 1.74 g/100 g and 62.88 ± 2.27 g/100 g, respectively. These values align with prediction values (15.37 ± 1.86 g/100 g, 17.68 ± 3.88 g/100 g and 57.58 ± 5.90 g/100 g) based on the TWP's moisture content and the proximate composition of TWL from Table 3.4 as shown in Chapter 3, which indicated the crude protein, total ash and total carbohydrate contents were approximately 0.38 ± 0.05 g/100 g, 0.43 ± 0.05 g/100 g and 1.76 ± 0.23g/100 g, respectively. However, the accuracy of total carbohydrate content may be limited by the measurement precision of the other components.

Additionally, after drying the tofu whey liquid – in other words, increasing the concentration of total solids in the tofu whey sample, the tests detected insoluble dietary fibre in tofu whey powder, whereas only soluble dietary fibre was detected in liquid tofu whey (refer to Table 3.4). However, the measured total fat content was 1.54 ± 0.29 g/100 g, which was lower than the expected value of 4.13 ± 0.58 g/100 g. This discrepancy in total fat content could be due to the method not being suited to the material. Studies indicate that the Soxhlet method is better for determining fat content as it relies less on technique skills, eliminating human error, and is slightly more suitable for dry products, such as TWP. In contrast, the Mojonnier method requires the complete digestion of the sample to release all fat content and technique skills when handling the liquid after digestion. If this digestion is incomplete, the results may vary. Although the fat content was lower than expected, the results appear to agree with data from Zhao et al. (2021), who reported protein and fat content in TWP of 13.26 and 2.47g/100 g. respectively. Variations between data could be due to differences in soybean cultivars, having different macronutrients and differences in

tofu manufacturing methods, which could affect the composition of tofu whey. If the fat content were as expected, the total carbohydrate content would be more in line with the predicted value reported earlier.

Table 4.1 Proximate and trace mineral composition of TWP with standard deviation (n= 6)

Component	***Value***
Moisture (g/100 g)	5.23 ± 1.04
Total Solids (g/100 g)	94.77 ± 1.04
Crude protein (g/100 g)	15.08 ± 0.35
Fat (g/100 g)	1.54 ± 0.29
Total Carbohydrate (g/100 g)	62.88 ± 2.27
Total Dietary Fibre (g/100 g)	2.55 ± 0.49
Insoluble Dietary Fibre (g/100 g)	0.55 ± 0.21
Soluble Dietary Fibre (g/100 g)	2.00 ± 0.28
Ash (g/100 g)	15.09 ± 1.74
Calcium (g/100 g)	0.07 ± 0.02
Magnesium (g/100 g)	1.37 ± 0.20
Potassium (g/100 g)	6.45 ± 0.64
Sodium (g/100 g)	0.04 ± 0.01
Phosphorus (g/100 g)	0.37 ± 0.01
Sulphur (mg/100 g)	0.39 ± 0.04
Iron (mg/100 g)	2.05 ± 0.49
Copper (mg/100 g)	3.55 ± 0.78
Manganese (mg/100 g)	0.51 ± 0.04
Zinc (mg/100 g)	1.00 ± 0.01
Chloride (g/100 g)	3.90 ± 0.71

Regardless of the relatively small amount of fat in TWP, the carbohydrate and protein contents were significant, 15.08 g and 62.88 g/100 g, respectively. This indicates that TWP is an important source of dietary protein and carbohydrates.

Trace mineral analysis was conducted to quantitatively assess the trace minerals in TWP, shown in Table 4.1. The results were as expected, with the outcomes being similar to the expected values calculated from the moisture content of 5.23 ± 1.04 g/100 g in TWP and the mineral content in Table 3.1 from Chapter 3. The highest trace minerals in TWP were potassium, chloride and magnesium at 6.45 ± 0.64, 3.90 ± 0.71. 1.37 ± 0.20 g/100 g, respectively. The high levels of these salts are believed to be due to the use of “Nigari” salt and soybeans. “Nigari” is used during tofu production and is made from seawater, mainly

containing magnesium chloride but also some potassium chloride (Kuda & Yano, 2014). Soybeans also contribute much potassium (Hanway & Johnson, 1985). Although this study does not explore the potential use of the high content of these trace minerals in TWP, there is potential for TWP to be used in the development of electrolytic sports drinks. TWP could provide the electrolytes needed for such drinks. In recent years, as reported by Leśniewicz et al. (2016), many of these drinks have been produced with high amounts of potassium, calcium, magnesium or sodium to boost water absorption in the body.

Furthermore, tofu whey has already been used to make alcoholic beverages (Chua et al., 2018) and is commercialised in Singapore under the brand Sachi. Therefore, its potential use in electrolytic sports drinks can be considered for further development. The current results provide valuable insight into the chemical composition of TWP, which was previously lacking. However, further studies on sugars and complex carbohydrates are needed to understand better the chemical composition of tofu whey and its potential as a functional ingredient, as polysaccharides are known to have functional applications in food.

Table 4.2 Trace mineral composition of tofu whey and whey protein (n= 6)

Mineral	**Tofu whey (g/100g)**	**Whey protein (g/100g) (González-Weller et al., 2023)**
Sodium	0.04 ± 0.01	0.34 ± 0.22
Potassium	6.45 ± 0.64	0.47 ± 0.17
Calcium	0.07 ± 0.02	0.38 ± 0.18
Magnesium	1.37 ± 0.20	0.08 ± 0.06

4.4.2 Physical Characteristics of TWP

4.4.2.1 SEM microstructure

An SEM analysis and a colourimeter scan were performed to examine TWP's physical structure and appearance, as shown in Figure 4.1 and Table 4.3. Compared to milk powder (Murrieta-Pazos et al., 2011), TWP exhibits irregular shapes and sizes and appears more crystalline around the edges. The crystalline appearance of TWP may be due to the chemical interactions between its protein and carbohydrate chains during the tofu cooking process. The SEM analysis of tofu has been shown to provide a crystalline uniform honeycomb-like

structure (Kao et al., 2003), which, if broken during the tofu production process and ending up in the whey, could look like that from Figure 4.1. However, it is unknown as no studies have contributed to the observed structural features. The SEM images of TWP in this study closely resemble those reported by Zhao et al. (2020), supporting the validity of these findings.

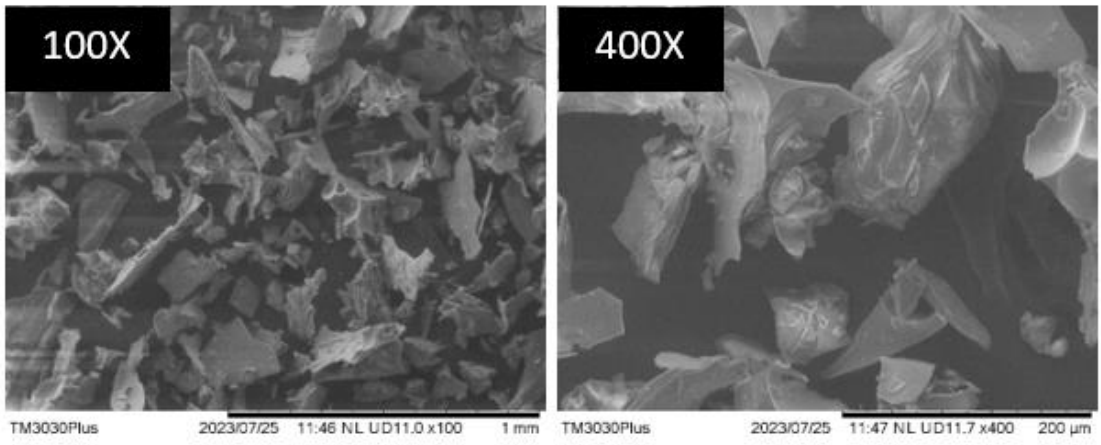


Figure 4.1 Scanning electron microscope (SEM) image of TWP.

4.4.2.2 Colour measurement

The colour results of tofu whey are L* (lightness) 87.95 ± 1.83, a* (red-green value) at -2.28 ± 1.33, b* (yellow-blue value) 33.50 ± 2.59, and the yellowing index 54.52 ± 5.40. Tofu whey powder showed a physical lightness attribute with an intense yellow colour and a shade of green, as suggested by the colour measurements.

The yellowness of TWP, as displayed in Table 4.3 with a yellowing index of 54.52 ± 5.40, could be due to a vitamin such as riboflavin, also known to impart an intense yellow colour. Riboflavin is believed to be the cause of this colour in tofu whey because it is found in soybeans (Huang et al., 2017) and is water-soluble (Pinto & Zempleni, 2016), meaning it would be present in tofu whey during tofu processing. Although the experimental results support and strengthen existing evidence regarding the physical characteristics of TWP, further studies on the particle size of the powder could provide a deeper understanding of the physical properties of TWP.

A study by Kao et al. (2003) reports an L* value of 50.78 ± 0.05 and a yellowing index of 22.80 ± 0.06. Our results show a significantly darker tofu whey that is much less yellow than

our tofu whey, possibly due to different soybean cultivars. Furthermore, studies on tofu and acidic tofu whey showed similar colour trends to our tofu whey. Tofu showed a colour measure of L* value of 84.3 ± 0.18, a* value of -1.9 ± 0.03, and a b* value of 21.6 ± 0.37, similar to tofu whey (Shi et al., 2020). However, acidic tofu whey showed an L* value of 97.30, a* value of 0.04, b* value of 1.65, which is significantly different from our value due to the fermentation of the pigment compound getting broken (Qiao et al., 2010).

Table 4.3. Colour properties of TWP (n= 6)

Colour property	***Value***
L*	87.95 ± 1.83
a*	-2.28 ± 1.33
b*	33.50 ± 2.59
Yellowing Index	54.52 ± 5.40

4.4.3 Phenolic Content and Antioxidant Activity in TWP

Total phenolic content and antioxidant activity in TWP were analysed using the methods Folin-Ciocalteu assay, DppH assay and ABTS assay, respectively, alongside the measurement of saponin content with the vanillin-sulphuric acid assay to assess their viability for health benefits along with their ability to be a functional food ingredient.

The results in Table 4.4 align with expectations based on Table 3.5, which used 5% TWP, approximately double the total solid content in TWL. This increase results in a doubling of DPPH (73.30 ± 2.06 μmol/g) and ABTS (82.04 ± 1.42 μmol/g) activity, as well as an increase in phenolic content (16.96 ± 2.72 mg/g) and saponin content (38.90 ± 2.51 mg/g) on a dry basis. This demonstrates a linear correlation between total solid content and antioxidant activity. The relationship between antioxidant activity and phenolic content further supports existing evidence and the theory of a linear relationship. Similar results have been reported in studies by Du et al. (2009); Kumar et al. (2014) on kiwifruit and plant extracts.

Table 4.4. Antioxidant activity, phenolic and saponin content in 5% TWP on a wet and dry basis with standard deviation (n=6).

	Wet basis	***Dry basis***
Antioxidant	**(µmol TE/100 mL)**	**(µmol TE/g)**
DPPH	362.12 ± 10.20	73.30 ± 2.06
ABTS	426.59 ± 7.40	82.04 ± 1.42
Phenolic	**(mg/mL)**	**(mg/g)**
Phenolics content	1.01 ± 0.01	16.96 ± 2.72
Saponin	**(mg/mL)**	**(mg/g)**
Saponin content	2.02 ± 0.13	38.90 ± 2.51

The total saponin content in 5% TWP was 2.02 ± 0.13 mg/mL (3.89 + 0.25 g/100g DW), which is slightly higher than the 1.26 ± 0.06 mg/mL reported by Sun et al. (2022) and similar to the 3.86 g/100g reported by W. Yang et al. (2016). However, the data reported by W. Yang et al. (2016) were measured using oleanolic acid as a standard compared to soya-saponin I. These findings help demonstrate the potential of tofu whey as a functional food ingredient. Fresh beans (chickpeas, haricot bans, and green beans) generally contain 40-70 mg/g of saponin, as reported by Fenwick and Oakenfull (1981, 1983), indicating that tofu whey has a considerable amount of total saponin content. The total saponin content could valorise tofu whey, as saponins have been shown to decrease blood lipids, lower cancer risk, and reduce blood glucose response (Shi et al., 2004). However, this is only a generalised statement, as different saponins have varying effects. Further research, such as GC-MS profiling of saponins and phenolic content in tofu whey, is needed to gain a more qualitative understanding of its composition.

4.4.4 Foamability of TWP

4.4.4.1 Effect of TWP concentration on foamability

The ability of TWP to foam is important for determining its capabilities as a foaming agent and part of its functionality. The effects of TWP at different concentrations at pH 5.58 are shown in Figure 4.2. There was no significant difference in foaming capacity, which ranged from 88.33% to 94.17%, among the three concentrations of TWP (3, 6, and 10%). However, foam stability and liquid drainage showed significant differences among the concentrations (3, 6, and 10%) over time (Figure 4.2 B, C). Notably, after 3 hours of standing, 10% TWP was

significantly different from 3% and 6% TWP, displaying better foam stability and liquid drainage in the first 2 hours compared to after that, as there was no significant difference between the different TWP concentrations.

These results did not align with the initial hypothesis, as most studies have shown that increasing protein concentration improves foam capacity and stability in soy protein (Li et al., 2022; Martínez et al., 2009). However, studies by Henao Ossa et al. (2020) showed that at around pH 5, there was no significant difference in foaming capacity for varying tofu whey concentrations (TW50 and TW80) under different temperatures. Additionally, increasing tofu whey concentration was observed to enhance foam stability. This discrepancy could be due to a change in soy protein, as denaturation could have occurred during the cooking process of tofu.

(Henao Ossa et al., 2020) Further explained that polysaccharides influence tofu whey foam stability in the soluble fraction, preventing the lamella film's thinning and reducing liquid drainage. Foam stability also benefits from higher particle content, as particles compact aggregates more effectively at the air/water interface, forming a viscoelasticity film.

Furthermore, the reliability of the current data set could also be impacted by whipping time. Previous studies reported by Shaari et al. (2018); Thuwapanichayanan et al. (2012) have shown that whipping time affects foam capacity, density and stability. This suggests that extending the whipping time may yield different results, potentially bringing the data in close alignment with the original hypothesis.

Additionally, freeze-drying TWL does not seem to affect the functionality of TWP when reconstituted in water, as the foaming capacity and stability of 3% tofu whey (pH 5.6) seem to be like the foamability results of TWL at pH 5.5 shown in Figure 3.4. Furthermore, when comparing tofu whey to aquafaba, although tofu whey exhibits lower foaming capacity, its foaming stability and resistance to liquid drainage seem to be significantly better than those of most aquafaba samples (Buhl et al., 2019; Choden et al., 2023; Lafarga et al., 2019). This suggests that tofu whey has a potential niche in the market as a vegan alternative foaming agent.

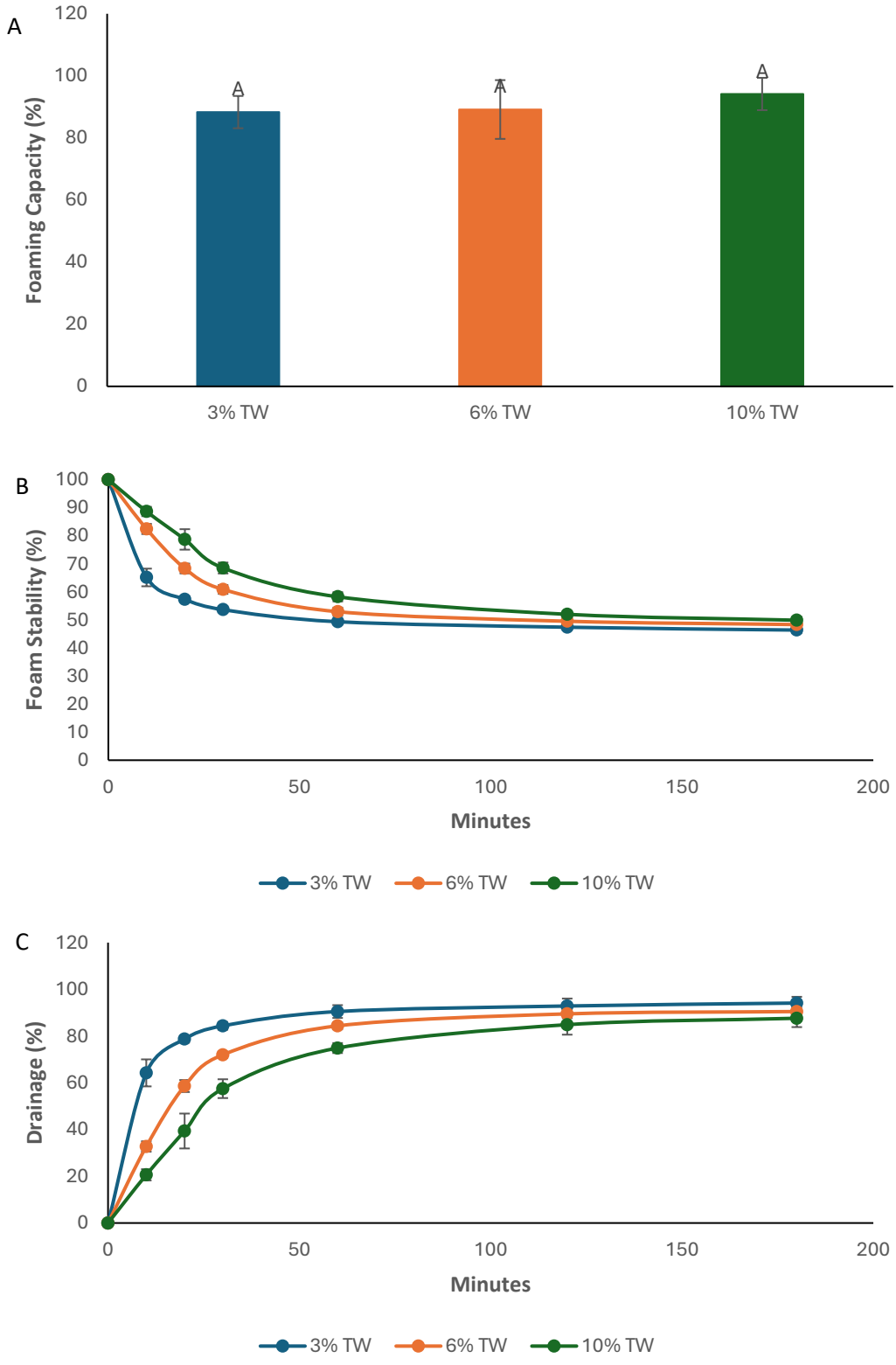


Figure 4.2. The effects of TWP concentration on foaming capacity (A), foaming stability (B), and liquid drainage (C) with standard deviation (n=6) ($p < 0.05$).

4.4.4.2 Effect of pH on foamability of concentrated TWP

The foamability of concentrated TWP at different pH values was examined within a pH 3-6 range to provide an in-depth analysis of foamability in a typical food pH range. The results in Figure 4.3 (A) show that pH 4 exhibited the highest foaming capacity at 103%, which was significantly greater than pH 3 (88%) and pH 6 (83%) ($p<0.05$). The second-best foaming capacity was observed at pH 5 (94%), which was also significantly higher than pH 6 ($p<0.05$).

For liquid drainage percentage (Figure 4.3), significant differences were observed over time ($p<0.05$), following a similar trend. However, for foam stability (Figure 4.3 B), this pattern held only up to 120 minutes ($p<0.05$). After 180 minutes, pH 3 exhibited significantly higher stability than pH 6 ($p<0.05$).

These results are consistent with the foamability observed in TWL, as shown in Figure 3.4 in the previous chapter. This outcome supports the hypothesis that the mechanism of foaming remains similar, as the concentration of tofu whey increases without altering the fundamental foaming properties.

The data also show that TWP can be an excellent alternative to egg white protein as a foaming agent when compared to other studies on the foamability of egg white (Lomakina & Mikova, 2006; Van der Plancken et al., 2007). Additionally, tofu whey at higher concentrations demonstrates similar foaming capacity to aquafaba (Buhl et al., 2019; He et al., 2021) but exhibits significantly better foaming stability over time. The improved foamability is likely primarily due to the interaction of saponins with protein side chains, which causes a change in conformation, affecting the air/water interface, reducing surface tension, and leading to better foam stability (Zhu, Yin, et al., 2020). These findings suggest that tofu whey would be an excellent foaming agent for food products. However, tofu whey must first be incorporated into food systems such as meringues and cakes and compared to current foaming agents to demonstrate its potential as a viable replacement.

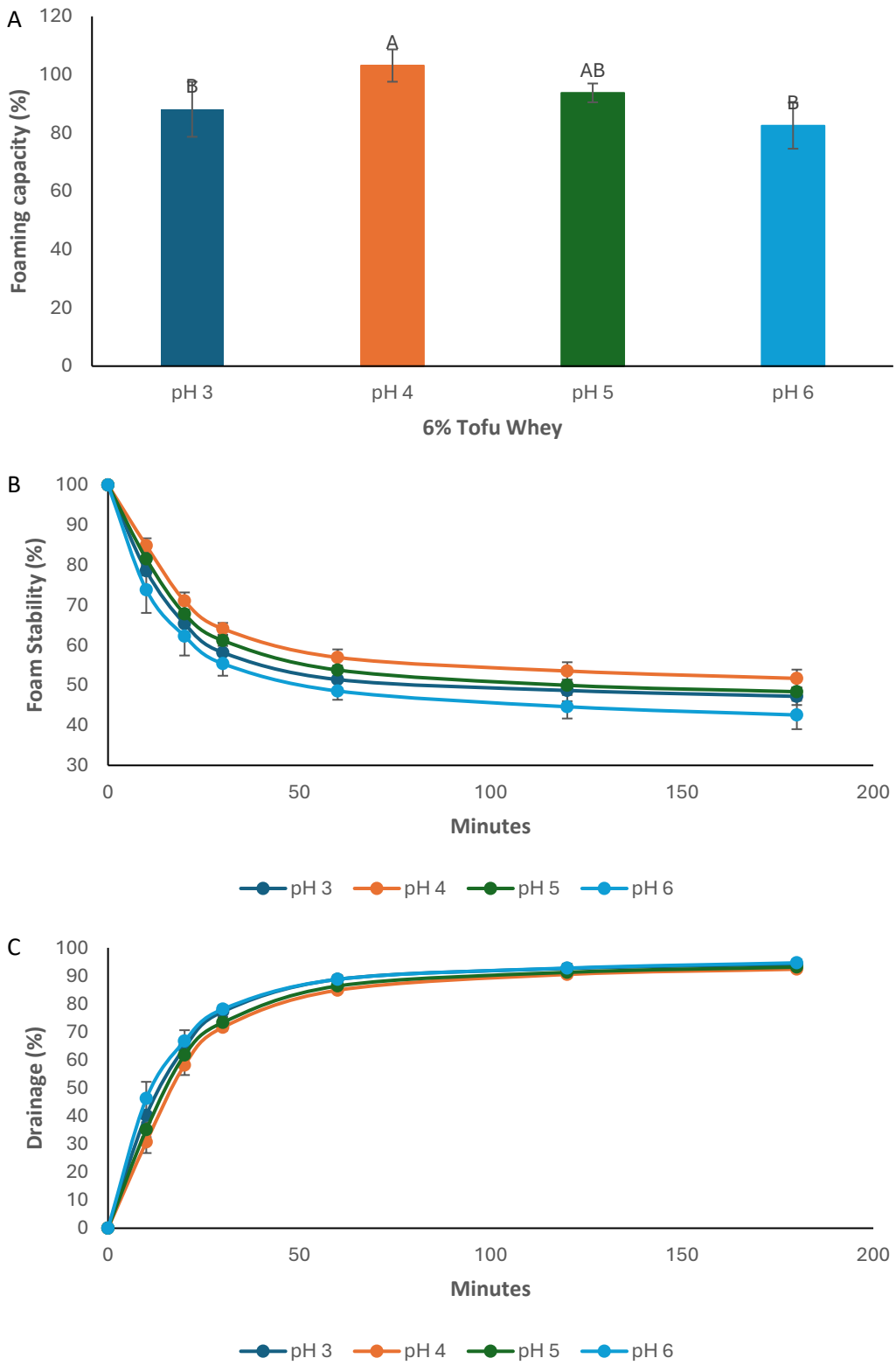


Figure 4.3 The effect of pH on the foamability of 6% TWP, showing foaming capacity (A), foaming stability (B), and liquid drainage (C). (the capital letters on the graph indicate a significant difference between pH for foaming capacity as determine by Fisher's test) with standard deviation (n=6) ($p < 0.05$).

4.4.5 Zeta potential on 5% TWP and TWP emulsion

The zeta potential value is important for understanding the stability of particle suspension as it has a significant role in stabilising particle suspension (Gaikwad et al., 2019) and can be used to measure tofu whey solubility and emulsification stability as a food ingredient at different pH levels. The zeta potential value was used to assess the electrostatic repulsion of 5% TWP and TWP emulsions, measured after dialysis. Dialysis was conducted to remove the excess salt in the tofu whey and tofu whey emulsions so an accurate analysis could be done, as the presence of excess salt can cause charge shielding by interaction with amino acids on the protein, affecting the overall zeta potential (da Silva et al., 2023). The results are shown in Figure 4.4. The zeta potential value for tofu whey was about -6.2 mV at pH 5.8, and as pH decreased, the zeta potential shifted to being more positive at around 7.86 mV at pH 2 and 0.71 mV at pH 3 while decreased pH shifted the zeta potential to be more negative and got as low as -14.20 and -13.57 mV at pH 8 and 9. The zeta potential value for tofu whey emulsion showed similar result as pH of tofu whey emulsion was -6.57 mV at pH 5.6 while as pH decrease the zeta potential value shifted to be more positive as the value was 10.18 mV at pH 3 and more negative as the value was -14.26 and -14.6 at pH 8 and 9.

The results indicate that the zeta potential shifts from negative to positive as the pH decreases. This aligns with the hypothesis, as adding acid and lowering the pH increases hydrogen ion concentration, which carries a positive charge and forms a positive cloud around the particle, causing a repulsion effect (Ossa et al., 2020). The same trend is observed when a base is added, as increasing the pH causes hydroxide ions to form negative clouds around the particle, leading to a repulsion effect (Thakur et al., 2020).

Although the zeta potential trend for tofu whey was as expected, the isoelectric point was not. The graph shows the isoelectric point to be between pH 3.0 and 3.5, while other studies on tofu whey and soy protein suggest that the isoelectric point should be around 4.5 (Henao Ossa et al., 2020; Xia et al., 2021; Yin et al., 2012). Two possible reasons for this shift in isoelectric point are proposed. First, dialysis may have interfered with the salt concentration and ion composition in tofu whey, leading to changes in ionic strength and causing a shift in the curve (Vargas et al., 2023). Second, the differences in coagulation processes in tofu may alter the salt and ion concentrations, leading to the shift in the zeta potential curve of tofu

whey. This highlights the complexity of tofu whey, as different coagulates used in tofu-making can affect and shift the chemical composition and properties in tofu whey, making it a unique material as a possible functional food ingredient. Even though the isoelectric point data did not align with expectations, the trend observed in the zeta potential data agrees with trends found in zeta potential studies for other protein solutions (Malhotra & Coupland, 2004; Ossa et al., 2020). This suggests that the generalizability of the data is not limited by the isoelectric point.

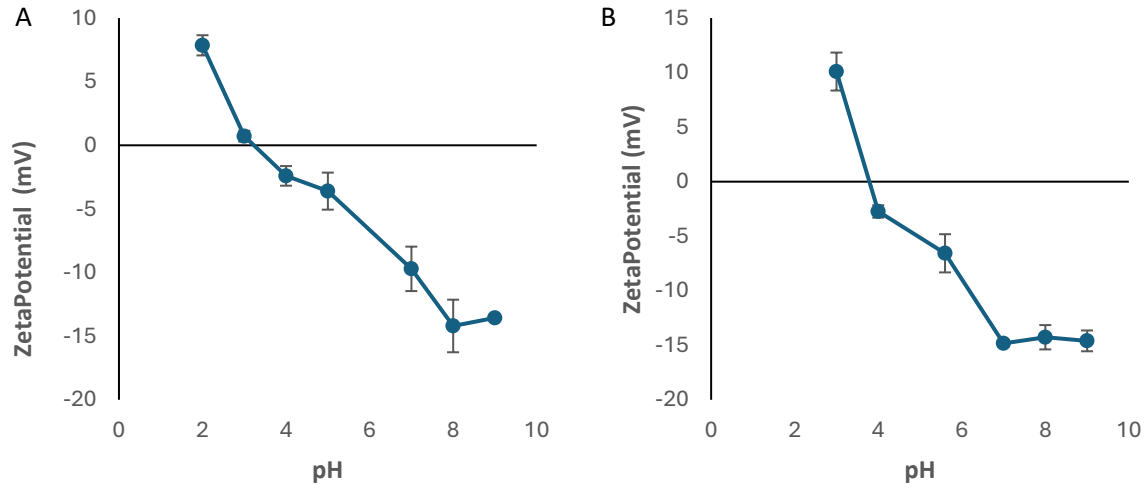


Figure 4.4 The zeta potential of 5% TWP solution (A) and 5% TWP emulsion (B) at different pH levels with standard deviation (n=6).

4.4.6 Effect of different TWP and oil concentrations on emulsions

4.4.6.1 Particle size and distribution of different TWP and oil concentrations on emulsions

To gain an in-depth understanding of the functionality of TWP as an emulsifier, TWP was reconstituted in water at various concentrations and used to emulsify oil at different concentrations. Four types of emulsions were prepared with varying concentrations: high TWP with low oil (20% TWP:5% oil), high TWP with high oil (20% TWP:20% oil), low TWP with low oil (5% TWP:5% oil) and low TWP with high oil (5% TWP:20% oil). The size and distribution of oil droplets in the emulsions were measured during storage for 7 days at 4^{o}C to assess the properties and stability of tofu whey emulsions. The results are shown in Figures 4.5 and 4.6.

The particle size of the emulsion oil droplet in Figure 4.5, represented by the diameters D(4,3) (A) and D(3,2) (B), shows that both low TWP emulsions were significantly different from the high TWP emulsions ($p < 0.05$). The particle size of the emulsion respectively for D(3,2) and D(4,3) at day 0 (Figure 4.5) were: 20% TWP and oil were 0.41 µm and 1.61 µm, 20% TWP and 5% oil were 0.52 µm and 2.47 µm, 5% TWP and 20% oil were 2.54 µm and 5.87 µm, 5% TWP and oil were 1.48 µm and 3.94 µm. Over the 7 days, only during the first day did 5% TWP and oil see a significant change in particle size for D (3,2), increasing from 1.48 µm to 1.97 µm. The other did not have any significant change during the 7 days. The particle size of the emulsion at different ratios was expected since low TWP with high oil showed the largest particle size, followed by low TWP and low oil, high TWP and low oil and then high TWP and high oil. This is because, at low TWP and high oil, there is not enough protein to emulsify the oil droplets, making them larger, but as we decrease the oil and then increase the protein solution, it becomes easier for the protein to emulsify the oil droplet making them have low particle size (Friberg et al., 2003). However, this rule does not seem to be the case when comparing high TWP and oil emulsion to high TWP and low oil emulsion, as high TWP and oil emulsion have a smaller particle size, which is due to having too much protein in high TWP and low oil emulsion as when the protein can fully emulsify the oil droplets the leftover protein will start to adsorbed to other protein forming a layer which could lead to bridging or coalescence and therefore cause an increase in particle size, which is what is been shown in the current emulsion system (Dickinson et al., 1997).

The particle size of oil droplets was significantly larger in the low TWP emulsions compared to the high TWP emulsions. Between the low TWP emulsions, there was a significant difference in particle size ($p < 0.05$), whereas the two high TWP emulsions did not exhibit a significant difference in particle size.

The particle size distribution curves in Figure 4.6 reveal notable trends. High TWP emulsions exhibited a single peak, ranging from 0.1 to 1.88 µm for high oil emulsions and 0.1 to 4 µm for low oil emulsions. In contrast, low TWP emulsions displayed a bimodal distribution, characterised by a small minor peak (0.188-0.767 µm) and a larger peak (1.65 – 14.5 µm). The particle size distribution range for low TWP emulsions was like that of the tofu whey liquid (TWL) emulsions, as shown in Chapter 3. Over the 7 days during storage, the particle

size distribution for 5% TWP and oil emulsion showed a slight shift to the right, indicating an increase in particle size, while the other emulsion showed minimal changes.

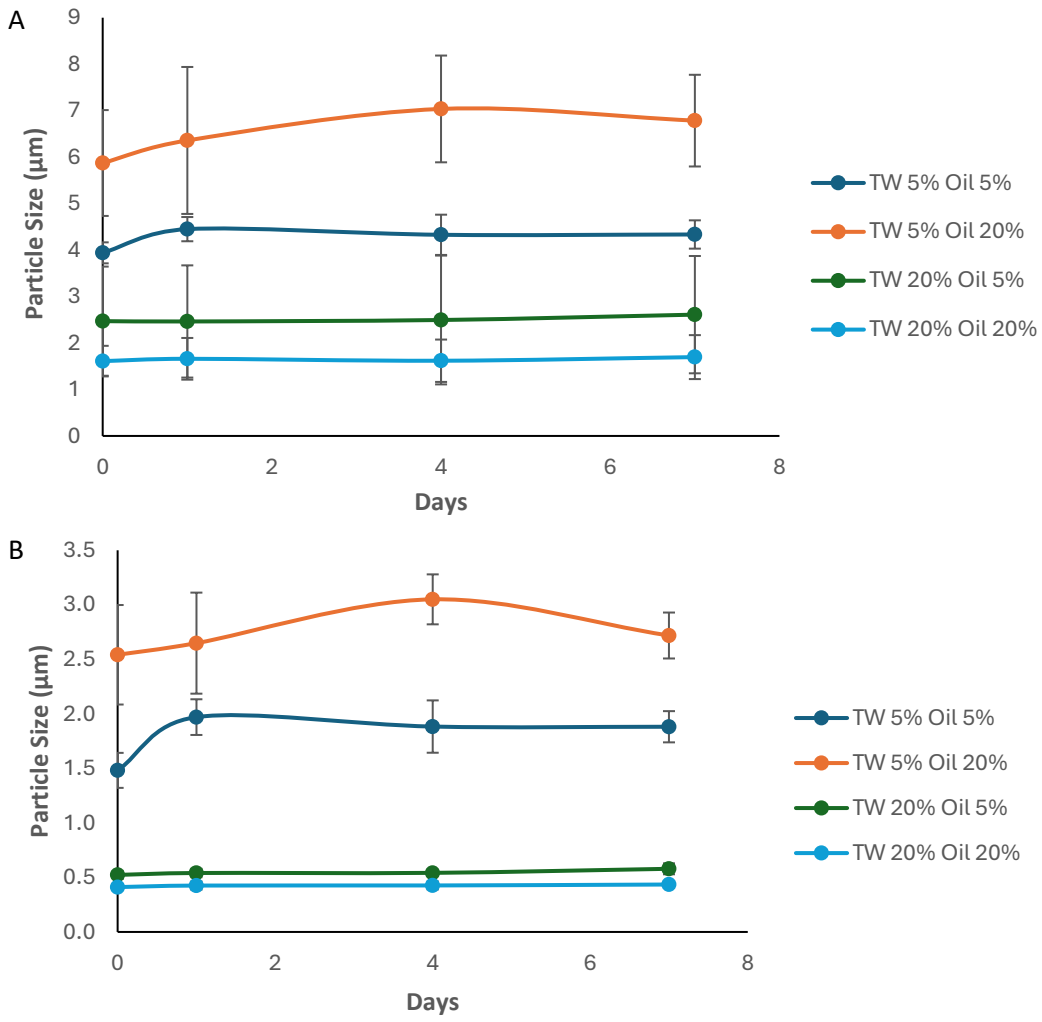


Figure 4.5 Particle size D(4,3) (A) and D(3,2) (B) of TWP emulsions prepared at varying TWP and oil concentrations and measured during 7-day storage at 4°C with standard deviation (n=6) ($p < 0.05$).

The low TWP emulsions in this study follow the similar trend observed in the TWL emulsions (5% TWP) from Chapter 3 (Figures 3.5 and 3.6), where an increase in oil concentration leads to a larger oil droplet size, thereby increasing the likelihood of flocculation. This can be expected, as increasing the tofu whey concentration by approximately 2% does not affect the droplet size significantly. However, for the high TWP emulsions, the trend was different. The high oil emulsion exhibited smaller particle sizes in both D(4,3) and D(3,2) and a narrower particle distribution than the low oil emulsion. The trend change seems to be due to depletion flocculation in the high TWP with low oil emulsion, as observed by Dickinson

Golding, (1997), who showed similar results when increasing protein concentration using sodium caseinates. As suggested by Dickinson, (2019); Langford et al., (2022), the depletion flocculation mechanism occurs when the oil content is low and not all dispersants from the protein solution adsorb onto the oil surface to form oil droplet particles. Some of the dispersants remain in the solution, which can cause an osmotic pressure gradient, which only occurs when the distance between the oil droplets is less than the length of the protein molecules. When this osmotic pressure gradient occurs, it causes particle attraction, resulting in an increase in particle size which causes a decrease in emulsion stabiliy. Thus, it seems that depletion flocculation was occuring in high TWP with low oil emulsions.

The experiment provides new insights into the ability of tofu whey to emulsify. It suggests that for low concentrations of tofu whey, using lower concentrations yields a more stable emulsion, whereas for high concentrations of tofu whey, increasing the oil concentration enhances emulsion stability. However, further studies are needed to determine tofu whey's emulsifying ability limits and the optimal tofu whey-to-oil ratio that provides the best stability at both high and low concentrations.

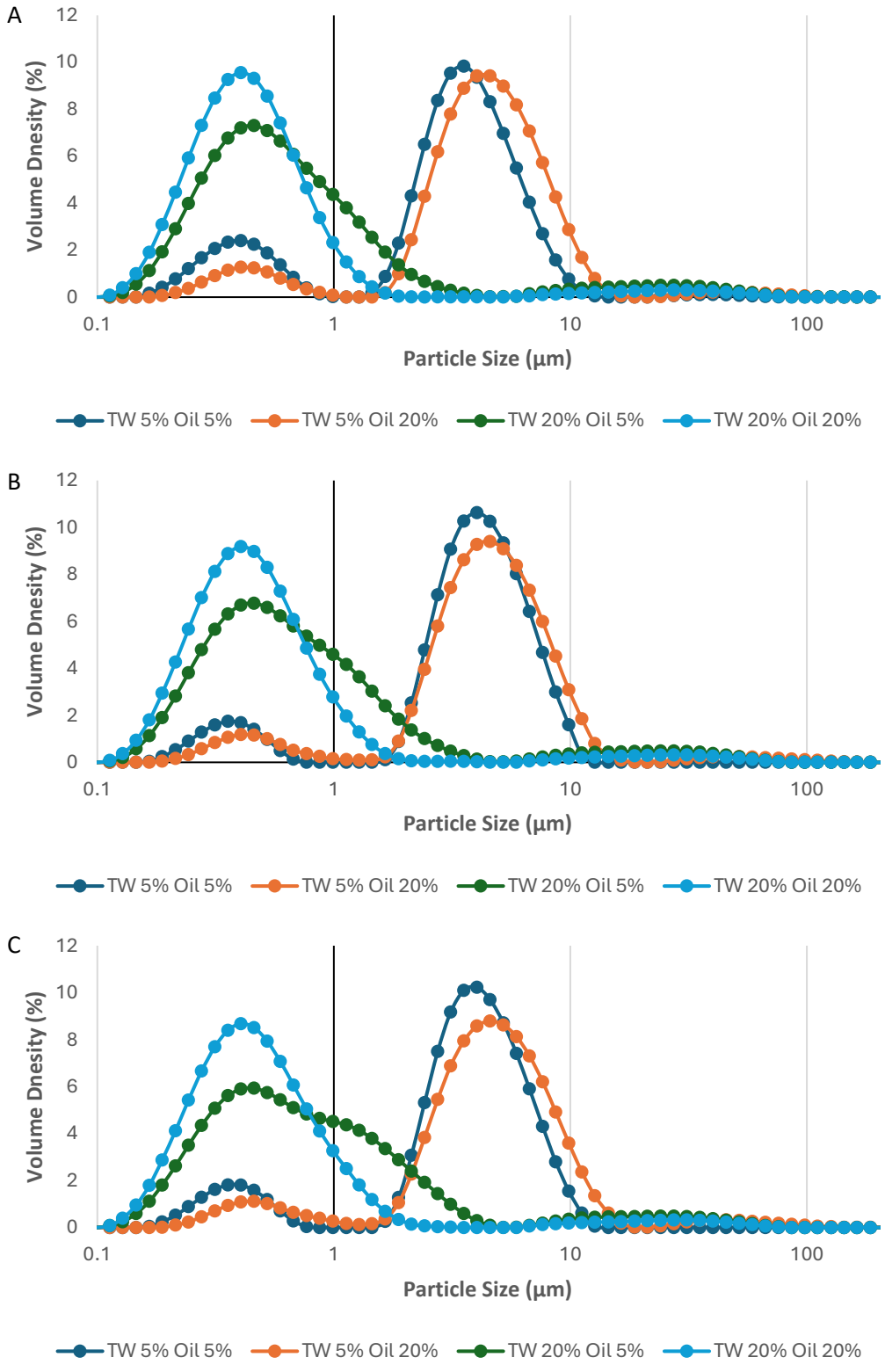


Figure 4.6. Change in particle distribution of TWP emulsions at different oil and TWP concentrations over 7 days (Day 0 (A), Day 1 (B), Day 7 (C)).

4.4.6.2 The stability of different TWP and oil concentrations on emulsions

The stability of the TWP emulsions was measured using the creaming index, which determines the aggregation of oil droplets and phase separation between oil and water in the emulsions. The results are shown in Figure 4.7. the creaming index of the high TWP with high oil emulsion on day 1 significantly differed ($p < 0.05$) from the high TWP with low oil and low TWP with high oil emulsions. On days 4 and 7, the high TWP with high oil emulsion was only significantly different ($p < 0.05$) from the low TWP with low oil emulsion, whereas the other samples were relatively similar over that period.

However, when comparing each emulsion to itself for significant changes over the days, only the low TWP emulsion with low oil concentration showed significant differences ($p < 0.05$) from day 0 to day 1, and the other emulsions showed no significant differences between each day.

The emulsion stability of most of the emulsions was in line with the hypothesis that particle size and distribution are strongly correlated with creaming rate, which has a substantial impact on stability (Binks & Whitby, 2005) and can be seen in Figure 4.8, where all emulsion started to phase separate after 1 day. However, the high TWP with low oil emulsion did not follow this trend. Despite having a smaller particle size, the high TWP with low oil emulsion exhibited a higher creaming index than the low TWP with low oil emulsion on both day 1 and day 4, which had a larger particle size. The reason for the faster creaming rate for the high TWP with low oil emulsion compared to the low TWP with low oil emulsion seems to be due to depletion flocculation. Depletion flocculation appears to influence phase separation more than the aggregation of oil droplets (Grundy et al., 2018).

One limitation of the method that might have affected creaming was that microbial spoilage was controlled by adding sodium azide and storage at 4^{o}C in the tofu whey emulsions, as stability should have been measured over 7 days at room temperature rather than at 4^{o}C. Studies by Kundu et al. (2015); Vanapalli et al. (2002) have shown that temperature affects the creaming rate in emulsions. Although the creaming measurement in tofu whey emulsions may have been flawed, the trend observed in the stability measurements can still

be used to assess the potential of tofu whey as a functional food with emulsification abilities.

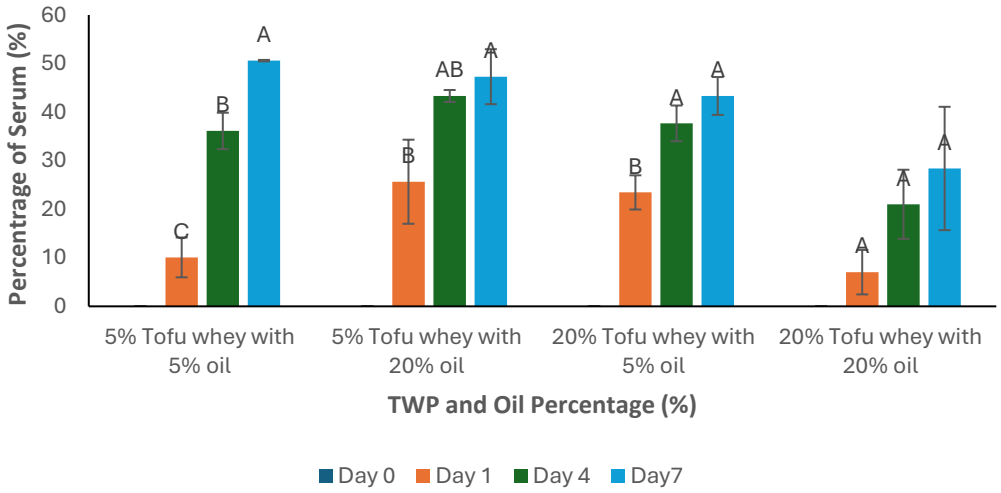
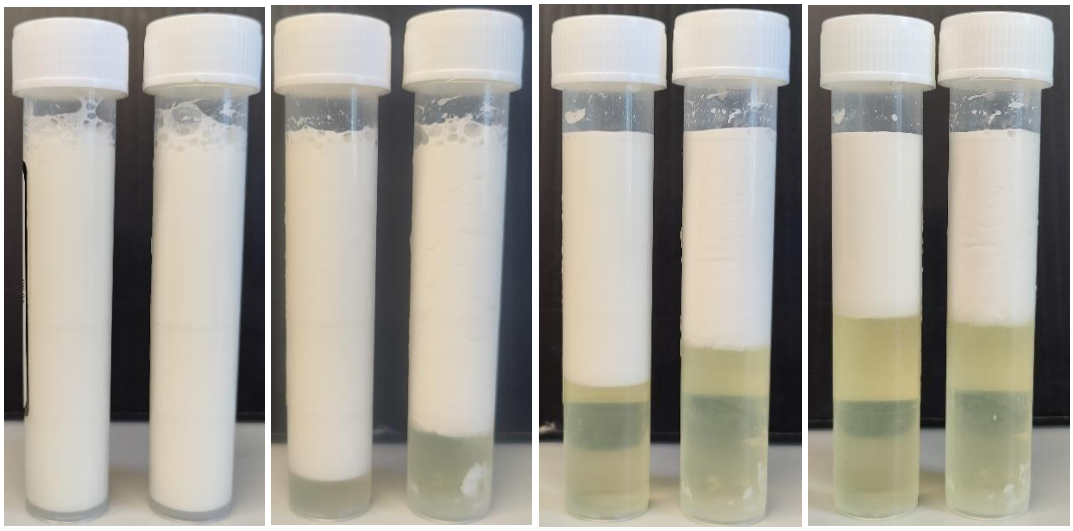


Figure 4.7 Creaming index of TWP emulsions at different oil and TWP concentrations over 7 days. The capital letters on the graph indicate significant difference between pH for foaming capacity as determine by Fisher's test with standard deviation (n=6) ($p < 0.05$).

5% TWP

Day 0 Day 1 Day 4 Day7



20% TWP

Day 0 Day 1 Day 4 Day 7

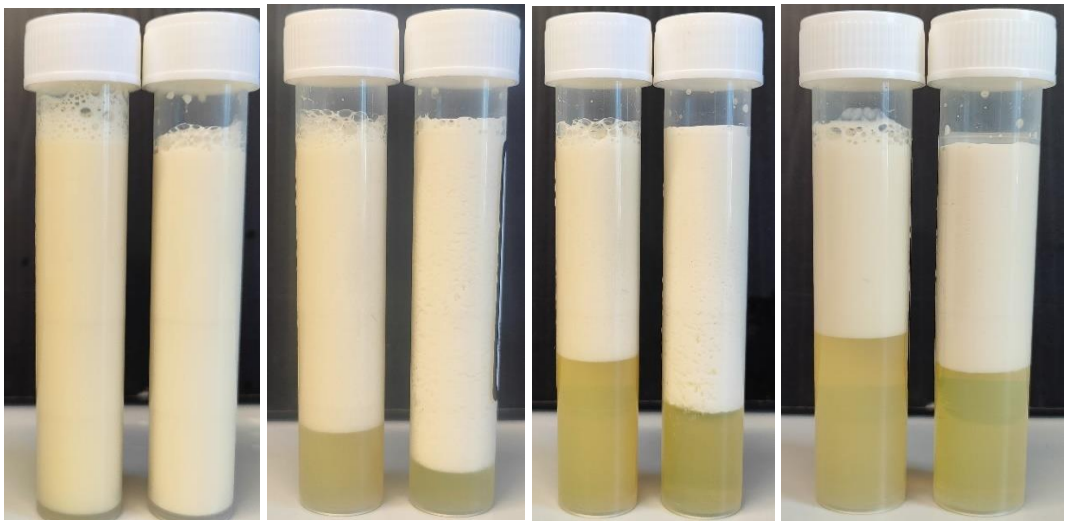


Figure 4.8 Picture of emulsion stability for 5%TWP and 20%TWP at different oil% (Left to right Oil% 5 to 20)

4.4.7 Effect of pH on tofu whey emulsion

4.4.7.1 Particle size and size distribution of pH on tofu whey emulsion

The initial pH of the original emulsion, prepared with 5% tofu whey and 5% oil concentrations, was 5.86. To evaluate the effect of pH on tofu whey emulsion, the pH was adjusted to 2, 3, 4, 5, 6, 7, 8, and 9. Particle size analysis was conducted following pH adjustment and during storage at 4^{o}C over 7 days to assess the impact of pH. This analysis

provides valuable insights into how pH influences tofu whey emulsion and is essential for understanding its potential as a functional ingredient in various food systems with differing pH ranges.

As shown in Figure 4.9 for D(4,3) (A) and D(3,2) (B) particle sizes, the particle size increased as the pH approached the pH 4-5 range and decreased as the pH deviated from this range. The increased particle size at the pH 4-5 range could be attributed to the aggregation of oil droplets near the isoelectric point of proteins at pH 4.5-5, where the electrostatic repulsive forces between protein molecules are minimal, promoting aggregation and larger particle formation. Initially, D(4,3) particle sizes were significantly different between pH 4-5, pH 2, 3, 6, and pH 7-9 ($p < 0.05$). Over time, pH 3 significantly differed from pH 2 and 6 ($p < 0.05$), while other pH levels remained unchanged. For D(3,2) particle sizes, pH 3, 4, and 5 were significantly different from one another, with the two distinct groups formed at pH 2 and 6 and pH 7-9 ($p < 0.05$). Over time, pH 2, 3, 4, 5, and 6 showed significant differences ($p < 0.05$), while pH 7-9 remained stable.

The changes in D(4,3) particle size are evident in Figure 4.10, which illustrates the particle distribution over time. On day 0 (Figure 4.10 A), the prominent peak for pH 4 and 5 overlaps between 2.42 - 35.2 µm for the first group, while pH 2, 3, and 6 form the second group at 1.65 -16.4 µm, and the third group is pH 7-9 at 0.1 -13 µm. Over time, from day 1 (Figure 4.10 B) to day 7 (Figure 4.10 C), all the groups remain unchanged, with a slight shift toward the right. However, pH 3 shifts notably more to the right than the other pH levels, causing a significant particle size difference between pH 2, 6 and 3.

The changes in D(3,2) particle size can be observed in the change in volume density between 0.1 - 1 µm on day 0 (Figure 4.10 A). At this point, pH 2 and 6 had a volume density of around 2%, while pH 3, 4, and 5 were at 0.7%. Over time, the volume density for pH 3, 4, 5, and 6 decreased as particles started to flocculate, shifting the graph to the right. While a similar shift was observed for pH 7, where the volume density decreased between 0.1 - 1 µm and increased over time from 1.2 - 3.6 µm, the shift was not significant enough to produce a difference in particle size between pH 7 and pH 8-9.

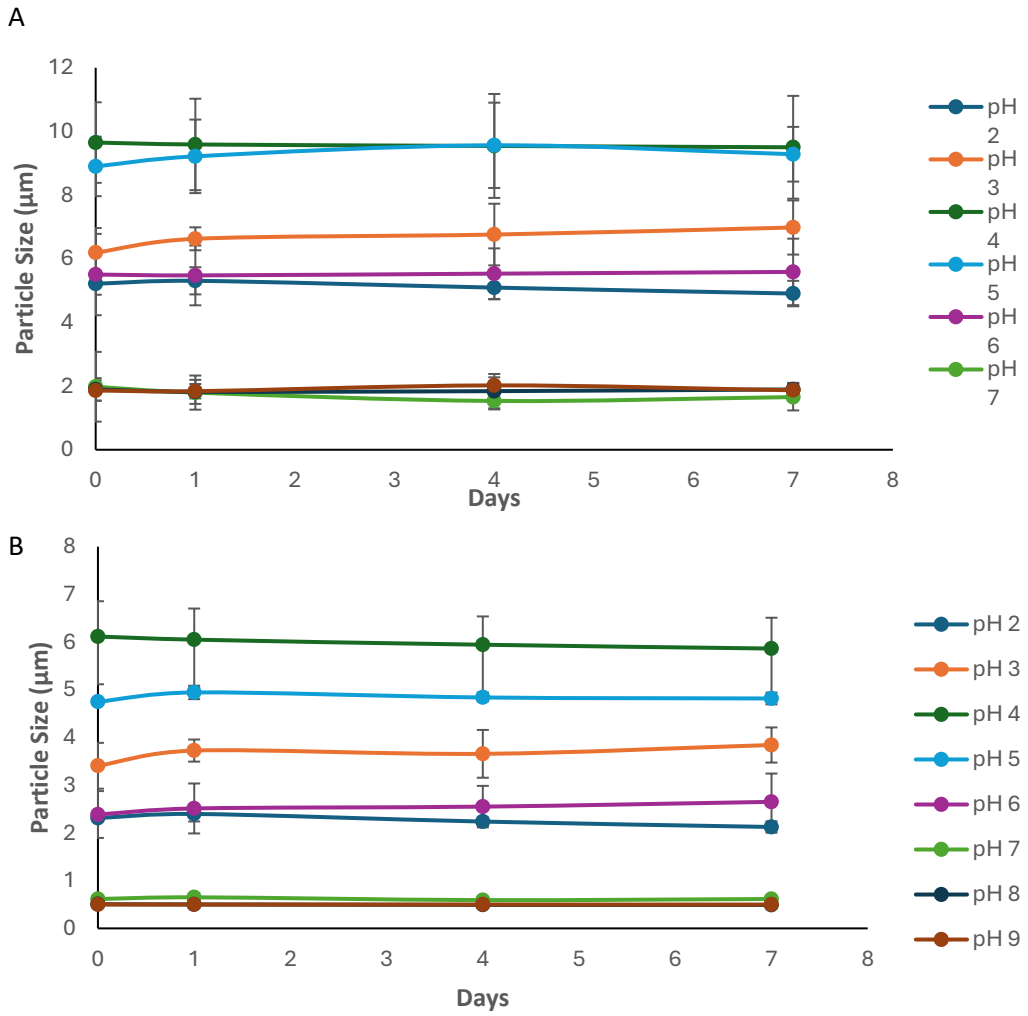


Figure 4.9. Particle size D(4,3) (A) and D(3,2) (B) in diameter of 5% TWP emulsion containing 5% oil at different pH levels over time with standard deviation (n= 6) ($p < 0.05$).

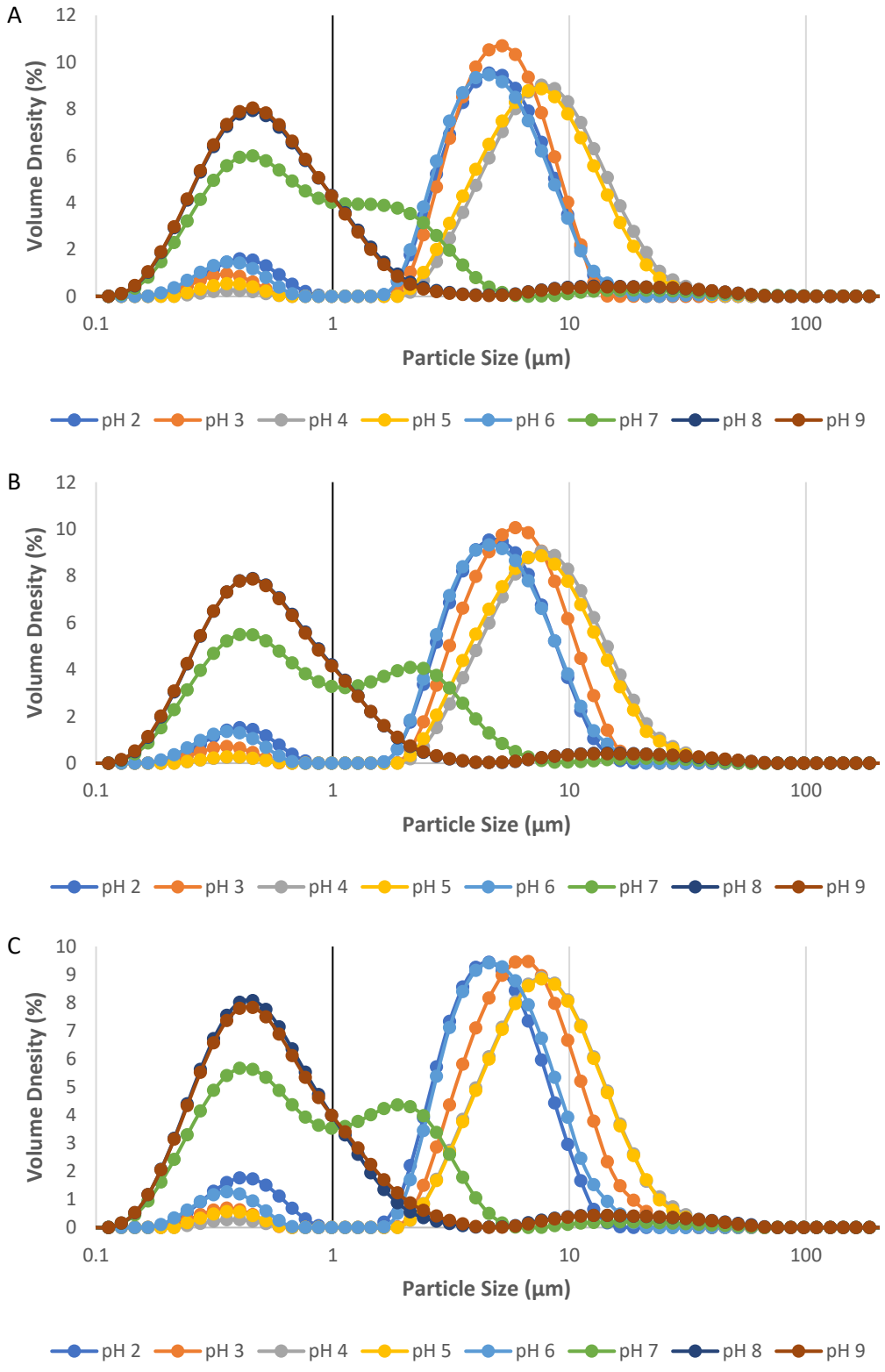


Figure 4.10. Change in the particle size distribution of 5% TWP emulsions containing 5% oil at different pH levels over 7 days: Day 0 (A), Day 1 (B), and Day 7 (C) with standard deviation (n= 6) ($p < 0.05$).

4.4.7.2 Zeta potential of pH on tofu whey emulsion

The results in Figures 4.9 and 4.10 are in line with studies on emulsions using soy protein isolate, which show that deviating from the isoelectric point (either by increasing or decreasing pH) decreases oil droplet size in the emulsion, making it more stable and less likely to flocculate or coalesce (Wang et al., 2020; Zhang et al., 2020; X. Zhang et al., 2021). The change in oil droplet size is due to the change in zeta potential as pH changes, defined as "a measurement of the effective electric charge on the nanoparticle's surface, quantifying the charges" (Selvamani, 2019). Table 3.5 shows the zeta potential values of emulsions at different pH levels. The zeta potential value of the particles in these emulsions dictates particle size: particles with a high zeta potential are more likely to have a more robust electrostatic cloud around them, causing electrostatic repulsion when other particles come near (Delahaije et al., 2017). Therefore, reducing the chance of flocculation and coalescence results in stable oil droplet size with no change.

Furthermore, the results in Figure 4.4 B) support existing evidence that a decrease in zeta potential values increases particle size. For instance, pH 4 and 5 show high D (4,3) and D(3,2) particle sizes with a zeta potential around -2.7 mV and -5 mV, while the next group (pH 2, 3, and 6 shows medium particle sizes with a zeta potential around +10 mV at pH 2-3 and -10 mV at pH 6. The final group (pH 7-9) show a zeta potential of – 15 mV with the small particle sizes.

Table 3.5 The zeta potential of TWP emulsion at different pH with standard deviation (n= 6) ($p < 0.05$).

pH	Zeta potential Average
3	10.10 ± 1.74
4	-2.73 ± 0.57
5.6	-6.57 ± 1.75
7	-14.83 ± 0.29
8	-14.27 ± 1.12
9	-14.60 ± 0.95

4.4.7.3 Microstructure of pH on tofu whey emulsion

In this study, CLSM was used to analyse the microstructure of emulsion samples. The particle size and distribution of the oil droplets at different pH levels are shown in Figure 4.11. The red areas represent the oil droplets, while the green areas represent the proteins. The results from CLSM further strengthen the relationship between increasing zeta potential and reducing flocculation or coalescence. At pH 4 and 5, the images show a high amount of flocculation and coalescence, while as the pH increased or decreased from this range, the emulsion displayed a reduction in flocculation, following the same order as the zeta potential values. The next group, at pH 2, 3, and 6, exhibited a low flocculation rate, while pH 7-9 showed little flocculation and smaller particles in the images.

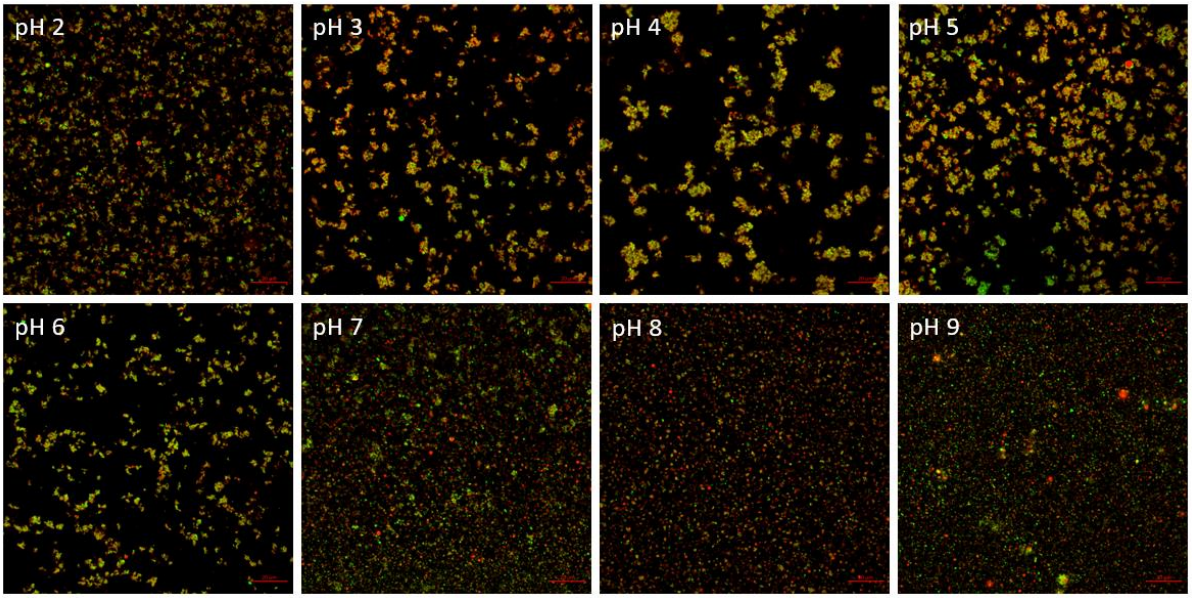


Figure 4.11. CLSM images of 5% TWP emulsions containing 5% oil at different pH levels.

4.4.7.4 Creaming of pH on tofu whey emulsion

The effect of pH on the emulsion's stability was determined using the creaming index, which exhibits the changes in creaming levels after the emulsion was left to sit for over a week. The results of the creaming index, as seen in Figure 4.12, displayed changes in emulsion creaming over 7 days. For pH 3, 4, and 5, there was a significant difference in creaming for each sample between day 1 and day 4, but no significant difference was observed on day 7. In contrast, pH 2 and 6 significantly differed between all three days ($p < 0.05$) for each sample. When comparing groups, there was significantly different creaming between the pH

groups ($p < 0.05$) on day 1 and day 4. The creaming rate followed this order: pH 4, 5 > pH 3, 6 > pH 2, 7-9 in terms of creaming. However, this changed after 7 days, where pH 5 showed significantly more creaming than the others. While pH 3, 4 and 3, 6 were no longer significantly different from each other, pH 4 and 6 remained significantly different ($p < 0.05$). pH 2 significantly differed from pH 7-9, resulting in the following order of creaming on day 7: pH 5 > pH 3, 4 ≥ pH 3, 6 > pH 2 > pH 7-9. These results support the theory that the creaming rate is reduced as particle size decreases (Robins & Hibberd, 1998), except for pH 5, which had significantly higher creaming than pH 4 despite having similar particle size and distribution. Based on the CLSM images in Figure 4.11, pH 5 does appear to have more particle aggregation than pH 4, which may explain the higher creaming index. However, further studies are needed to understand the underlying mechanism of tofu whey, as tofu whey consists of proteins, polysaccharides and saponins, which can interact to form a complex structure, affecting the behaviour of these emulsions at different pH levels.

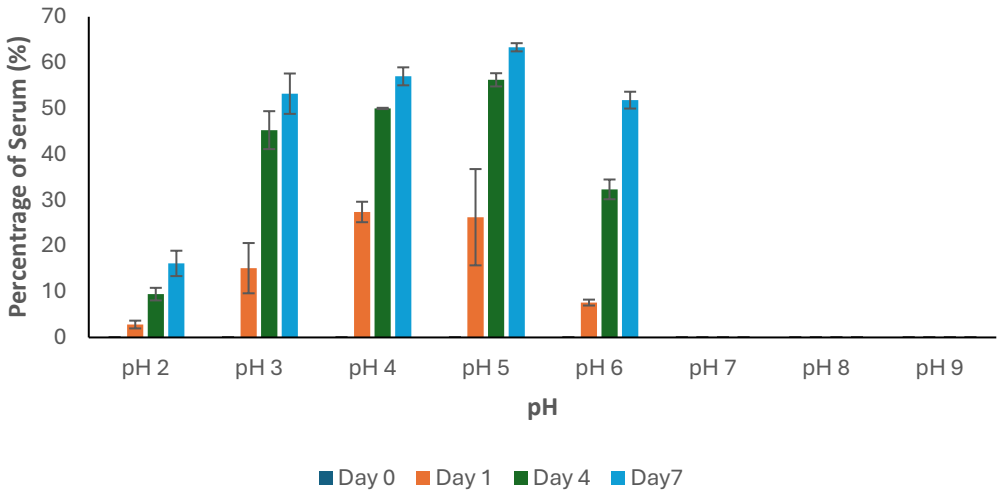
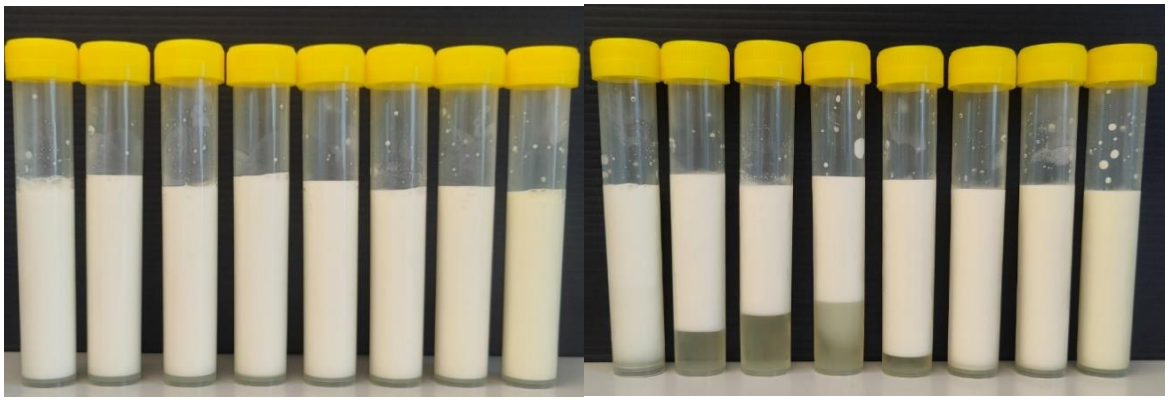


Figure 4.12 Creaming index of TWP emulsions at different pH levels over 7 days. The creaming index was measured and expressed as the percentage of serum phase height over the total liquid height in a test tube with standard deviation (n=6) ($p < 0.05$).

Day 0 Day 1



Day 4 Day 7

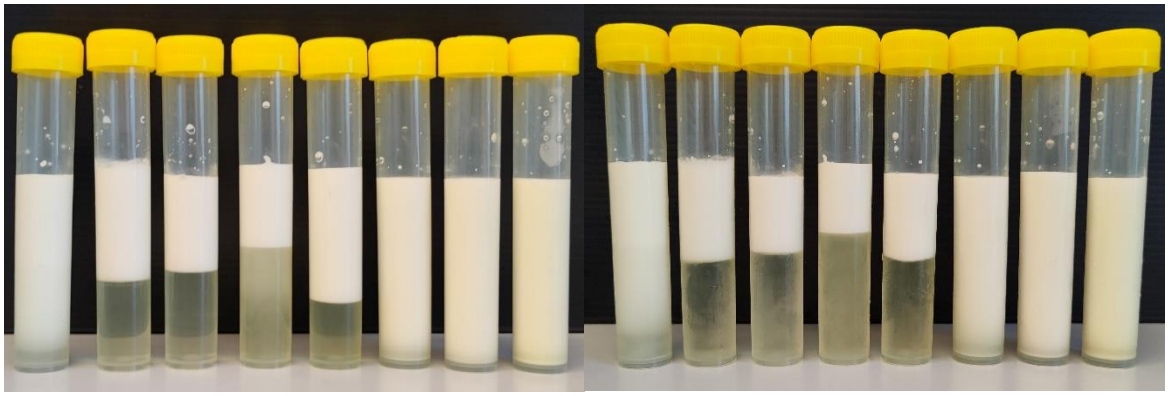


Figure 4.13 Picture of emulsion stability for 5%TWP at different pH (Left to right pH 2,3,4,5,6,7,8,9)

4.4.8 Effect of salt on tofu whey emulsion

4.4.8.1 Particle size and size distribution of salt on tofu whey emulsion

Salts are commonly used in food as flavour, binders or preservatives. Understanding their effect on tofu whey emulsion is crucial for exploring it as a functional food ingredient. Particle size and distribution are key indicators for assessing the impact of salt on the stability and structure of TWP emulsions.

Figure 4.14 shows the D(4,3) (A) and D(3,2) (B) particle size measurements, while Figure 4.15 presents the particle size distribution of tofu whey emulsions at different concentrations of NaCl and $CaCl_2$. The results in Figure 4.14 reveal significant differences in particle size for both salts compared to no-salt emulsion at day 0 ($p < 0.05$). Over time, while the particle size of the no-salt emulsion increases, the particle size of the 20 mM $CaCl_2$ emulsion remains relatively stable. By the end of the study, the particle size of 20 mM $CaCl_2$

was no longer significantly different from the no-salt emulsion, while the 10 mM $CaCl_2$ emulsion still showed a significant difference ($p < 0.05$).

NaCl's particle size remained significantly different from the no-salt emulsion over the 7 days ($p < 0.05$). Furthermore, the D(4,3) particle size of the 500 mM NaCl emulsion was significantly different from both the 100 mM and 200 mM NaCl emulsions over the 7 days ($p < 0.05$). A similar trend can be seen in Figure 4.15, where the no-salt emulsion shifts its volume density peak from 0.1-1 µm to the 1-10 µm range over time, aligning more closely with the $CaCl_2$ peak. For NaCl, the 500 mM concentration showed no shift in distribution over the 7 days, while the 100 mM and 200 mM concentrations exhibited a reduction in volume density between 0.1-1 µm and a shift toward the right, like the no-salt emulsion.

Overall, the results suggest that $CaCl_2$ increases the particle size in tofu whey emulsion, while NaCl decreases the particle size, especially at a high concentration of 500 mM, where the emulsion appears more stable in particle size with less distribution shift in the oil droplets.

The effect of $CaCl_2$ on tofu whey emulsion appears to be in line with studies on emulsions stabilised with soy and whey proteins (Keowmaneechai & McClements, 2002; Kulmyrzaev et al., 2000; Wang et al., 2023; X. Zhang et al., 2022), where increasing $CaCl_2$ concentrations lead to increased particle size and distribution in the emulsions. The aggregation of droplets with higher $CaCl_2$ concentrations seems to be caused by flocculation rather than coalescence (Wang et al., 2023). This flocculation is believed to be driven by Ca2+ ions, which blind proteins on different droplets, forming a bridge between oil droplets and causing aggregation (X. Zhang et al., 2022). However, another study by Mu et al., (2009) suggests that Ca2+ ions cause protein-protein interactions, reducing protein-oil interactions and thus increasing particle size. Both mechanisms may be occurring in tofu whey emulsion. As shown in Figure 4.16, the large red oil droplets may be due to reduced protein-oil interactions, while the smaller green protein clumps could represent bridges forming between oil droplets, causing aggregation.

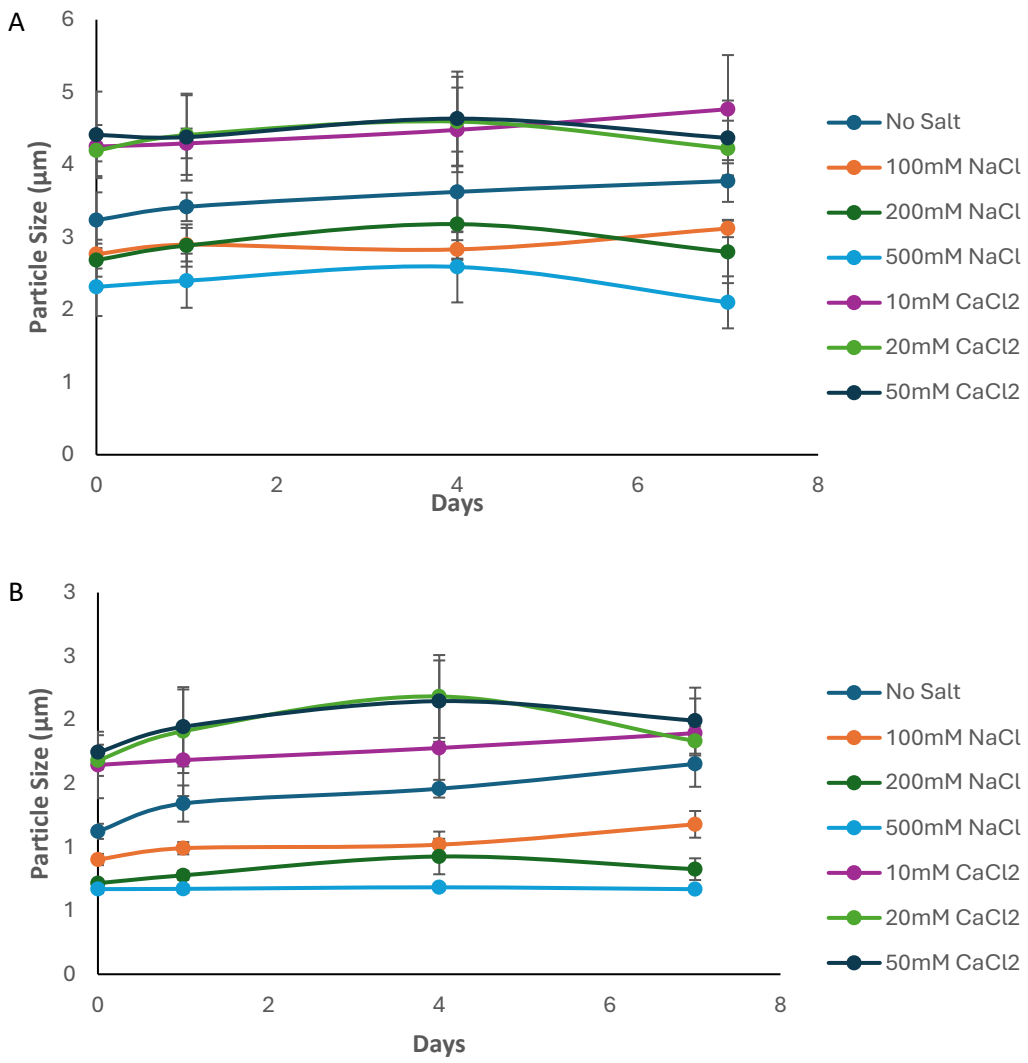


Figure 4.14. Particle size D (4,3) (A) and D(3,2) (B) of 5% TWP emulsions at different salt types and concentrations over time during storage at 4°C for 7 days with standard deviation (n= 6) ($p < 0.05$).

However, the reliability of the data was impacted by the results in Figures 4.14 and 4.15, where no significant change was observed in the concentration of $CaCl_2$. This may be due to the low concentration of $CaCl_2$ used. Increasing the concentration of $CaCl_2$ in future studies is recommended to determine whether it affects the particle size and distribution. Currently, the effects of $CaCl_2$ can only be generalised, as its in-depth effect cannot be demonstrated.

The effect of NaCl on tofu whey emulsions aligns with the hypothesis since studies on soy protein isolate, soy glycinin, and chickpeas display similar trends. These studies report that increasing NaCl concentration decreases particle size in emulsions (Xu et al., 2015; L. Zhang et al., 2022; Zhang et al., 2009). The decrease in droplet size seems to be due to the

hydrophilic nature of soy proteins, which promotes the formation of double Na^+-water layers around the protein. This double hydration layer, along with the electrostatic screening provided by Na^+ ions, seems to form a cloud barrier that prevents bridging flocculation (L. Zhang et al., 2022), thereby decreasing particle sizes as the concentration of NaCl increases.

In contrast, another study (Shao & Tang, 2014) reports that adding NaCl causes an increase in particle size for soy protein molecules. This could be due to the high amount of soy conglycinin, as research done by L. Zhang et al. (2022) exhibits that NaCl promotes particle size increases in soy conglycinin due to aggregation but decreases particle size in soy glycinin under similar conditions. Given that the SDS-PAGE analysis in Figure 3.1 reveals that tofu whey contains more soy glycinin, this may explain why tofu whey shows different results from soy protein.

These results contribute to existing evidence that salts can have varying effects on different types of soy proteins, and generalising soy protein may lead to misleading conclusions in some research. Furthermore, it appears that in tofu whey emulsion, monovalent salts (e.g. NaCl) decrease particle size and distribution, while divalent salts ($CaCl_2$) seem to have the opposite effect, increasing particle size and distribution. Understanding these effects is important as they can be used to help understand the role of tofu whey as a functional ingredient in food production.

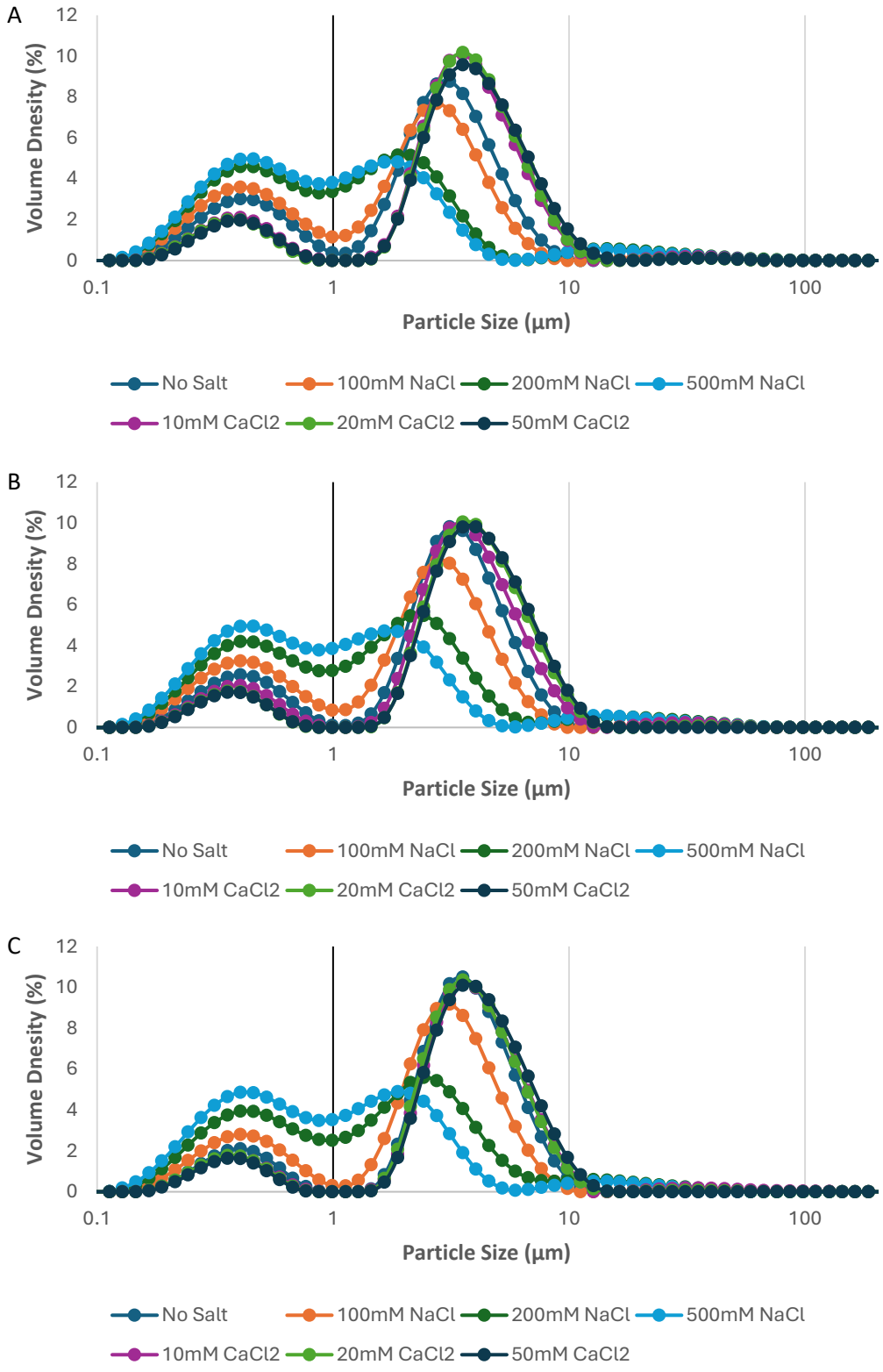


Figure 4.15. Change in particle distribution of 5% TWP emulsion with different salts and salt concentrations over 7 days: Day 0 (A), Day 1 (B), and Day 7 (C).

4.4.8.2 Microstructure of salt on tofu whey emulsion

The effect of salt on emulsion particle size and distribution is exhibited through CLSM in Figure 4.16. The red and green areas represent fat and protein, respectively. As shown in Figure 4.16, the addition of $CaCl_2$ appears to induce the flocculation of particles, evident from the clumping of the green areas, and increased coalescence, indicated by the enlargement of the red dots. At low NaCl concentrations (100 mM and 200 mM), coalescence becomes apparent after 7 days, while at a higher concentration (500 mM), increased particle aggregation is observed in the image.

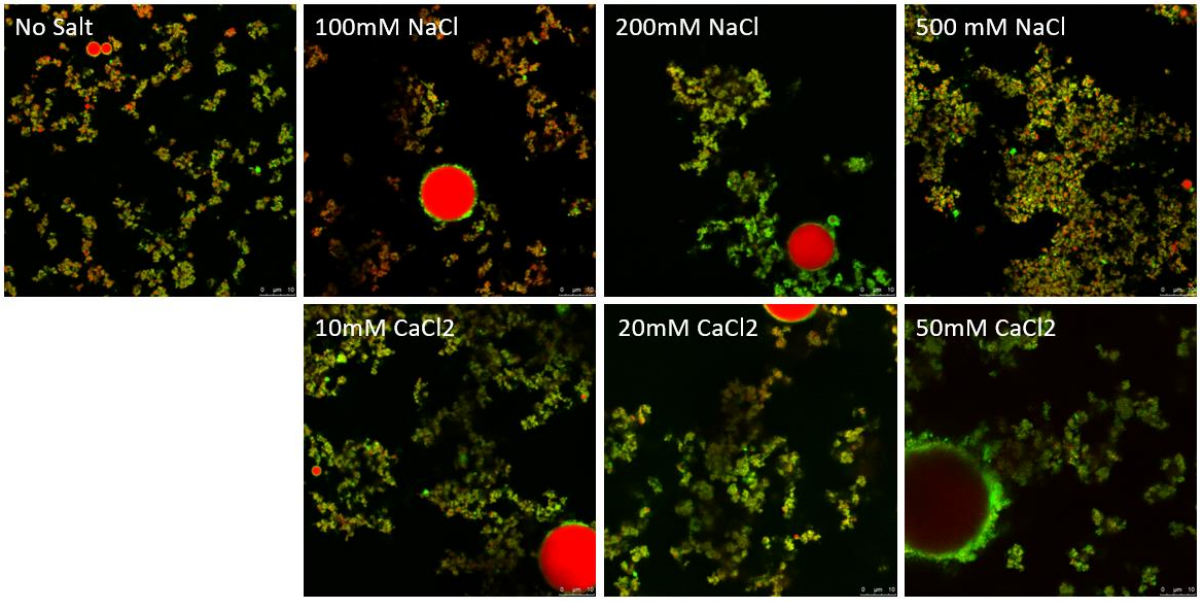


Figure 4.16. Representative CLSM images of 5% TWP emulsions with different salts and salt concentrations.

4.4.8.3 Creaming of salt on tofu whey emulsion

The creaming index shown in Figure 4.17 was used to determine the stability of emulsions with added salt. The results exhibit that emulsions without salt and those with NaCl were significantly different ($p < 0.05$) and had less creaming than emulsions containing 20 mM and 50 mM of $CaCl_2$. However, over time, the emulsion with 500 mM NaCl demonstrated significantly less creaming than all other emulsions, including the no-salt emulsion. The emulsion with 100 mM NaCl also differed significantly from those with 200 mM NaCl and $CaCl_2$ ($p < 0.05$). Additionally, only the 500 mM NaCl emulsion showed no significant

changes in creaming over the 7 days, suggesting that adding 500 mM NaCl significantly improved emulsion stability and prevented creaming.

However, these results contradict those reported by L. Zhang et al. (2022), which showed that increasing NaCl concentration increased the creaming index in emulsions stabilised by globular soy proteins. However, the $CaCl_2$ results align with another study on soy protein emulsions, which found that increasing $CaCl_2$ concentration decreased emulsion stability (X. Zhang et al., 2022). A possible explanation for the enhanced stability observed in the tofu whey emulsion with 500 mM NaCl could involve protein-saponin interactions. A study reported by de Faria et al. (2017) displayed that adding NaCl to β-lactoglobulin and saponin emulsion Increases the zeta potential and emulsion stability. A similar effect may occur in tofu whey emulsions, where NaCl concentrations reaching a certain threshold could significantly alter the zeta potential, thereby increasing emulsion stability by providing a zeta potential barrier and reducing flocculation. However, the precise mechanisms of how the saponins and protein in tofu whey interact are unclear, and further research is required to understand these mechanisms better.

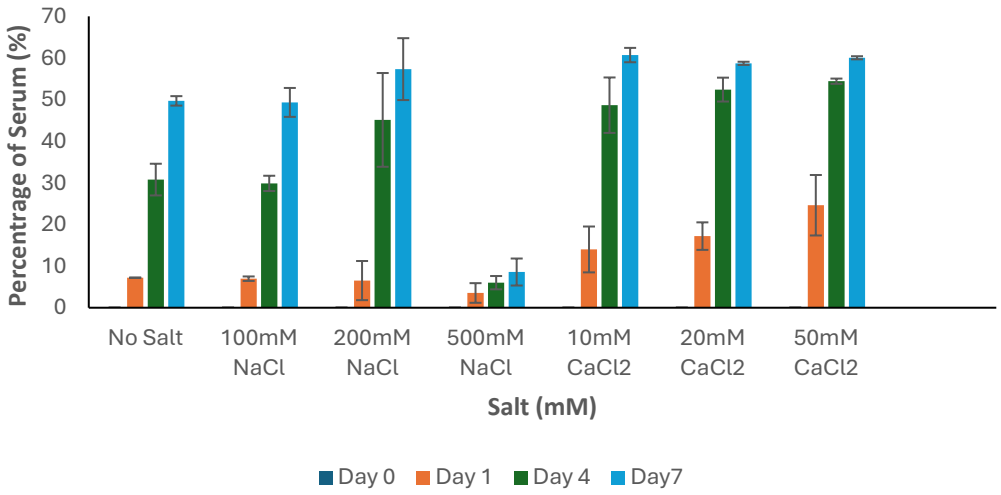


Figure 4.17. Creaming index of TWP emulsions with different salts and salt concentrations over 7 days with standard deviation (n= 6) ($p < 0.05$).

4.5 Conclusion

The chemical composition of TWP was found to be 5.23 ± 1.04% moisture, 15.08 ± 0.35% crude protein, 1.54 ± 0.29% total fat, 15.09 ± 1.74 ash and 62.88 ± 2.77 carbohydrates. TWP appears to provide a high amount of dietary fibre when concentrated, with magnesium,

potassium, and chloride as the main minerals. The SEM structure of TWP was crystalline, likely due to interactions between proteins and carbohydrates. Its intense yellow suggests a decent number of bioactive compounds, such as riboflavin, a vitamin responsible for this colour in TWP. Additionally, TWP's antioxidant, phenolic, and saponin contents were proportionately similar to those in TWL, suggesting that freeze-drying does not significantly affect these bioactive compounds. This enhances TWP's potential as a functional food ingredient.

The foaming functionality of TWP exhibited significant changes in foam stability and liquid drainage as concentration increased, though there was no difference in foam capacity at different concentrations, possibly due to blending time. Furthermore, the effect of pH on foaming showed optimal results between pH 4 and 5. However, it is not certain that tofu whey may display better surface tension near its isoelectric point. Regarding emulsion functionality, low TWP concentrations were most effective with low oil concentrations, while high TWP concentrations worked best with high oil concentrations due to flocculation at low TWP concentrations and depletion flocculation at high TWP concentrations. Furthermore, the effect of pH on emulsions exhibited excellent results at pH of 7-9 and pH 2, while middle pH ranges showed increased particle clumping and larger particle sizes. Regarding salt effects, $CaCl_2$ increased particle size in tofu whey emulsions, while NaCl decreased particle size, possibly due to differences in ion interactions. Interestingly, increasing NaCl concentration up to 500 mM stabilised tofu whey emulsions, though the underlying mechanism remains unclear and requires further investigation. Furthermore, based on the research presented in Chapters 3 and 4, the state of tofu whey whether its dry form (TWP) or wet form (TWL) does not significantly influence functionality. Rather, the concentration of tofu whey appears to be the more critical factor affecting functionality.

This research provides fundamental insights into the characteristics and functional properties of tofu whey, which inform future studies and demonstrate its value as a functional food ingredient.

Chapter 5. Application of tofu whey in meringues

5.1 Abstract

In a growing world population, the demand for more cost-effective and environmentally friendly alternative in food production are higher and higher due to customer and activist. As foods are getting more expensive due to the growing world population, upcycling ingredient could be a cheap alternative to provide the food industry with new material which can be used to produce alternative product. This allow tofu whey, a by-product which is derived from soybeans to be used in alternative food product like meringue. As it contains plant proteins and saponins which can be used as surfactants to help improve food functionally in meringue such a foam stabilizer. Incorporating tofu whey into meringue, as a possible EW replacement was done using TWL and TWP. The meringue was analysis by rheology and air fraction for the meringue batter and textural and physical properties for the cooked meringue. The meringue batter for TWP when compared to EW display no significant changes in density and ϕ while TWL showed higher density and lower ϕ, which was seen in the change in viscosity of the batter. The texture analysis of cooked meringues shows no impact on texture properties when minimal TWL or TWP was added.

5.2 Introduction

In recent times, there has been a progressive improvement in living standards for a growing world population, leading to a high demand for high-quality foods. This demand leads to faster agricultural production growth, raising environmental concerns and challenging the sustainability of the food industry (Khan et al., 2021). Industries are meeting the growing demand for animal-derived products in the market, driven by their desirable sensory properties. For example, animal proteins such as egg white (EW) demonstrate strong functional properties such as foaming, making them indispensable in many foods such as cakes, chocolates, mousses and meringues (Ellis & Lazidis, 2018; Wouters et al., 2018). However, the production of these food products is getting more expensive and are contributes significantly to environmental impacts such as global warming and pollution. The rise in consumer demand for high quality products raises these changes, pressuring industries to either meet these demands or shift toward an alternative source.

Furthermore, consumers and activists are placing significant demands on agricultural production. For example, in New Zealand, starting in early 2023, battery caging of hens was banned due to animal welfare concerns (Morris, 2006). However, this change caused an egg shortage in the market and an increase in egg prices. Consequently, changing from animal proteins to plant-based proteins could offer a more cost-effective and environmentally friendly alternative for the food industry.

Tofu whey, a by-product of tofu production, presents an opportunity to utilize plant proteins. As a waste product, tofu whey incurs disposal costs but could serve as a valuable resource of plant proteins. It is derived from soybeans and contains soy proteins and saponins, which can function as surfactants. Foams used in food products are aeration that trap gas dispersions within a liquid. These foams are typically immiscible and thermodynamically unstable, but they can be stabilized using surfactants or proteins. Surfactant, such as saponin from tofu whey and proteins like soy protein diffuse into the foam interfaces to lower surface tension. This process provides steric and electrostatic barriers, which can prevent gas bubbles from merging and thereby reducing foam coarsening and collapse over time (Martínez et al., 2009; Ossa et al., 2020; Zhu, Yin, et al., 2020).

To the best of our knowledge, no research has been conducted on the application of tofu whey or extensive studies on soy proteins in the production of meringues. This chapter aims to investigate the potential of tofu whey as a substitute for egg white in producing meringues with textural characteristics similar to traditional ones. Additionally, it examines the impact of tofu whey on the rheological properties of meringue foam. The study seeks to provide significant insights into the application of plant-based alternatives in meringue production while valorising tofu whey as a functional food product. Utilizing tofu whey not only enhances its value but also reduces its treatment costs through upcycling.

The study focuses on two components: the meringue batter and the cooked meringues. For the meringue batter, key parameters include air incorporation (ϕ), density, rheology and viscosity. For the cooked meringues, the emphasis is on specific volume and textural properties. By addressing these factors, this research aims to contribute to the development of sustainable, plant-based alternatives in the food industry.

5.3 Methods and Materials

5.3.1 Materials

Fresh eggs and Pam White sugar were purchased from a local grocery store. Tofu whey liquid (TWL) was prepared as described in Section 3.3.1. Additionally, a solution containing 10% tofu whey powder (TWP) was also prepared from freeze-dried tofu whey powder following the method outlined in Section 4.3.1. This is denoted as 10% TWP in the ingredient formulations for making meringues. In this study on meringue production, it should also be mentioned that sodium azide was not added to the TWL and TWP solution as an antimicrobial agent.

5.3.2 Tofu whey liquid (TWL) and Tofu whey powder (TWP)

As mentioned in the material TWL is the liquid that is collected from Tonzu and used straight away in the making of meringues without any changes to the material meaning that it is mostly water as shown in chapter 3 in Table 3.4. While TWP is powder that is collected from freeze drying TWL and then is made to a 10% solution using 10g of TWP and 90g of water.

5.3.3 Preparation of meringues

The meringue batter was prepared with slight modifications to the method described by Yüceer & Caner (2021). Table 5.1 presents the ingredient formulations used in meringue production, where EW and TWL (or TWP) indicates the combined weight (30 g) of egg white (EW) from fresh hen eggs and tofu whey liquid (TWL) or 10% tofu whey protein (TWP) solution used in each formulation. The proportions of EW and TWL (or TWP) varied based on the tested ratios or combinations. Additionally, 50 g of sugar was incorporated into each batter. EW from fresh hen eggs contains approximately 88% water and 11% protein (Wouters et al., 2018).

Formulations using mixtures of EW and TWL (or 10% TWP solution) at four different ratios (100:0, 75:25, 50:50, 0:100) are denoted as EW 100, EW:TWL (75:25), EW:TWL (50:50) and TWL 100, respectively. Similarly, formulations containing mixtures of EW and 10% TWP solution at the same ratios are referred to as EW 100, EW:TWP (75:25), EW:TWP (50:50), and TWP 100, respectively.

The preparation process began by whisking the 30 g mixture using a Breville Wizz mix 320w at speed 3 for 2 minutes. Sugar was gradually added starting 30 seconds into whisking. After all the sugar was added, the speed was reduced to speed 2, and the batter was whipped for an additional 8 minutes.

The meringue batter was transferred to a piping bag and piped onto a tray lined with baking paper, with each meringue weighing approximately 3-4 grams and roughly around the same volume. The tray was then placed into a preheated Turbofan 32 Max oven at 100^{o}C for 10minutes and then baked for an hour and 30 minutes. After baking, the oven was turned off, and the meringues were allowed to cool for 2 hours inside the oven. After cooling, pictures of the samples were taken to capture a representative image of the changes in the formulation.

Table 5.1. Ingredient formulations for making meringues.

Meringue Samples	**Egg White (g)**	**TWL or TWP (g)**	**Sugar (g)**	**Total (g)**	**Egg white (%)**	**Tofu whey (%)**	**Sugar (%)**	**Total (%)**
EW (100)	30	0	50	80	37.5	0	62.5	100
EW:TWL (75:25)[a]	22.5	7.5	50	80	28.1	9.4	62.5	100
EW:TWL (50:50)[b]	15	15	50	80	18.7	18.8	62.5	100
TWL (100)[c]	0	30	50	80	0	37.5	62.5	100

[a]EW:TWL (75:25) or EW:TWP (75:25)
[b]EW:TWL (50:50) or EW:TWP (50:50)
[c]TWL (100) or TWP (100)

5.3.4 Air fraction (ϕ) of meringue batters

The volume of air fraction (ϕ) in the meringue was measured using the methods described by Wouters et al. (2018), with slight modifications. The air fraction was calculated using the equation below.

$$\varphi = 1 - \frac{\rho_b}{\rho_0} \qquad \text{(Equation 5.1)}$$

Where ρ_b is the density of the batter after whipping and ρ_0 is the density of the batter before whipping

The ρ_b was measured by filling a known volume contain with the whipped meringue and its weight was measured using a balance The ρ_0 was determined by measuring the weight of the unwhipped batter using a graduated cylinder and a balance.

5.3.5 Rheological and viscosity measurements of meringue batters

The rheology and viscosity properties of meringue batters were measured using the methods described by Nastaj et al. (2021), with slight modifications. The measurements were determined using an AR550 Rheometer (Alpha Tech) equipped with a 40 mm steel plate geometry. The viscosity was measured over a shear rate range of 0.1 – 250 s^{-1} at 25^{o}C. The rheological measurements were also conducted using the same geometry, with a frequency sweep test carried out at a range of 0.1-10 Hz at a strain of 0.1 s^{-1}. Changes in the storage modulus (G') and loss modulus (G'') were recorded during the frequency sweep. The loss tangent (Tan δ) was calculated at a frequency of 1 Hz using the following equation.

$$Tan\ (\delta) = G''/G' \qquad \text{(Equation 5.2)}$$

5.3.6 Specific volume measurement of meringues

The specific volume of meringue was measured using a method adapted from the bread volume measurement method. The meringue was placed in a container of known volume, and rapeseed was added to fill the gaps around the meringue. The volume of the meringue was determined by calculating the difference between the volume of rapeseed in the container without the meringue (V_M) and the volume of rapeseed in the container with meringue (V_R). The following equations were used to calculate the volume and specific volume of the meringue.

$$Volume\ of\ Meringue\ (mL) = V_M - V_R \qquad \text{(Equation 5.3)}$$

$$Specific\ Volume\ of\ Meringue\ \left(\frac{mL}{g}\right) = \frac{Volume\ of\ Meringue\ (mL)}{Weight\ of\ Meringue\ (g)} \qquad \text{(Equation 5.4)}$$

Here, V_M is the volume of rapeseed in the container without the meringue, while V_R is the volume of rapeseed in the container with the meringue. The weight of the meringue was measured to calculate its mass for determining the specific volume.

5.3.7 Texture measurement of cooked meringues

The texture profile analysis (TPA) of cooked meringues was conducted on different meringue formulations using a texture analyser (TA-XT plus 11390 Alphatech system). The parameters measured included hardness, fracturability, adhesiveness, springiness,

cohesiveness, gumminess, chewiness and resilience. Texture measurements were performed within 30-minutes after the meringues had cooled after baking. The texture analyser settings were adapted from the method described by Yüceer and Caner (2021) with slight modifications. A 35R probe with a 35 mm diameter and a contact radius of 962.11 mm^2 was used to deal with the irregular shape of meringues. The test settings included: pre-test speed of 5.0 mm/s, test speed of 1.0 mm/s and post-test speed of 5.0 mm/s, trigger force of 5 g and 25% strain. The texture analysis was carried out using the texture exponent software program to measure changes in the meringues' physical properties.

5.3.8 Statistical analysis

All experiments and measurements were carried out in triplicate, and the data are expressed as means ± standard deviations (SD). Minitab software (Version 21.1.0.0, Pennsylvania, USA) was used for a one-way analysis of variance (ANOVA), and Fisher's Least Significance Difference (LSD) test was used to identify significant differences between results.

5.4 Results and Discussion

5.4.1 The density and air fraction of meringue batters

The density and air fraction (ϕ) are critical factors in food systems such as meringues and ice creams, as air volume contributes significantly to the texture and mouthfeel of these foods (Wierenga et al., 2012). Although not much is known about the effect of air on the texture and mouthfeel of meringues, it is more common in ice cream that higher overruns are associated with lighter textures and softer mouthfeel (Sofjan & Hartel, 2004). This suggests that air may similarly affect the texture and sensory characteristics of meringues.

Table 5.2 show the density and ϕ of meringue batters prepared with different ratios of EW and TWL or 10% tofu whey protein (TWP) solution. For meringues containing 10% TWP at all different ratios, ϕ showed no significant changes compared to EW meringues. However, density measurements revealed significant differences ($p < 0.05$) between EW and TWP meringues at 100% and 50% TWP but not at 25% TWP. In contrast, meringue batters made with TWL exhibited significant differences ($p < 0.05$) in both density and ϕ at all ratios compared to EW meringues. This outcome was in line with the hypothesis that higher

protein content is associated with lower density and improved ϕ in meringue batters. Notably, 100% EW contained around 11% protein, while 10% TWP contained about 1.5% protein, and TWL contained only 0.38% protein.

EW meringues exhibited better density and ϕ than the EW/TWP meringues, indicating a lower density and higher aeration. Interestingly, despite its lower protein content (e.g. 1.5% protein), 10% TWP meringues showed no significant difference in ϕ compared to EW meringues. This result may be attributed to the presence of a protein-polysaccharide-saponin network matrix in tofu whey, as these component have been shown to enhance foamability (Ossa et al., 2020; Zhu, Yin, et al., 2020). However, the mechanism underlying the formation of this matrix is still unknown and is a possible avenue for further research in tofu whey. Understanding these mechanisms is crucial for leveraging tofu whey as a functional food ingredient.

Table 5.2 The density and air fraction (ϕ) of meringues.

SAMPLE	DENSITY (g/ML)	AIR FRACTION (Φ)
EW (100)	0.431 ± 0.004^{e}	0.649 ± 0.009^{a}
EW:TWP (75:25)	0.440 ± 0.019^{de}	0.646 ± 0.009^{a}
EW:TWP (50:50)	0.452 ± 0.003^{cd}	0.642 ± 0.006^{a}
TWP (100)	0.459 ± 0.002^{cd}	0.638 ± 0.009^{ab}
EW:TWL (75:25)	0.466 ± 0.006^{bc}	0.626 ± 0.001^{bc}
EW:TWL (50:50)	0.481 ± 0.008^{b}	0.615 ± 0.007^{c}
TWL (100)	0.566 ± 0.017^{a}	0.560 ± 0.014^{d}

$^{a\text{-}e}$ the same letter within the column indicates no significant differences between samples (n= 6) ($p < 0.05$).

5.4.2 Physical appearance and specific volume of meringues

The dimensions of the cooked meringues are displayed in Figure 5.1 and Table 5.3. The meringue diameter was measured at the base, and the height was measured vertically from the centre. For 10% TWP meringues, slight variations in dimensions were observed between different ratios, but no significant differences were seen.

In contrast, TWL meringues showed a noticeable change as the ratio of EW decreased. The height of the meringues decreased, while their diameter increased, resulting in a flatter, more cookie-like shape. This phenomenon can be attributed to the composition of TWL,

which contains significantly lower levels of protein, carbohydrates, and saponins needed to form a stable structure in the meringue. At lower ratios of EW to TWL, the batter has a higher water content and fewer solid components, leading to a weaker meringue structure. Consequently, when the meringue batter is piped, it does not hold its shape, causing the meringues to spread and flatten.

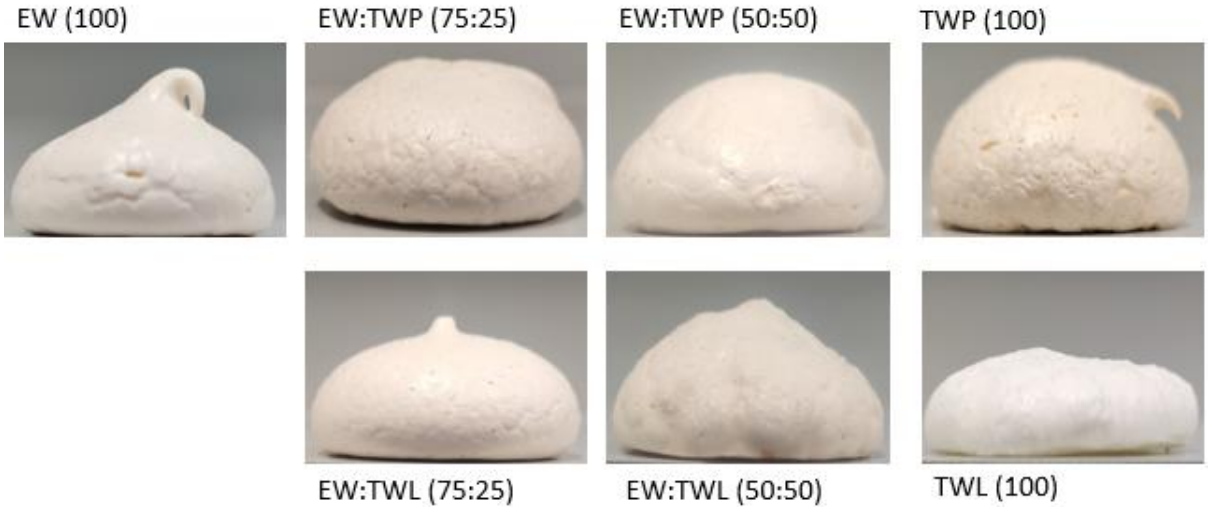


Figure 5.1. Images of cooked meringues prepared with various formulations using fresh egg white (EW), tofu whey liquid (TWL), a 10% solution of tofu whey powder (TWP), or combinations of EW/TWL or EW/TWP at different ratios.

Table 5.3. Dimensions of meringues measured with a ruler.

SAMPLE	DIAMETER (CM)	HEIGHT (CM)
EW (100)	3.53 ± 0.15^{bc}	1.80 ± 0.10^{a}
EW:TWP (75:25)	3.37 ± 0.21^{c}	1.67 ± 0.06^{ab}
EW:TWP (50:50)	3.33 ± 0.12^{c}	1.70 ± 0.10^{ab}
TWP (100)	3.40 ± 0.10^{bc}	1.60 ± 0.10^{ab}
EW:TWL (75:25)	3.53 ± 0.06^{bc}	1.50 ± 0.20^{b}
EW:TWL (50:50)	3.63 ± 0.15^{b}	1.20 ± 0.10^{c}
TWL (100)	3.97 ± 0.15^{a}	0.5 ± 0.10^{d}

$^{a-d}$ the same letter within the column indicates no significant differences between samples ($n= 6$) ($p < 0.05$).

A specific volume is the ratio of volume to mass that an object holds. It is commonly used in baking to compare samples of different weights. The results for the specific volume of meringues shown in Table 5.4 indicate that there was no significant difference between EW and 10% TWP at any ratio. For TWL, at 75:25 EW to TWL ratio, there was no significant

difference, but at the other two ratios, significant differences were observed ($p > 0.05$). These results align with our hypothesis, based on the ϕ data, as the specific volume of meringues should correspond with ϕ, as suggested by.

Furthermore, the specific volume of 100% EW (7.16 ± 0.65 mL/g) was not within the range reported by Wouters et al. (2018), which was 3.5-4 mL/g, even though the ϕ values were similar (around 0.65-0.68). However, the specific volume of EW was consistent with the 7-8 mL/g range reported by O'Charoen et al. (2014), who used egg white protein from fresh hen eggs, compared to the EW protein powder used in this study. This suggests that 10% TWP at all ratios and TWL at the 75:25 EW to TWL ratio could be used as a substitute for making meringues, as the specific volume of meringues made with tofu whey and egg white seem to be not significantly different.

Table 5.4 Specific volume of cooked meringues

SAMPLE	SPECIFIC VOLUME (ML/G)
EW (100)	7.16 ± 0.65^{a}
EW:TWP (75:25)	7.13 ± 0.25^{a}
EW:TWP (50:50)	7.10 ± 0.34^{a}
TWP (100)	7.06 ± 0.51^{a}
EW:TWL (75:25)	6.80 ± 0.33^{a}
EW:TWL (50:50)	6.37 ± 0.25^{b}
TWL (100)	5.52 ± 0.41^{c}

$^{a-c}$ the same letter within the column indicates no significant differences between samples ($n= 6$) ($p < 0.05$).

5.4.3 Viscosity of meringue batters

The viscosity of the meringue batters was measured to determine their resistance to deformation as a fluid. Figure 5.2 illustrates the effect of 10% TWP and TWL on the viscosity of the meringue batters. The results show that as the shear rate increases, the viscosity of the solution decreases. A noticeable difference between the samples was observed at a shear rate of around 25 s^{-1}, where meringue batters containing higher levels of EW (100% or 75%) exhibited better viscosity than those with lower EW content. Beyond this point, 10% TWP (100%) showed viscosity values similar to the other samples, while TWL (100%) exhibited a significantly lower viscosity at the start.

The lower viscosity of the TWL meringue batter can be attributed to its high moisture content and lower total solid content, which resulted in less structure formation during mixing compared to the other meringue batters. (Belén et al., 2013) similarly observed that as the concentration of tofu whey decreased, so did its viscosity. The viscosity results of the meringue batter aligned with our hypothesis, as the trend was similar to the density and ϕ data in Table 5.2. The TWL sample was much liquid-like and had less resistance to deformation when piped.

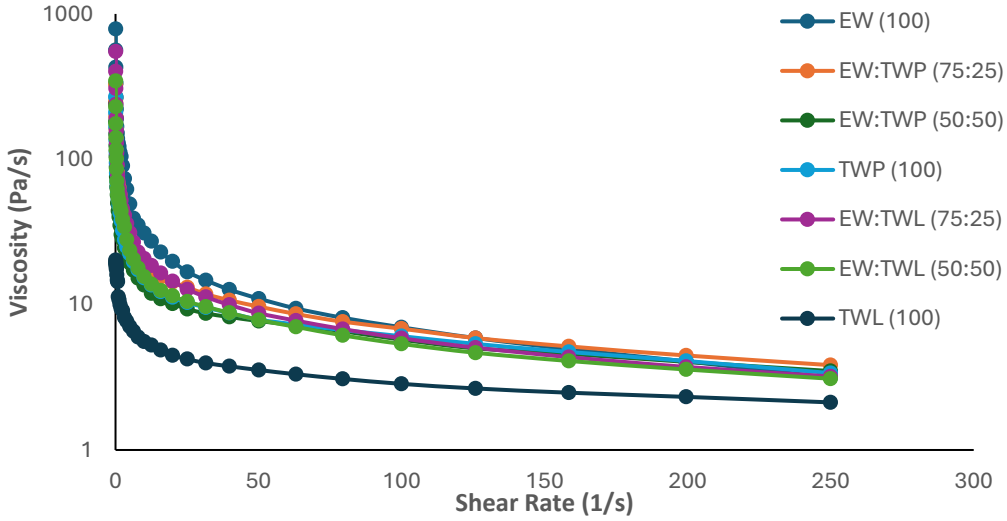


Figure 5.2 Viscosity of the meringue batters with various formulations after mixing.

5.4.4 Rheology of Meringues

Rheology was measured on the meringue batters to identify their flow and deformation, which helps characterize the fundamental properties of the batters. Figure 5.3 demonstrates the effect of TWL and 10% TWP on the meringue batter's G' (storage modulus), G'' (loss modulus) and tan (δ).

Tan (δ) measures the ratio between the loss and storage energy during the deformation cycle, which is used to identify the elastic and viscous properties of a sample. When tan (δ) is greater than 1, G'' is greater than G', indicating a more viscous (liquid-like) structure. Conversely, when tan (δ) is less than 1, G' is greater than G'', indicating a more elastic (solid-like) structure (Murata, 2012; Tabilo-Munizaga & Barbosa-Cánovas, 2005).

Figure 5.3 (A) shows that at lower frequencies (0.1-1 Hz), G' dominated over G'', indicating that the foam showed a solid-like behaviour. However, as the frequency increased (1-10 Hz),

at lower ratios of EW and high ratios of TWL and 10% TWP (50:50 and 0:100), G'' began to dominate, displaying more liquid-like behaviour in the foam. For the 1-10 Hz range, TWL samples started displaying the liquid-like behaviour earlier than 10% TWP. No significant changes occurred at higher ratios of EW and lower ratios of TWL and 10% TWP (100:0 and 75:25), and the foams continued to display solid-like behaviour. However, for the 75:25 ratio of EW to TWL at 10 Hz, an increase in tan (δ) was observed, bringing it closer to 1 and indicating more viscous properties in the TWL foam. Although tofu whey has not been previously used to make meringue batter foam, the results for the EW meringue batter were similar to those for 10% native egg white protein meringue batter (Alavi et al., 2020), where G'' approached G' over a frequency range of 0.1 – 10 Hz, displaying more viscous-like properties.

Furthermore, (Alavi et al., 2020) explained that the spherical-shape of native egg white protein (EWP) led to it being more easily tangled, resulting in poor shear thinning behaviour. This theory is also in line with why tofu whey meringue foam shows a similar trend in terms of G'' and G', as the 11S and 7S protein components in tofu whey are also spherically shaped (Kao et al., 2003; Zhao et al., 2011), which could lead to the poor shear thinning behaviour. Additionally, the results of strong foam elasticity and its solid-like behaviour correlated with increased air incorporation, as shown in Figure 5.3 (B and C). The TWL meringue batter had significantly lower G' and G'' values compared to EW, while 10% TWP showed similar G' and G'' values to EW due to the increased solid content, which provided more structure to the foam and allowed higher air incorporation. Higher air incorporation in foam enhances elasticity and solid-like behaviour, which is consistent with previous studies (Nastaj et al., 2021; Wouters et al., 2018).

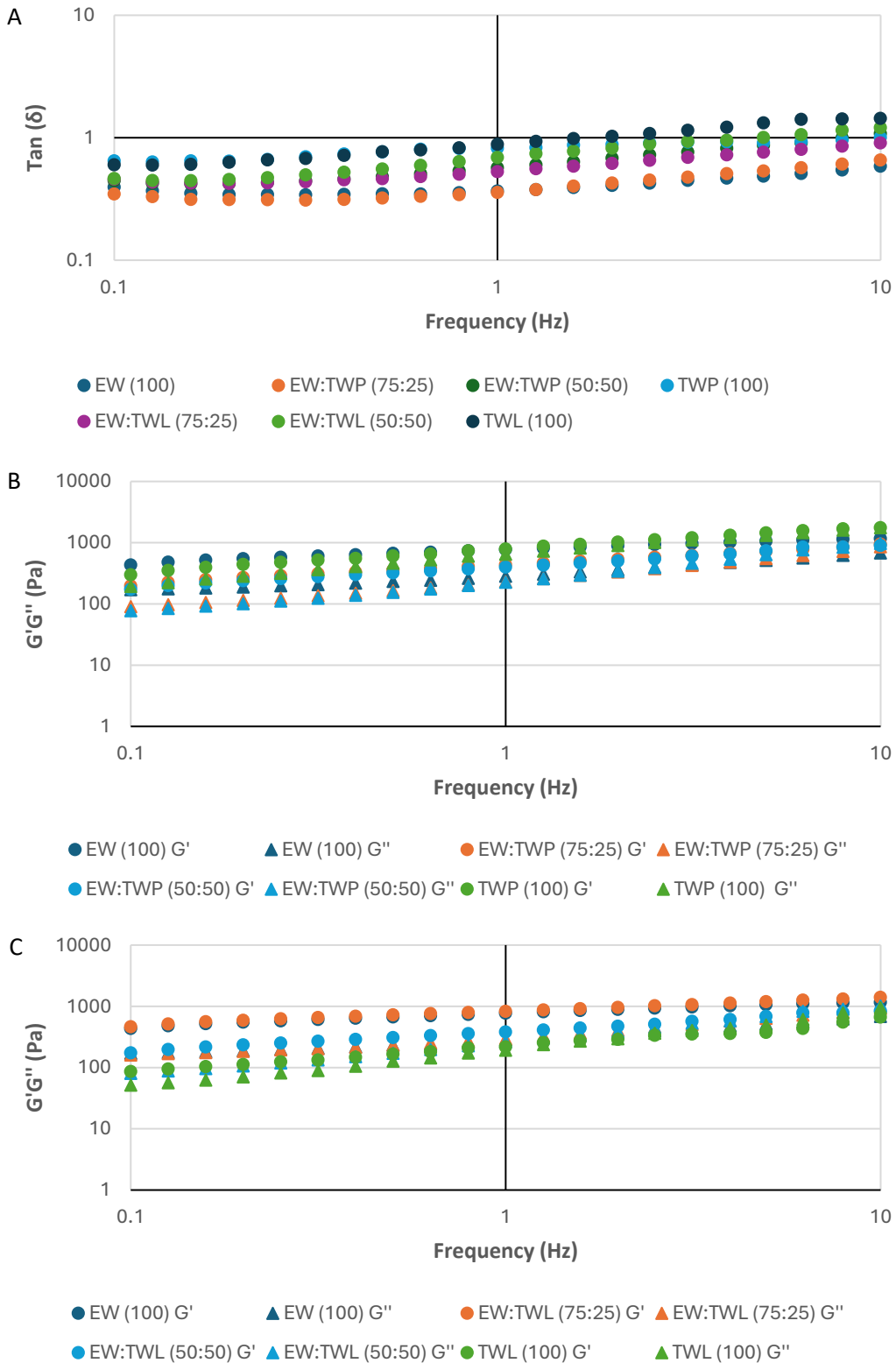


Figure 5.3: Frequency sweep of meringue batter for Tan (δ) (A) and G'/G'' of TWP (B) and TWL (C)

5.4.5 Texture analysis of meringues

Texture plays a crucial role in consumer acceptance of food, significantly influencing food intake and providing insights into the quality of the food being consumed (Kadam et al., 2015; KILCAST & LEWIS, 1990). The texture of meringues made with TWL and 10% TWP was measured using texture profile analysis (TPA).

The data in Table 5.5 show that as the ratio of EW decreases, the hardness value of the meringues also decreases ($p < 0.05$). Specifically, meringues made with 10% TWP (1623 ± 167) exhibited lower hardness than those made with EW (3558 ± 711). Regarding fracturability, 10% TWP meringues were comparable to EW meringues, showing no significant difference. In contrast, TWL meringues (at all ratios: 535 ± 251, 468 ± 241) exhibited significantly lower fracturability than EW meringues (705 ± 148).

Adhesiveness increased significantly as the proportion of EW decreased ($p > 0.05$). Meringues with high ratios of EW (100% and 75%) displayed adhesive values of -40.74 ± 9.36, -38.03 ± 5.05 and -40.70 ± 7.85, respectively, for 100% EW, 75:25 EW:10% TWP, and 75:25 EW: TWL, respectively. In contrast, meringues with low ratios of 10% TWP, 50:50 EW:10% TWP and 50:50 EW:TWL displayed values of -0.62 ± 0.74, -3.35 ± 4.96 and -2.39 ± 4.61, respectively.

The chewiness values were interesting. There was no significant difference between meringues made with EW (34.23 ±18.25) and TWL (31.29 ± 24.91) and 10% TWP (39.55 ± 37.06) at the 75:25 ratio and 50:50 ratio for TWL (30.94 ± 29.71). However, a significant decrease in chewiness for 10% TWP (2.23 ± 2.45) and 50:50 ratio of 10%TWP (15.92 ± 9.11). The change in chewiness values might be due to the differences in the soy protein content, which could affect the structure of the meringue. Increased soy protein may influence chewiness when compared to 100% EW or higher ratios of EW. TWL, which contains a lower amount of soy protein compared to 10% TWP, may explain why no significant effect on chewiness was observed at the 50:50 ratio.

While no previous texture profile analysis has been done on tofu whey or soy protein meringues, the texture profile analysis of EW meringues in this study aligns with findings from (Yüceer & Caner, 2021), with the exception of significantly lower adhesiveness.

Furthermore, it seems that adding TWL and 10% TWP in low ratios does not significantly alter the texture of meringues. However, further research involving consumer acceptability and sensory evaluation is required to assess the potential of using tofu whey as a functional food ingredient in industrial meringue production.

Table 5.5 The texture profile analysis (TPA) values of meringues with EW, TWL and 10% TWP.

SAMPLE	HARDNESS (G)	FRACTURABILITY (G)	ADHESIVENESS (G/S)	SPRINGINESS	COHESIVENESS	GUMMINESS	CHEWINESS	RESILIENCE
EW (100)	3557 ± 710^{a}	705 ± 147^{ab}	-40.74 ± 9.36^{b}	0.10 ± 0.04^{a}	0.10 ± 0.04^{a}	394 ± 212^{a}	34.23 ± 18.25^{ab}	0.08 ± 0.03^{a}
				10% TWP				
EW:TWP (75:25)	3011 ± 327^{b}	775 ± 250^{a}	-38.03 ± 5.05^{b}	0.16 ± 0.16^{a}	0.09 ± 0.02^{ab}	269 ± 101^{b}	39.55 ± 37.06^{a}	0.06 ± 0.02^{ab}
EW:TWP (50:50)	2112 ± 397^{c}	592 ± 130^{abc}	-3.35 ± 4.96^{a}	0.18 ± 0.27^{a}	0.06 ± 0.04^{b}	154 ± 117^{b}	15.92 ± 9.11^{bc}	0.05 ± 0.03^{b}
TWP (100)	1622 ± 166^{d}	729 ± 228^{ab}	-0.62 ± 0.74^{a}	0.10 ± 0.09^{a}	0.01 ± 0.01^{c}	18 ± 10^{c}	2.23 ± 2.45^{c}	0.01 ± 0.00^{c}
				TWL				
EW:TWL (75:25)	3160 ± 439^{ab}	534 ± 251^{bc}	-40.70 ± 7.85^{b}	0.11 ± 0.07^{a}	0.08 ± 0.02^{ab}	268 ± 79^{b}	31.29 ± 24.91^{ab}	0.06 ± 0.01^{ab}
EW:TWL (50:50)	2427± 398^{c}	468 ± 241^{c}	-2.39 ± 4.61^{a}	0.20 ± 0.15^{a}	0.06 ± 0.03^{b}	159 ± 77^{b}	30.94 ± 29.71^{ab}	0.05 ± 0.02^{b}
TWL (100)	N.D	N.D	- N.D	N.D	N.D	N.D	N.D	N.D

$^{a-c}$ the same letter within the column indicates no significant differences between samples (n= 6) ($p < 0.05$).

5.5 Conclusions

The results suggest that 10% TWP had less impact on the meringue batter compared to TWL, when being used as an egg replacement in meringues. The density and ϕ values of the meringue batters with 10% TWP and EW at different ratios were not significantly different, while TWL at high ratios showed higher density and lower ϕ values. These results were reflected in the viscosity and rheology of the meringue batters, where only the 100% of TWL meringue batter showed a significantly lower viscosity curve. Additionally, high ratios (100% and 50:50) of TWL and 100% of 10%TWP meringue batters showed tan (δ) values greater than 1, meaning a more liquid-like behaviour.

Regarding physical appearance, 10% TWP at all ratios appeared similar to EW meringues, with no significant changes in specific volume measurements. In contrast, meringues with high ratios of TWL showed a reduction in size, particularly in height, which was also reflected in the significantly different specific volume measurements compared to EW meringues.

For the texture of meringues, only at high ratios of EW were there no significant changes or only slight changes across various attributes, including hardness, fracturability, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience. These results suggest that further studies focusing on the sensory attributes of these meringues are necessary. However, from a physical perspective, adding tofu whey as a functional ingredient in meringues at low percentages appears to have minimal impact on their properties.

Chapter 6. Overall Conclusions & Recommendations

6.1 Research Outcomes

This research project was carried out to investigate the chemical and physical properties of tofu whey and its effect as a functional food ingredient. The chemical composition of tofu whey liquid (TWL) was 97.34 ± 0.23% moisture, 0.38 ± 0.05% crude protein, 0.10 ± 0.06% fat, 0.43 ± 0.05% ash, 1.76 ± 0.23% carbohydrate, and 0.10 ± ND% dietary fibre. The chemical composition of tofu whey powder (TWP) was found to be 5.23 ± 1.04% moisture, 15.08 ± 0.35% crude protein, 1.54 ± 0.29% total fat, 15.09 ± 1.74% ash and 62.88 ± 2.77% carbohydrates, which was roughly what was predicted beside the fat which was a bit on the higher side. The main crude protein components were β-conglycinin subunits β and β', glycinin acid polypeptide chain, essential polypeptide chains, and low molecular weight protein of 15 kDa, while the main mineral components were magnesium, potassium, and chloride and lastly, the primary sugar components were sucrose.

Regarding functionality, tofu whey exhibits adequate antioxidant activity, with the phenolic and saponin content found in tofu whey, which can also be seen in its potential effect as an anti-browning agent. It showed promising results against low concentrations of citric acid at stopping polyphenol oxidase activity but was not comparable against ascorbic acid and sodium bisulphite. Besides its effect as an anti-browning agent, it showed high potential as an emulsifying agent and foaming agent in TWP, as it shows upwards of 88.33% to 94.17% foaming capacity at three different concentrations of TWP (3, 6, and 10%) which was further enhanced at pH 4 showing 103.13%. At the same time, TWP expressed good foam stability at a concentration of 6%, 10% and a pH of 4 and 5 since they were above 60% foam stability after 30 minutes. However, regarding tofu whey as an emulsifying agent, low TWP concentrations were most effective with low oil concentrations, while high TWP concentrations worked best with high oil concentrations due to flocculation at low TWP concentrations and depletion flocculation at high TWP. The effect of pH can be seen at very low or very high pH, enhancing the emulsification of tofu whey in terms of particle size and even stability as displayed by high negative or positive electrostatic energy, as seen in the

zeta potential. As for salts, NaCl decreased the particle size, and $CaCl_2$ increased the particle size while interestingly increasing NaCl concentration up to 500 mM stabilised tofu whey emulsions.

The study on meringues demonstrates that incorporating TWL and 10% TWP into meringues impacts batter and foam properties. At lower incorporation ratios, 10% TWP performs comparably to egg white (EW) meringues, with minimal effects on batter density, specific volume, and textural attributes, making it a viable functional ingredient. Conversely, high ratios of TWL result in denser batter, reduced foam stability, and significant changes in texture, highlighting limitations in its application, while high ratios of TWP might not result in denser batter, reduced foam stability, but its textural aspect is left to be desired when compared to EW.

6.2 Recommendations

This research aims to upcycle or valorise tofu whey so that it can be used instead of treated as wastewater. With the physical attributes of tofu whey shown, tofu whey has a high salt content naturally due to the primary coagulation technique being salt. This allows tofu whey the ability to be upcycled and developed into a sports drink with electrolytes, as it is already shown when undergoing fermentation, can be turned into an alcoholic beverage that is on the market. Aside from high salt, tofu whey seems to have decent protein and sugar content for fermentation and showed a new frontier of research in tofu whey to further valorise as an ingredient in foods or even as a food source for microbes to promote further or enhance the growth of another resource.

This study only looked at the functionality of tofu whey on the tofu whey liquid after being strained, and the small tofu whey curds were lost. However, instead of straining out the curds from tofu whey, blending them into the tofu whey could be a way to valorise tofu whey as it provides high protein, carbohydrate, and even more fat. Furthermore, the further effects on tofu whey, such as ultrasonication and fermentation, should be studied to get a good overview of the best way to valorise tofu whey functionality as a food ingredient. Different drying methods to concentrate the tofu whey and valorise it as cheaply as possible will help it get into the mainstream food production industry faster.

Sensory evaluation and consumer acceptability studies are recommended to explore tofu whey's potential as a substitute for egg whites in meringues. Additionally, research on optimizing processing methods, such as enzymatic modification or blending with other functional ingredients, could improve the performance of tofu whey in meringues and other food systems. These findings provide insights into tofu whey's role in sustainable food formulations and encourage its utilization as a by-product in value-added applications.

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