Development of edible packaging film from whey protein phospholipid concentrate

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Teguh Santoso, 1st July 2022

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Abstract

In an attempt to utilise the natural fat content in whey protein phospholipid concentrate (WPPC), this study formulated, optimised, characterised, and compared edible films made from WPPC with films made from whey protein concentrate (WPC) and whey protein isolate (WPI). The formulation and processing utilised techniques that are considered generally safe for consumption. It was possible to produce a WPPC film using 16% (w/w) WPPC solution and glycerol at a 2:1 WPPC:GLY ratio. In terms of appearance and transparency, all films had good transparency with greater than 50% transparency at 600 nm. WPPC and WPC film were also good at blocking the UV wavelength. The WPPC and WPC films were yellow, while the WPI film was opaque/colourless. Out of the three films, the WPI film had the best appearance. As for the films' mechanical properties, WPPC films had significantly lower tensile strength and elongation at break than the WPC and WPI films. In addition, WPPC films were very brittle compared to the WPC and WPI films. The water barrier properties of the films were interesting. WPPC films retained more water in the film matrix and performed better as a water barrier, as demonstrated by the swelling index and water vapour permeability (WVP) values, which were lower for WPPC films than WPC and WPI films. The higher moisture content, lower swelling index, and WVP suggest that either fat (phospholipid) or lactose influences the interaction between WPPC film and water and should be investigated further. Based on the film's characteristics, the best film was from WPI. WPPC films are not practical with the current formulation and processing techniques.

Keywords: edible packaging material, edible film, protein films, whey protein phospholipid concentrate, physical properties, water vapour permeability

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List of Abbreviations

EAB	Elongation at break
FFS	Film-forming solution
GLY	Glycerol
TG	Transglutaminase
TS	Tensile strength
WPI	Whey protein isolate
WPC	Whey protein concentrate
WPPC	Whey protein phospholipid concentrate
EPM	Edible packaging material
WVP	Water vapour permeability

1. Introduction

Petroleum-based packaging materials are used as packaging material in almost every product. They are popular materials because they are cheap to produce and durable whilst providing excellent protection (Muscat et al., 2012). However, despite its popularity, petroleum-based packaging contributes to serious environmental problems due to its resistance to degradation. In addition, the demand for petroleum-based packaging is increasing due to the rise in the human population, which significantly stresses our limited resources (Krochta, 2002). Therefore, researchers have focused on developing more eco-friendly packaging to mitigate environmental damage caused by synthetic and non-biodegradable packaging. Consumers support the eco-friendly trend as they gain more awareness of the environmental problems caused by non-biodegradable packaging (Tyagi et al., 2021). Subsequently, the industry responded to the consumers' demand by utilising edible packaging material (EPM). EPMs are biopolymers made from polysaccharides, proteins, and lipids (Janjarasskul & Krochta, 2010). These materials are usually sourced from agricultural commodities or food processing by-products. Since they are food products, EPM can be used to create edible film packaging (edible films), defined as 'packaging films, sheets, coating, or pouches that consumers can eat as part of the product' (Guilbert et al., 1995). Edible films are desirable as packaging because they are biodegradable, edible, and non-toxic (Çakmak et al., 2020).

The development of edible films has been complex as an ideal EPM has extensive requirements. First, the EPM must be abundant, renewable, environmentally friendly, and could be used as an alternative to bioplastics in packaging applications (DeJong & Koppelman, 2002). Further, the EPM must have neutral organoleptic properties (I.e., clear, transparent, and odourless) so that it is not easily detected when consumed (Guilbert et al., 1995). Finally, the EPM must also have mass transfer properties suitable for its product to prevent significant loss of food quality caused by the transfer of moisture, gases, aroma, flavour, or colour to and from the environment (Wihodo & Moraru, 2013).

Whey protein is a suitable EPM for edible packaging due to its transparency, lack of aroma, and flavour (Ket-On et al., 2016). It is also eco-friendly and economically efficient because whey protein products are by-products of dairy production (Price, 2019). Currently, there are three commercially available whey protein by-products: whey protein concentrate (WPC), whey protein isolate (WPI), and whey protein phospholipid concentrate (WPPC). As packaging materials, WPC and WPI are well-studied (Cinelli et al., 2014). On the other hand, WPPC is still not well studied due to its limited use. Therefore, the production method and composition have not been standardised (Levin et al., 2016). As a result, the important nutritional composition (protein and fat) of WPPC varies depending on the processing and

treatment. The American Dairy Products Institute regulated the WPPC protein content with only a minimum requirement of 50%. By contrast, the protein content for WPI and WPC has a defined range of 90.0–92.0% and 80.0–82.0%, respectively. In terms of fat, WPPC has the highest amount at a minimum of 12%, while WPI and WPC fat content can range from 0.5–1.0% and 4.0–8.0%, respectively (American Dairy Products Institute, 2015a, 2015b, 2015c).

The fat content in WPPC is of interest in this study because adding lipids is a common approach to increasing water barrier properties (Pérez-Gago & Krochta, 2000). Following this trend, it may be possible to utilise the natural fat content in WPPC to make a novel edible film that offers a better water barrier property without the extra addition of water-repelling ingredients. However, the use of WPPC as a base material for an edible film has not been investigated. Fortunately, edible films made from WPI and WPC have been previously studied. So, to fill in the missing knowledge, this study aimed to achieve two goals: (1) to create an edible film using WPPC as a base material; and (2) to test the practicality of the film made from WPPC based on its physicochemical and water barrier properties. The approach this study took was by applying the processing techniques for WPI and WPC films and adapting them for WPPC film. Then, the study compared the water barrier and physicochemical properties of WPI, WPC, and WPPC films.

2. Literature review

2.1. Whey protein phospholipid concentrate as an EPM

Figure 1 illustrates the pathway in which WPPC is produced. To summarise the pathway in writing: The fluid whey waste from cheese making is refined via microfiltration and separates whey concentrate and whey cream. Further refinment of the whey concentrate or cream via ultrafiltration results in WPC or WPI and WPPC respectively (Levin et al., 2016). In regards to the refining process of WPPC, Levin et al. (2016) noted that there are variations between processing plants due to the lack of standardised method. As a result, the composition of WPPC varies between sources. Although American Dairy Products Institute (2015c) recognised these variations, the organisation did not impose a standardised method nor established a defined range for WPPC composition. Instead, compostions are defined with a minimum or maximum amount requirement. The standardised WPPC components are protein (min. 50%), fat (min. 12%), ash (max. 8%), and moisture (max. 6%). It is interesting to note that, despite the presence of lactose in WPPC, the American Dairy Products Institute (2015c) did not set any standard for WPPC's lactose content.

The protein composition in WPPC is similar to other whey protein products. Levin et al. (2016) reported that the globular proteins β-lactoglobulin and α-lactalbumin comprise most of the protein content, but the exact amount is unknown. Other native proteins include immunoglobulins, lactoferrin, and bovine serum albumin (BSA), as filtration is not 100% efficient. When applying WPPC as EPM, β-Lactoglobulin is the primary structural element for the film. This is because as α-lactalbumin cannot polymerise. Nevertheless, thiol-disulfide bond exchange reactions between the α-lactalbumin and β-lactoglobulin can help β-lactoglobulin polymerise better (Qian et al., 2017). In addition to being the main structure of the edible film, whey protein antioxidant capability provides protection with its ability to inhibit free radicals and provide excellent barriers to lipids and aromas (Hammam, 2019; Lara et al., 2020). The Lactoferrin in wheys also provides protection with its ability as an antimicrobial agent that is effective against against Gram-positive and Gram-negative bacteria (Dinika et al., 2020). A weakness of whey protein is its hydrophilic nature. Subsequently, whey protein strongly interacts with water and exhibits poor moisture barrier properties (Seiwert et al., 2021). Whey protein polymerisation also results in a brittle structure, which often needs to be modified with plasticisers to increase their application as a food packaging material (Huntrakul & Harnkarnsujarit, 2020).

The fat components in WPPC are similar to other whey protein products: myristic, oleic, palmitic, and stearic acid are the major components (Levin et al., 2016). Otherwise, out of the minimum 12% fat in WPPC, 10–30% are phospholipid (Li, 2017). Phospholipid provides nutritional value and health benefits and other functionalities such as carriers for bioactive

compounds, emulsification, surfactants, texture improver, and moisture retention (Huang et al., 2020). For that reason, research efforts surrounding WPPC have focused on isolating and concentrating its fat and phospholipid over other functional properties (Li et al., 2016; Price et al., 2018). Unlike the usual research efforts, this study is interested in utilising the phospholipid's ability to interact with water. Phospholipids are amphiphilic with a hydrophilic head and hydrophobic fatty acid tail (Contarini & Povolo, 2013). Thus, its application in an edible film could enhance or weaken the film's performance. More specifically, while the hydrophobic fatty acid tail could improve the barrier against water, at the same time, the hydrophilic head could retain moisture, making the film more brittle. The effect of phospholipid on the performance of WPPC film as a water repellent is still unknown. Nonetheless, other fat components in WPPC, myristic, oleic (major component at 19.1–22.5%), palmitic, and stearic acid are all hydrophobic fats and have been shown to reduce water vapour permeability (Debeaufort & Voilley, 2009). Thus, edible film made from WPPC should offer water barrier properties to some degree.

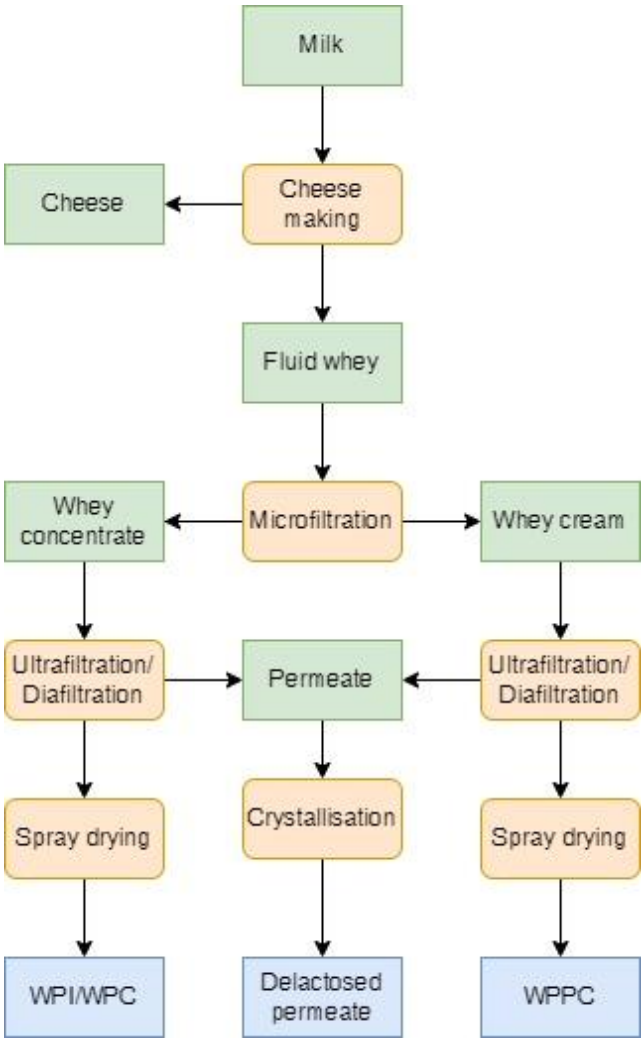

Figure 1. Flow diagram showing the production of WPPC, WPC, and WPI from milk.

2.2. Processing of EPM into edible film and its application

An edible film can be produced by two different processing methods, which the industry classifies as dry and wet processes (Coltelli et al., 2016). Dry processing is commonly achieved by compression moulding, extrusion, heat pressing, or injection (Aguirre-Joya et al., 2018). For dry processing, extrusion is more commonly applied to thermoplastic materials like starches combined with a plasticiser (Jiménez et al., 2012). During extrusion, high temperature is used to shape the packaging into the desired shape. In many biopolymers, high-temperature processing may alter the quality of the end product (Silva-Weiss et al., 2013). However, starches can be processed this way due to the unique microstructure of different starches when subjected to different temperatures (Liu et al., 2009).

Wet processes are more commonly used to produce films and can be applied widely to all EPM types. There are two applications for the film, casting and coating, and their usage depends on the protected product. Coating is the application of film directly on the product's surface intended to be protected or enhanced, where it remains and becomes part of the product through use and consumption (Krochta, 2002). The coating can be applied to the product by spraying, dipping, rolling, spin-coatings, and physical vapour deposition (Coltelli et al., 2016). Coatings are widely used and studied for fruits and vegetables like mangoes (Minh et al., 2019), spinach leaves (Abedi et al., 2021), and frozen strawberries (Muley & Singhal, 2020). For fruits and vegetables, being directly in contact with the product, the edible film coating can protect the product from a harmful environment and regulates its food system by limiting the transfer of moisture, oxygen, carbon dioxide, aroma, and taste compounds. Further, coatings can provide structural integrity for frozen fruits (Lin & Zhao, 2007).

Casting results in a stand-alone product, separately formed before it is used for packaging (Krochta, 2002). Film-forming solution (FFS) is poured over a mould and allowed to dry in a controlled condition to form the edible film (Aguirre-Joya et al., 2018). As a stand-alone product, edible films offer more variety of applications and versatility, like covers, wraps, layers, or pouches (Chen et al., 2019). Furthermore, casting is an especially common technique for academic research since it is a quick and simple production method that can evaluate the EPM potential as packaging (Schmid & Müller, 2019). Therefore, this study aims to test WPPC film-forming capability from casted films rather than coating it on a product.

There are many factors to consider in the processing and application of edible films. The most important is that only food-grade ingredients and processing techniques conforming to food-product regulations can be used (Krochta, 2002). Some factors that must be considered in the processing technique are the properties of the packaging, for example, the amount of polymer in the matrix, processing temperature, amount of plasticiser, and other additives

(Jiménez et al., 2012). For application, the essential factors are the protected product, the packaging type, ease in processing and packaging, and consumer perspective on the product (Zhang et al., 2018).

In summary, it is essential to have synergy between the protected product, the EPM, and the packaging application. Taking the example from above, when packaging fruits and vegetables, packaging made from proteins or polysaccharides would be preferable over lipids because lipids may give a waxy taste to the product (Mohamed et al., 2020). Further, a coating film is preferred over a casted film, as coating provides additional structural integrity while allowing mass transfer across the membrane. In contrast, a large air pocket may be present in a pouch film, resulting in less quality protection.

2.3. Selecting an ideal EPM for an edible film

Section 2.2 commented on the importance of synergy between the product, the EPM, and the application. This is because each biopolymer has strengths and limitations regarding its physical and mechanical properties (Fernandes et al., 2020). Proteins have strong oxygen and aroma barrier properties at low relative humidity and moderate temperatures, but, due to their hydrophilic structure, proteins interact with water strongly and are brittle (Hong & Krochta, 2006; Huntrakul & Harnkarnsujarit, 2020). Polysaccharides have low water barrier properties but have strong gas barrier properties, especially against oxygen and carbon dioxide. Lipids react with gases but are better at limiting water movement across their structure due to their hydrophobic nature (Aguirre-Joya et al., 2018; Debeaufort & Voilley, 2009; Mohamed et al., 2020).

Due to the different nature of EPMs, the protected product may require additional protection that an EPM cannot provide as a sole base material. Thus, to overcome the issue, a 'composite film' made of combinations of materials can be used to help enhance the film's properties (Xu et al., 2021). As mentioned briefly in the introduction, adding lipid to EPM can increase water barrier properties. Several studies have examined various lipids and polysaccharide/protein combinations (Bravin et al., 2004; Cerqueira et al., 2012; Fabra et al., 2008; Monedero et al., 2009; Valenzuela et al., 2013). However, this study is more interested in studies conducted specifically on lipids and whey protein (Kim & Ustunol, 2001; McHugh & Krochta, 1994; Pérez-Gago & Krochta, 2000; Shellhammer & Krochta, 1997). Previous studies that combined lipids and whey protein noted the films had complex interactions, and the effect depends on the material's respective nature. As such, films made by combining many materials can result in both enhancing and weakening the edible film's structure and mechanical properties (Liaotrakoon & Raviyan, 2018). Other modifications such as adding plasticiser or

nano-materials, cross-linking, and pH modification are standard techniques to enhance film structure and mechanical properties (Wihodo & Moraru, 2013). The effect of this addition will be discussed further in Section 2.5.

2.4. Formulation of edible film

Choosing a versatile material for an edible film is a challenging task. Therefore, considering the complexity surrounding the formulation of edible film, selecting a product before formulating the packaging is more effective. In that way, additions are tailored for the specific application and nature of the product (Di Pierro et al., 2018). Ramos et al. (2012) and Schmid and Müller (2019) noted that factors impacting the film properties are the base material, the addition of lipids, polymers, and other additives, as well as the crosslinking process (Ramos et al., 2012; Schmid & Müller, 2019). Considering that this study does not want to apply extensive additions, the important formulation parameters are protein concentration and plasticiser concentration, which directly correlate with film-forming ability and thickness (Gounga et al., 2007). The natural fat content in WPC and WPPC becomes an additional variable. Therefore, protein and plasticiser concentrations must be formulated carefully to form a perfect film with minimal defects, while also acknowledging the role of fat in the overall formulation.

2.5. Processing and treatment of edible film by casting

The processing of edible film by casting can be summarised into four major steps: (1) dispersion, (2) homogenisation, (3) casting, and (4) drying (Aguirre-Joya et al., 2018). Although there are only four major steps, the processing is complex due to various factors influencing the final film during its processing and treatment (Schmid & Müller, 2019). This section discusses those factors using whey protein film as an example.

2.5.1. Dispersion

Dispersion is necessary for the EPM to be distributed evenly when the film-forming solution (FFS) is dispensed into the moulds. For polar EPMs, the solvent used for dispersion is usually water. Non-polar EPMs (e.g., lipids or resins) can be dispersed in an organic solvent (Janjarasskul & Krochta, 2010). The dispersion of whey protein powder involves suspending the powder in water while continuously mixing the solution for some time. During this process, rehydration occurs; rehydration is a process where water is added to the dry protein powder to regain its original functionalities (Khaire & Gogate, 2021). The rehydration of food is dependent on time and temperature. Thus sufficient time must be allowed to rehydrate the protein powder before further processing (Al-Jassar et al., 2020). When rehydrating, it is important to consider

the powder's dispersibility as it represents its ability to disperse quickly and evenly without forming lumps (Berk, 2009). Lumps affect powder's solubility, leading to sedimentation and the solution's aggregations (Khaire & Gogate, 2021). For that reason, a sufficient amount of water and time is needed for the protein powder to rehydrate and regain its functional properties. Any clumps, sedimentation, and aggregations could mean that the protein powder does not have its full functional properties and could weaken the final produced film.

2.5.2. Homogenisation

Homogenisation aims to create similar particle sizes and help disperse the whey protein powder and other additions evenly throughout the solvent. When there are additions, homogenisation can happen twice depending on the added ingredients. For example, if the added ingredients are heat sensitive, e.g., phenolic compounds, homogenisation must be done again after heat treatment to disperse the phenolic compounds into the matrix evenly. In this step, consideration needs to be made as shearing could cause foaming. Levin et al. (2016) noted that the foamability of whey protein is high due to the strong adsorbed layer at the water interface. Foams or air bubbles could result in a film with air bubbles, cracks, or pores, subsequently decreasing the film's structural integrity (Sanches et al., 2021). In previous studies, bubbles and foams have been removed by ultrasound, scooping, or degassing using a vacuum chamber (Lara et al., 2020; Schmid et al., 2014b; Seiwert et al., 2021). Each of these methods has its strength and weaknesses. Ultrasound is quick and readily available, but can destroy the physical and chemical interactions, weakening the aggregation of proteins (Schmid & Müller, 2019). Scooping can remove foams but does not remove any dissolved gasses (although dissolved gasses may dissipate over time). On the other hand, it is also the most economical and most straightforward method; using a vacuum chamber is probably the most effective method, but vacuum chambers are expensive and not readily available.

2.5.3. Crosslinking: chemical, enzymatic, physical crosslinking

Crosslinking is the formation of covalent bonds between a polymer. The bonds can occur internally, between polypeptide chains within a polymer (intramolecular crosslinking) or between polymer chains (intermolecular crosslinking) (Gerrard, 2002). Biopolymers can form crosslinking naturally. Nevertheless, by maintaining and increasing the crosslinking network, the mechanical and barrier properties of the film can be improved further (Xu et al., 2021). The protein film crosslinking is commonly enhanced by chemical, enzymatic, and physical crosslinking techniques. Xu et al. (2021) suggested that combining the various crosslinking techniques results in better film mechanical properties (i.e., tensile strength and elongation). However, it does not mean that all the modification techniques are necessary. The following

paragraphs discuss the basics of each crosslinking technique and considerations that may influence the final film.

Chemical crosslinking can be done by chemical additions or pH modification. Chemical modifications involve the reaction of protein side chains using chemicals through alkylation, acylation, acetylation, succinylation, and grafting (Zink et al., 2016). Chemical crosslinking is not popular as most chemical modification reagents are toxic and unsuitable for edible films (Xu et al., 2021). pH modifications (combined with thermal treatment) are a more common and suitable way to crosslink whey protein, credited to its unique properties for its good solubility in water over a wide range of pH (pH2–9) (Burrington, 2012). pH changes the protein's net charges, subsequently impacting protein-to-protein interaction. High pH has been associated with a negative net charge in whey protein, while low pH results in a positive net charge. Díaz et al. (2017) and Zink et al. (2016) reported that alkaline pH improves mechanical properties (tensile strength, elongation break, and elastic modulus). Burrington (2012) reported that, at pH 3, whey protein loses its film formatting ability due to the inhibition of protein interactions caused by the positive net charge. The author also observed an increase in water vapour permeability (WVP) at pH 5, while no significant differences were found at higher pH values. Overall, high pH is more advantageous for producing whey protein film.

Enzymatic crosslinking of protein is commonly catalysed by transglutaminase (TG). The use of TG is common as it is a versatile enzyme that is stable at a range of pH values (pH 4–9) and temperatures (10–60 °C) (Seguro et al., 1996). TG catalyses the reaction between the γ-carboxamide (NH_2-CO) of glutamine and an ε–amine group of lysine to form an ε-(γ-glutamyl)-lysin bond (Schmid et al., 2014b). This reaction is affected by temperature, pH, and calcium content (Dickinson, 1997). Another factor affecting the rate of crosslinking is the protein structure, with protein containing glutamine residues being the most efficient substrates (Gerrard, 2002). Globular proteins such as ovalbumin and β-lactoglobulin are poor substrates in their native form, but crosslinking can be increased by disrupting the intermolecular disulphide bonds (denaturation) (Dickinson, 1997). Many studies reported a significant increase in whey film's mechanical properties when treated with heat and TG (Di Pierro et al., 2006; Schmid et al., 2014a; Schmid et al., 2014b; Seiwert et al., 2021; Xu et al., 2021). Therefore, consideration needs to be given to deactivate the enzyme after the desired reaction time to avoid extensive cross linking (refer to physical crosslinking).

Physical crosslinking by thermal treatment is the most commonly used method in edible film production due to its simple and economical application (Zink et al., 2016). Most crosslinking steps require thermal treatment to a certain degree to drive the reaction. Denaturation and crosslinking are closely associated with globular proteins as the unfolding of protein exposes the internal functional groups, which allow crosslinking to occur (Schmid et al., 2014a). Specifically

for β-lactoglobulin, the protein can unfold when heated at approximately 78 °C (denaturation temperature) (Burrington, 2012). Then, hydrophilic groups are exposed to the solvent, including a large number of hydroxyl (-OH) and sulfhydryl (-SH) (Cruz-Diaz et al., 2019; Pérez-Gago & Krochta, 2001). In turn, the proteins rearrange by forming new intermolecular disulphide bonds and reforming with a more tightly packed film network (Xu et al., 2021). Thus, the unfolding of protein is an essential step for globular protein for crosslinking. In regards to temperature, Burrington (2012) reported that higher temperatures may result in aggregation. Schmid and Müller (2019) noted that films processed at higher temperatures are more brittle due to extensive crosslinking and less plasticisation due to water loss. Still, higher temperature treatment does not necessarily negatively affect the film. WPI thermally treated at higher temperature (80–100 °C, 5–20 min) has been shown to form a denser and stronger film (Pérez-Gago & Krochta, 2001; Schmid et al., 2013). Interestingly, Schmid et al. (2014a) suggest that films with a degree of denaturation of greater than 25% did not provide further improvement in the film properties. In addition, mixing native protein to adjust the degree of denaturation (and therefore the degree of crosslinking) could reduce production costs and avoid extensive heat treatment.

In brief, time and temperature are important factors in this processing step. Furthermore, the denaturation of protein by heat treatment is necessary for crosslinking. Adjusting pH and using TG could also be used simultaneously to increase further the crosslinking rate. However, proper adjustment is needed to ensure that extensive crosslinking does not negatively affect the film. Many studies have reported the enhancement in film properties when combining crosslinking techniques.

2.5.4. Addition of a plasticiser

A plasticiser is a substance or material used in film formulation to increase flexibility, workability, or distensibility (Vieira et al., 2011). By adding plasticiser, films can enhance moisture transfer resistance and flexibility. However, the film usually results in low tensile strength and high moisture uptake. The weakening of the film is because most plasticisers are hydrophilic and hygroscopic, enabling them to draw water molecules (Suhag et al., 2020). Nevertheless, plasticisers are still necessary ingredients for polysaccharides and protein films since these films are stiff and rigid due to the extensive interactions between the polymeric chains (Suhag et al., 2020). Furthermore, it has been reported that the increasing concentration of plasticisers in an edible film positively impacts the barrier properties, mechanical strength, and thermal properties of whey protein films (Sanyang et al., 2015; Sothornvit & Krochta, 2005). When choosing a plasticiser, the structure of the plasticiser needs to be compatible with the polymer. Typically, a plasticiser with solubility, polarity and hydrogen bonding characteristics

similar to the polymer would have good compatibility (Godwin, 2000). Some plasticisers may be toxic due to the migration of phthalates. Hence, natural and biodegradable plasticisers must be used in edible films (Fernandes et al., 2020). Several plasticisers have been previously studied in conjunction with whey protein-based film, including glycerol, oleic acid, PEG 200 & 400, sorbitol, and xylitol (Huntrakul & Harnkarnsujarit, 2020; Shaw et al., 2002; Sothornvit & Krochta, 2001). Huntrakul and Harnkarnsujarit (2020), Sothornvit and Krochta (2001), and Xu et al. (2021) concluded that glycerol is one of the most efficient plasticisers. This efficiency is attributed to glycerol's small molecular size (Sanyang et al., 2015; Sothornvit & Krochta, 2005). Furthermore, glycerol has a high boiling point, is water-soluble, polar, non-volatile, and protein miscible. These properties make glycerol a suitable plasticiser for use with a compatible water-soluble polymer (i.e. whey protein) (Gounga et al., 2007).

2.5.5. Other additions: antioxidant, antimicrobial agents, and prebiotics

Ingredients such as antioxidants, antimicrobial agents, and probiotics are active ingredients that can improve the film's ability to increase food shelf life or improve the nutritional quality of the food product (Ramos et al., 2012). When tailoring a film for a specific product, these additions are essential to enhance the film's functionality as packaging. This section lists additional ingredients that have been used in previous studies to enhance whey protein film's ability to enhance shelf life.

Antioxidants incorporated into whey protein films are phenolic compounds such as anthocyanins from red cabbages (Sanches et al., 2021), tannins from pecan nut extract (Arciello et al., 2021), and catechin from rambutan peels (Chollakup et al., 2020). These are natural plant extracts that have high antioxidant capacity. Alongside antioxidants, some natural plant extracts are a rich source of active compounds exhibiting antimicrobial activity. Red cabbage, for example, has been shown to have both antioxidant and antimicrobial effects (Sanches et al., 2021). However, not all antioxidants exhibit antimicrobial activities. Therefore, it is also common to see the ingredients with antimicrobial and antioxidant activities paired together during the development of a film (Chollakup et al., 2020).

Antimicrobial agents incorporated into whey protein films are extracts or oils from plant-based ingredients such as cinnamon, rosemary, lemon, and bergamot (Abedi et al., 2021; Bahram et al., 2014; Çakmak et al., 2020). In addition to providing protection, essential oils also offer health benefits, making their use more relevant (Aguirre-Joya et al., 2018). Other sources of antimicrobial agents, such as silver nano-particles and titanium oxide, have also been incorporated into whey-based films (Çağrı Mehmetoğlu et al., 2021; Zhou et al., 2009).

Prebiotics are incorporated in edible films via (1) direct addition to enhance the nutritional properties of the film and (2) incorporation of prebiotics and probiotics to create a

symbiotic effect that maintains the viability of probiotic microorganisms (Paulo et al., 2021). Incorporating prebiotics is common in food; therefore, prebiotics are generally recognised as safe (GRAS). A recent study has explored combining galactooligosaccharides and xylooligosaccharides in whey protein films (Fernandes et al., 2020). Aside from nutritional benefits, some prebiotics such as mannitol, xylitol, and sorbitol also have plasticising effects (Janjarasskul & Krochta, 2010; Mohanty et al., 2018). Sorbitol, in particular, has received much attention. Sorbitol was reported to provide the whey protein film with similar or better tensile strength than glycerol. Nevertheless, glycerol was reportedly better in terms of efficiency of water-absorbing ability, plasticising effect, and temperature stability (Huntrakul & Harnkarnsujarit, 2020; McHugh & Krochta, 1994; Sanyang et al., 2015; Shaw et al., 2002).

In summary, additional ingredients in the film create bioactive films that provide functionalities for consumers or the product. Some of these additional ingredients are multi-functional, making them a valuable addition to the film. However, the prebiotic's solubility and ability to be suspended should be considered when incorporating such ingredients. If the ingredients disrupt the interaction between polymers, it could negatively affect the film (Aguirre-Joya et al., 2018).

2.5.6. Casting

The type of moulds and the amount of FFS dispensed are essential aspects of casting. The mould type matters as it needs to be able to release the edible film efficiently. For that reason, moulds with a very low coefficient of friction like Teflon are preferable (Anker et al., 1998). Present studies utilised moulds made of Teflon-coated glass plates or polystyrene (Suhag et al., 2020). The moulds should also have (and be placed on) a flat surface so that the film thickness is consistent. The mould's volume also needs to be considered as the amount of FFS dispensed into the mould is also a factor of thickness variation. Usually, the thickness of a film can be mathematically calculated (Fernandes et al., 2020; Schmid et al., 2014b).

2.5.7. Drying

Drying is probably one of the most important processes for improving the intra-molecular relationship between polymer chains and defining the film's microstructure (Suhag et al., 2020). Relative humidity, temperature, and time control the drying rate (Embuscado & Huber, 2009). The most common drying technique for whey protein films is ambient temperature with humidity of ~50% for 24–48 h (Schmid et al., 2014b; Seiwert et al., 2021; Xu et al., 2021). Alternative methods for ambient drying are hot-air drying using a conventional oven at around 30 °C (Agudelo-Cuartas et al., 2021; Sanches et al., 2021) and microwave drying (Kaya & Kaya, 2000). Utilising hot-air drying without controlling humidity can increase the drying rate, but hot-

air drying methods can negatively impact the edible films' physical and structural properties (Suhag et al., 2020). Pérez-Gago and Krochta (2000) supported this idea and reported that whey protein film dried at ambient temperature and humidity performs better than films dried at a higher temperature (40 °C and 80 °C). Nevertheless, considering upscaling and commercialisation, drying at ambient temperature is not economical due to the drying time and risk of microbial growth. Therefore, the film's overall cost performance should be considered a trade-off for the production to be more effective.

2.6. Summary

Based on the information in the literature review, substituting the whey protein source from WPI or WPC with WPPC is promising. However, the formulation and processing steps greatly influence the final film. Furthermore, as many modifications can be made to the films to enhance their ability to protect food, it is best to formulate the packaging based on the product to be selected. The theme of this study is "edible" film, so the ingredients and processing need to be safe for consumption. However, this study aims to investigate whether WPPC films can be made and then compare their performance to films made from WPC and WPI. Thus, sensory testing was not conducted. Considering the points above, the formulations and processing need to have the following requirement: (1) The formulation needs to be kept simple and consistent to decrease variables, i.e., ensure the main components, protein concentration and plasticiser are kept constant. (2) Modification should be kept minimal, i.e., only crosslinking by heating, and no other modifications were used.

3. Materials & Methodology

3.1. Materials

The base materials used in this study were: (1) WPPC and (2) WPI powder, sourced from Mullins Cheese, USA; (3) WPC powder was sourced from Bin Inn Wholefoods, New Zealand; (4) Glycerol was supplied by Home Essentials, New Zealand. Sigma-Aldrich and ThermoFisher Scientific New Zealand supplied all other chemicals and reagents.

3.2. Whey products component analysis

3.2.1. Fat content

Fat content was determined by manual Soxhlet extraction. Three grams of whey powder were weighed into an extraction thimble and covered with glass wool. A distillation flask containing anti-bumping granules was weighed and recorded. Then, approximately 125 mL of petroleum spirit was poured into the flask. The extraction was allowed to run for 3 h. Additional petroleum spirit was added when needed to prevent the extraction from running dry. After 3 h, the distillation flask was removed, and the remaining petroleum spirit was distilled using a water bath at 90°C. Once the flask was visibly dry, it was dried further in the oven at 105°C for 30 minutes to remove any remaining moisture. The flasks were weighed again once cooled. Fat content was determined by calculating the weight difference between the flask before and after the extraction with reference to the sample weight.

3.2.1. Protein content

Protein content was determined using an Elemental Analyzer (CE-440, Exter Analytical INC.). Acetanilide was used for conditioning and standards. About 2000 to 3000 µg of the sample was weighed in the silver capsule. Then, it was placed in a nickel sleeve before going through the Elemental Analyzer, which produced the nitrogen content as a percentage. The protein content of the samples was then calculated by multiplying the percentage nitrogen content with the N factor for milk, 6.38.

3.2.2. Other components

Moisture content was estimated to be around 5% in all the whey powders. Lactose and ash content was calculated by subtracting 100% from total protein, fat, and moisture. These components were not accounted for in the formulation. Therefore, they were not tested.

3.3. Experimental plan

This study starts with proof of concept that an edible film can be made using an existing formulation. First, formulation and processing techniques were studied. Then, trials were conducted to confirm the viability of formulation and processing techniques using WPC as the base material. After successfully adjusting the formulation and processing technique, it was applied to WPPC. The WPPC films did not behave as WPC films. Thus, optimisation of the formulation was required. A possible explanation for this observation was (1) insufficient amount of water in the film, causing the film to shrink; (2) insufficient amount of protein preventing sufficient crosslinking; (3) there is an interruption at a molecular level that prevents sufficient crosslinking, e.g., the extra fat in the WPPC. These possibilities were also explored in the trials. Below is the list of trials conducted:

Trial 1: Initial formulation by adapting several studies (WPC)
Trial 2: Optimising formulation by changing the drying mechanism (WPC)
Trial 3: Optimising formulation by decreasing the denaturation temperature (WPC)
Trial 4: Optimising formulation by increasing the amount of plasticiser (WPC)
Trial 5: Applying formulation to WPPC
Trials 6 & 7: Improving formulation by increasing the protein concentration of WPPC
Trial 8: Finding the optimum protein concentration for WPPC
Trial 9: Reconfirm the optimum WPPC concentration for film formation
Trial 10: Finding the optimum amount of plasticiser for film formation using WPPC
Trial 11: Investigating the possibility of intermolecular interruption by defatting WPPC solution
Trial 12: Final formula and processing techniques for WPPC films

Figure 2 visually outlines the trials explored in the formulation of WPPC film. The methodology for each trial is recorded in the following sections. After successfully creating a WPPC film with minimal defects, films made from WPPC, WPC, and WPI were characterised and compared (Section 3.5).

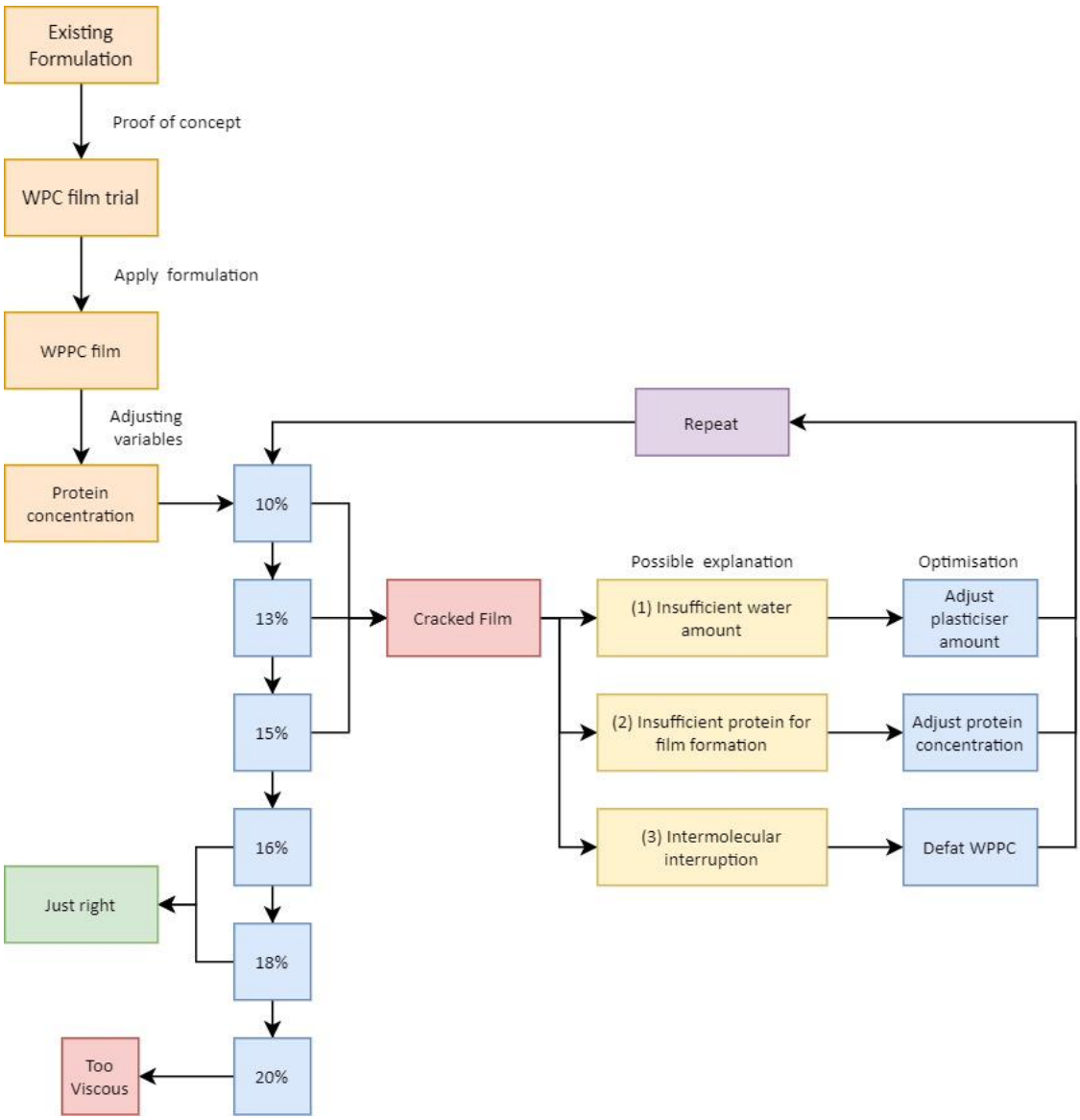

Figure 2. Flow diagram visualising the initial formulation process of the WPPC film.

3.4. Film optimisation trials

3.4.1. Trial 1: Initial formulation by adapting several studies (WPC)

The initial formulation was adapted from Fernandes et al. (2020), Schmid et al. (2014b), and Xu et al. (2021). An aqueous WPC solution (300 mL) with a 10% (w/w) concentration was made by mixing the WPC powder with room temperature (~22 °C) deionised water in a thermal cooking kitchen machine (Thermomix) (K2304, Anko, Australia) for 1 h (speed 3). The solution was then heated to 90 °C and maintained for 30 min while continuously stirring (speed 3). After moving to a cool container, the solution was allowed to cool to room temperature. Glycerol (GLY) (1:15 GLY:WPC) was added to the solution and allowed to mix again in the Thermomix for 5 min at speed 3. Any foam and bubbles were skimmed using a spoon, and the FFS were allowed

to rest for 3 h at room temperature. Then, varying amounts of FFS were dispensed into the Petri dish (ø = 8.5 cm): 6 g, 8 g, 10 g, 12 g, and 14 g, coated with vegetable oil, and dried in an incubator at 25 °C with relative humidity (RH) ~50 ±5%. . Films were casted in duplicates. After preparation the film remained the same throughout Trials 1–4 with modification listed in each trial.

3.3.2. Trial 2: Optimising formulation by changing drying mechanism (WPC)

Instead of allowing the film to dry at ambient temperature, the films were dried on a levelling table placed on top of a food dehydrator and covered with a lid lined with water-absorbing materials. The temperature was set at 50, 45, and 40 °C, and the films were dried for 20 h. RH was not monitored in this formulation.

3.3.3. Trial 3: Optimising formulation by decreasing denaturation temperature (WPC)

The denaturation temperature was reduced from 90 °C to 80 °C, and the stirring speed was decreased from speed 3 to speed 2.

3.3.4. Trial 4: Optimising formulation by increasing the amount of plasticiser (WPC)

The amount of glycerol was increased from a WPC:GLY ratio of 15:1 to ratios of 1:7.5 (double) and 1:2. The new ratios were adapted from Shaw et al. (2002).

3.3.5. Trial 5: Applying formulation to WPPC

The optimised formulation was applied to WPPC. In trials 5–12, the following methodology was used. A WPPC aqueous solution (300 mL) with a 10% (w/w) concentration was made by mixing the powder with room temperature (~22 °C) deionised water in the Thermomix for 1 h (speed 2). The solution was then heated to 80 °C, maintained for 30 min while continuously stirring (speed 2), and cooled to room temperature. Glycerol (2:1 GWPC:GLY ratio) was added to the solution and allowed to mix again in the Thermomix for 30 min at speed 2. Any foam and bubbles were skimmed using a spoon, and the FFS was allowed to rest overnight at 4 °C. The next day, the FFS temperature was readjusted to room temperature while stirring in the Thermomix for 30 min. FFS was dispensed into Petri dishes (ø = 8.5 cm) coated in vegetable oil. Varying amounts were dispensed into the Petri dish: 6 g, 8 g, 10 g, 12 g, and 14 g. Films were cast in duplicates. After dispensing, the FFS was dispersed in the Petri dish by placing the Petri dish on a flat surface and gently moving the plate in a figure-eight motion. The films were placed on a levelling table on top of a food dehydrator and then covered with a lid. The temperature was set at 40 °C, and the film was dried for 20 h. The internal temperature inside the food dehydrator was 30 °C. RH was not monitored in this formulation.

3.3.6. Trial 6 & 7: Improving formulation by increasing protein concentration of WPPC

Trial 5 was repeated to confirm the observation. The trials used the same methodology in Section 3.3.4, except the films were made with increased concentration WPPC solutions, 13% (w/w) (Trial 6) and 15% (w/w) (Trial 7). Trials 6 and 7 were repeated twice to confirm the observation.

3.3.7. Trial 8: Finding the optimum protein concentration of WPPC

In Trial 8, the concentration of WPPC was increased further to find the optimum protein concentration. An aqueous WPPC solution (1200 mL) with a 20% (w/w) concentration was made according to the method recorded in Section 3.3.4. Then, the solution was divided into three equal portions, and their concentrations were adjusted to 20%, 18%, and 16% (w/w), respectively, by adding deionised water. The solutions were then re-homogenised using the Thermomix (speed 2) for 5 min. Foam and bubbles were removed using a spoon, followed by resting, casting, and drying.

3.3.8. Trial 9: Reconfirm the optimum WPPC concentration for film formation

Trial 8 was repeated to confirm the observation. The trial used the same methodology in Section 3.3.4 to ensure dilution factors did not affect the observation. WPPC solutions with 18% and 16% (w/w) concentrations were in separate batches.

3.3.9. Trial 10: Finding the optimum amount of plasticiser for film formation using WPPC

Trial 10 utilised the method recorded in Section 3.3.34 with modifications to the ratios of WPPC: GLY (4:1, 3:1, 2.5:1, 2:1). This trial aimed to find the optimum plasticiser concentration.

3.3.10. Trial 11: Investigating the possibility of intermolecular interruption by defatting WPPC solution

The defatting was accomplished by the centrifugation method. An aqueous WPPC solution (300 mL) with a 13% concentration was made. Then, after rehydration and homogenisation, the solution was transferred into 50 mL centrifuge tubes and was centrifuged at 5000× G at 10 °C for 30 min. The fat became visible and was skimmed using a Rayon filter cloth. After skimming the fat, the solution was made into films using the method recorded in Trial 5. In addition, the Petri dishes were not coated with vegetable oil to avoid the effect of additional fat.

3.3.11. Trial 12: Final formula and processing techniques for WPPC films

The purpose of this trial was to optimise the production rate and increase the quality of the films produced. An aqueous WPPC solution (300 mL) with 16% (w/w) concentration was

made by homogenising the powder with room temperature (~22 °C) deionised water. The solution was homogenised at 5,000 rpm for 10 min until clumps were no longer visible using an overhead homogeniser (L4RT, Silverson). Then, the solution was allowed to rehydrate further in the Thermomix for 1 h with stirring (speed 2). The solution was then heated to 80 °C, maintained for 30 min while continuously stirring (speed 2), and cooled to room temperature in an ice bath. Glycerol (1:2 GLY:WPPC ratio) was added to the solution and homogenised at 5,000 rpm for another 10 min, followed by resting overnight at 4 °C. The next day, the FFS temperature was readjusted to room temperature while stirring in the Thermomix for 30 min. Any foam and bubbles were skimmed using a spoon. FFS was dispensed into Petri dishes (ø = 8.5 cm) at a 10 g sample size. The FFS was dispersed by placing the dish on a flat surface and gently moving the plate in a figure-eight motion. Any bubbles that formed were removed by gently tapping the Petri dish on the surface or using a skewer. The films were allowed to dry on a levelling table placed on top of a food dehydrator and covered with a lid. The temperature was set at 40 °C, and the film was dried for 20 h. The internal temperature inside the food dehydrator was 30 °C. RH was not monitored in this formulation.

3.5. Film characterisation

3.5.1. Final film preparation for characterisation

For the final film preparation, the methodology from **Trial 12** was applied to WPC and WPI with some protein concentration and sample size changes. WPC films were made using 10% WPC solution and 15 g of FFS. WPI films were made using 10% WPI concentration and 12 g of FFS. These concentrations and sample sizes were calculated to ensure each film contained the same amount of protein and the same protein:glycerol ratio. The formulation of each film can be seen in **Table 1.**

Table 1. The estimated composition of WPPC, WPC, and WPI film per 100 mL.

Composition	WPPC	WPC	WPI
Whey concentration (%)	16	10	10
Sample size (g)	10	15	12
Protein (g)	10.8	10.8	10.8
Fat (g)	1.81	0.14	0.06
Moisture (g)	0.8	0.75	0.6
Lactose and ash (g)	2.6	3.3	0.5
Glycerol (g)	5.4	5.4	5.4
Total solid (g)	21.4	20.4	17.4

The formulation was based on the compositional analysis result listed in Table 2.

3.5.2. Colour

The film colour was measured with a portable colour sensor (NixPro, Nix Sensor) to record the CIELAB colour parameters L*, a*, and b*. Where L* represents the lightness of the sample, varying from black (0) to white (100); a* represents red/greenness, (+) value represents redness, while (-) value represents green; and b* represents yellow/blueness, (+) value represents yellow, while (-) value represents blue. The films were placed on the white paper (L* = 88.7, a* = -1.83, b* = -1.67). Then, the colour difference (ΔE) was calculated using Equation 1. Where L_0, a_0, and b_0 are the colour parameter values of the standard reference (white paper) and L*, a*, and b* are the colour parameter values of the sample.

$$\Delta E = \sqrt{(L_0 - L^*)^2 (a_0 - a^*)^2 (b_0 - b^*)^2}$$

Equation 1. The equation for calculating the colour difference.

3.5.3. Light transmission rate

The methodology for measuring light transmission rate was adapted and modified from He et al. (2022) and Schmid et al. (2014b). Films were cut to 12.5 mm × 50 mm so that it was able to support its own structure in the cuvette holder without the use of a cuvette. Then, using a spectrophotometer (Genesys 150, Thermo Fisher Scientific), the reading for % Transmission at wavelengths of 250, 300, 306, 310, 320, 250, 400, 500, 600, 700, 700, 900 and 1000 nm were recorded. Air was used as a reference to compare the transmission of the films.

3.4.4. Moisture content

The film's moisture content was determined by the gravimetric method, as noted by AOAC (2001). The film was weighed and placed in crucibles of known weight. The film was then dried in a conventional oven at 105 °C for 24 h and cooled in a desiccator before reweighing. The film's moisture content was determined by the difference in weight between the dried film and crucibles, expressed in percentage.

3.4.5. Swelling Index

The swelling index was determined by the method noted by Galus and Kadzińska (2016) with modifications. The films were cut into 20 × 20 mm strips and weighed. They were then immersed in room temperature (~22 °C) deionised water for 2 min. A filter paper was used to remove excess liquid before reweighing. The swelling index was calculated by expressing the amount of absorbed water in percentage.

3.4.6. Water Vapour Permeability (WVP)

The methodology for measuring WVP was adapted from Fernandes et al. (2020) with modification. The set-up of the samples can be visualised in **Figure 7**. A small glass jar (fitted with plastic lid), 125 mL volume and ⌀ = 45 mm opening were used in this experiment. The plastic lid was cut into ⌀ = 40 mm, which determines the area of permeation (A, Equation 2). Approximately 20 g of silica gel were weighed into the jars, dried in the oven at 105 °C for 3 h, and then cooled in a desiccator chamber. Films similar to the average thickness were cut into a circle (ø = 50 mm). Once the jars had cooled, the film (smooth side facing outside) and lid were fitted on the jar and sealed with paraffin film. The samples were stored in an incubator (Heracell Vios 160i) at 25°C, 98% RH. Over time, water vapour will move from the air (98% RH) into the jar (~0% RH) via osmosis. The weight of the jar was recorded at 24 h intervals for seven days, and WVP was determined according to Equation 2, where WVP is water vapour permeability ($g.mm.hr^{-1}.m^{-2}.kPa^{-1}$); g is the weight gain (g); t is the total time in hour; A is the permeation area (mm^2); x is the mean film thickness (mm); and ΔP is the vapour pressure difference between the surface of desiccant (silica gel; 0 kPa at 25°C) and pure water (3.167 kPa at 25°C).

$$WVP = \left(\frac{g}{t \times A}\right) \times \frac{x}{\Delta P}$$

Equation 2. The equation for calculating water vapour permeability (WVP).

Figure 3. Set up of film sample for measuring WVP.

3.4.7. Thickness

The film thickness was measured using an electronic digital calliper (0-150 mm calliper, Craftright). The sensitivity of the callipers was ±0.01 mm. Ten different random parts were measured from a single film to represent the film's thickness clearly.

3.4.8. Mechanical properties: tensile strength, elongation, and Young's modulus

The methodology for measuring tensile strength was adapted from Fernandes et al. (2020), which followed the standard D882-02 method proposed by the American Society for Testing and Materials (ASTM). Films similar to the average thickness were cut into 50 mm by 20 mm and stored for 48 h at 25°C, 55% RH. Tests were conducted using the texture analyser (Ta-XT plus, Stable Micro Systems). A clamp speed of 1 mm/s and an initial clamp distance of 25 mm were used. Before each testing, the actual thickness of the film was recorded and used in the resulting calculations. Five repetitions were performed on the mechanical testing. The amount of force was measured to obtain the mechanical properties. Then, tensile strength at break (TS) (MPa), elongation at break (EAB) (%) and Young's modulus (MPa) were calculated from the data obtained using Equation 3-3. In Equation 3, F is the total force (N) applied to the film, and A is the total cross-section area applied with force (width × thickness). In Equation 4, L_1 is the length at break (mm), and $L_{0\ is}$ the initial length (mm). In Equation 5, σ is tensile strength (Pa), and ε is the strain calculated by Equation 2 without conversion to percentage.

$$TS = \frac{F}{A}$$

Equation 3. The equation for calculating tensile strength.

$$\%EAB = \frac{L_1 - L_0}{L_0} \times 100$$

Equation 4. The equation for calculating the film's elongation at break.

$$E = \frac{\sigma}{\varepsilon}$$

Equation 5. The equation for calculating Young's modulus.

3.5. Statistical analysis

Each test was performed in triplicate unless specified otherwise. Tests were conducted using films from different batches. All statistical analyses were calculated using XLSTAT 2022. One-way analysis of variance (ANOVA) was used to evaluate differences between parameters obtained from WPPC, WPC, and WPI. Tukey's multiple range test with the criteria $p < 0.05$ was used to determine if there were any significant differences between the parameters.

4. Results & Discussion

4.1. Whey protein components

Based on the values set by the American Dairy Products Institute (American Dairy Products Institute, 2015a, 2015b, 2015c), the protein content of the WPPC powder was within the expected standard (66.9%) (**Table 2**). However, the WPC and WPI protein content (70.5% and 87.4%, respectively) was slightly lower than what was set by the standard (80–82% and 90–92%, respectively). This variation was attributed to two factors, (1) batch variation during the production of whey powders or (2) the unusual methodology used in this study to analyse the protein content. Thie method was used over the Kjeldahl method to analyse the protein as the instrument was broken during the study.

Regarding the fat content, this study also recorded variations in the fat content. Levin et al. (2016) reported variation in fat content between 10.85–38.11%, while the WPPC powder in this study had a fat content of 11.3% (**Table 2**). The fat content in this study was in the lower end of the range but still within the standard set by American Dairy Products Institute (2015c). As noted in Section 2.1, WPPC production is not fully standardised. Therefore, variations in composition were expected. The fat in WPC powder was slightly lower at 0.90% compared to 4–8%, as set by American Dairy Products Institute (2015a). The WPI powder had a fat content of 0.53%, which was within an acceptable range set by the American Dairy Products Institute (2015b). It is noteworthy that the whey powder used in the study was not produced recently, and due to the impact of the COVID-19 pandemic, a new batch was unavailable during this study.

Mentioned above, lactose and ash are part of WPPC. They were not studied thoroughly in this study as it was not considered as part of the formulation. As such, **Table 2** only presented lactose and ash in theoretical value. While there are limited data is available on the interaction between minerals and protein in edible films, lactose has been known as a crosslinking agent (Venkatachalam et al., 1993). A study on gelatin film supplemented with lactose reported that maillard crosslinking had a positive impact on the tensile strength of the films (Bhat & Karim, 2014). Considering the propotion of lactose in WPC and WPPC in ccomparison to WPI, this may impact the tensile strength of the film, and is a limitation of this study and should be considered in future studies to optimise the films strength.

Table 2. Component analysis of WPPC, WPC, and WPI powder.

Composition	WPPC	WPC	WPI
Protein (%)	66.9 ± 1.02	70.5 ± 0.49	87.4 ± 0.33
Fat (%)	11.3 ± 0.27	0.90 ± 0.04	0.53 ± 0.03
Moisture (%)	~5.0	~5.0	~5.0
Lactose and ash (%)	~16.8	~23.6	~7.07

Moisture and lactose/ash were calculated based on theoretical values where moisture is around 5%, so the powder's remaining components could be lactose or ash.

4.2. Film optimisation trials

The performance of the final films depends on the formulation and processing (Schmid & Müller, 2019). Therefore, optimising the formulation and processing technique was a significant portion of this study. This section summarises the findings from the trials.

In **Trial 1**, an issue with drying was encountered, and the film was not completely dry after 25 °C and 50% RH for 48 h. This observation was not consistent with many studies. As mentioned in Section 2.5.7, they have utilised ambient drying temperature under controlled humidity. The drying issue was possibly due to the amount of FFS dispensed in the mould or the high total solids of the mixture. The attraction of water molecules to the protein and lactose could also explain this observation. As seen in **Figure 4**, the WPC film was green and reddish, which could be caused by bacterial growth. Therefore, in **Trial 2**, a food dehydrator was used as a drying mechanism to accelerate drying and mitigate the possibility of microbial growth.

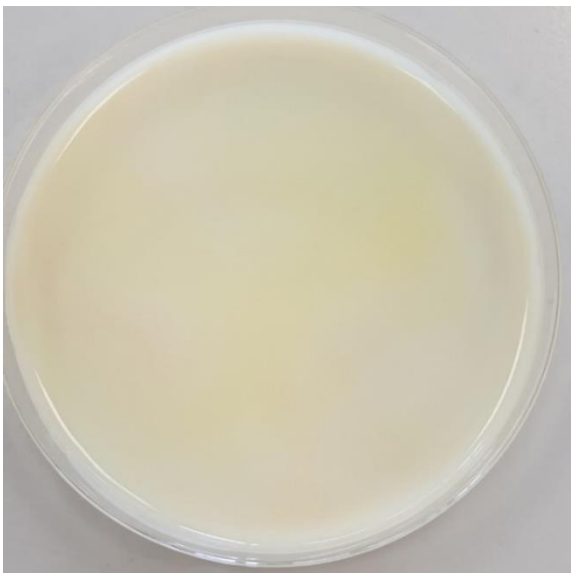

Figure 4. WPC film after drying at 25 °C and 50% RH for 48 h (Trial 1).

In **Trial 2**, a range of temperatures was also tested to find the optimum drying conditions to mitigate microbial growth but still at a low temperature that does not significantly affect the film's mechanical properties. Films with a lower amount of FFS dried faster regardless of the amount of FFS dispensed. More importantly, all the films cracked (**Figure 5**). Furthermore, there was an issue with the uniformity of the film's thickness. This issue was associated with the flatness of the food dehydrator. A level table made from acacia wood was crafted for future

experiments. However, films still cracked despite temperature control. Shellhammer and Krochta (1997) noted that film cracking is caused when the protein shrinks during drying. But in this case, the observed cracking may be caused by insufficient material, degree of crosslinking, or insufficient water in the film matrix. Adjustments to the thermal treatment and the concentration of plasticisers were explored in **Trials 3 and 4** to investigate the cause.

Figure 5. WPC film cracked after drying at 40 °C for 20 h (Trial 2).

The change in thermal treatment was explored first in **Trial 3** because aggregates were observed in the processing and did not break down during homogenisation. Further, some WPC aggregates were stuck to the side and bottom of the Thermomix (**Figure 6.** Aggregates on the side of Thermomix after 10% WPC solution was denatured at 90 °C for 30 min (Trial 1).**Figure 6**), so the mixing speed was also decreased. Both changes should adjust the degree of crosslinking and the amount of material able to crosslink. It could be argued that the heating mechanism was also unsuitable. However, the Thermomix has been used previously by Schmid et al. (2014b) to produce WPI films. Furthermore, considering upscale production, tools like the Thermomix are more realistic than using a beaker and magnetic stirrer on a hot plate. Note that the capacity of Thermomix is large. Thus, the amount of FFS was always noted in the methodology. The decrease in temperature was based on the fact that extensive crosslinking does not positively impact the final film (Schmid et al., 2014a), and the new temperature (80 °C) was based on the denaturation temperature of whey proteins. The new temperature ensures the occurrence of denaturation while preventing extensive crosslinking. The decrease in temperature and the stirring speed was sufficient to prevent aggregation during the denaturation of WPC film. Despite the changes made, the casted films still cracked. Thus, the concentration of plasticiser

was explored in **Trial 4**. Doubling the amount of plasticiser from the original formulation was not successful. But, using the WPC:GLY ratio (1:2) adapted from Shaw et al. (2002) resulted in a film for the first time, so the ratio was utilised until optimisation (**Trial 10**).

Figure 6. Aggregates on the side of Thermomix after 10% WPC solution was denatured at 90 °C for 30 min (Trial 1).

The adapted formulation of the WPC method did not work well with WPPC (**Trial 5**). Although uniform films were formed, they were cracked (**Figure 7).** Therefore, in **Trials 6 to 9,** the concentration of WPPC was increased to find the optimum WPPC concentration for film formation. Films made from 12–15% WPPC concentration cracked regardless of the amount dispensed. Films could be made with minimal defect using higher WPPC concentrations (16–20%), regardless of the amount dispensed. However, the FFS made with 20% WPPC concentration were too viscous and difficult to handle, and the films produced significantly shrunk and cracked (**Figure 8**). This observation was interesting because the films made from low WPPC concentration (12–15%) and a large amount of FFS (12–14g) should have more total solid than films made with high WPPC concentration (16–20%) and a small amount of FFS (6–8 g). **Figure 8** illustrates the effect of different total solids within WPPC with a 20% concentration. There was no noticeable difference in the film's appearance regardless of the FFS dispensed, and the thickness was also similar (data not presented).

The films made with 16% WPPC concentration were used for the characterisation experiments as it has the optimum amount of WPPC concentration to form a whole film with minimal defects. The properties of the WPPC powder could explain the need to increase the WPPC concentration: (1) the fat may be interfering with the protein interactions as the

denatured protein bound to the phospholipid could not form strong protein-to-protein interactions; and (2) the quantity of denatured protein in WPPC is high because it has gone through rigorous processing (Levin et al., 2016). In **Trial 11**, defatting the WPPC at 13% concentration resulted in cracked films. However, the film's appearance after drying was similar to films without modification (**Figure 7**). This observation suggests several possible reasons: (1) the fat was not sufficiently defatted, or (2) there were a lot of denatured proteins that were not able to crosslink.

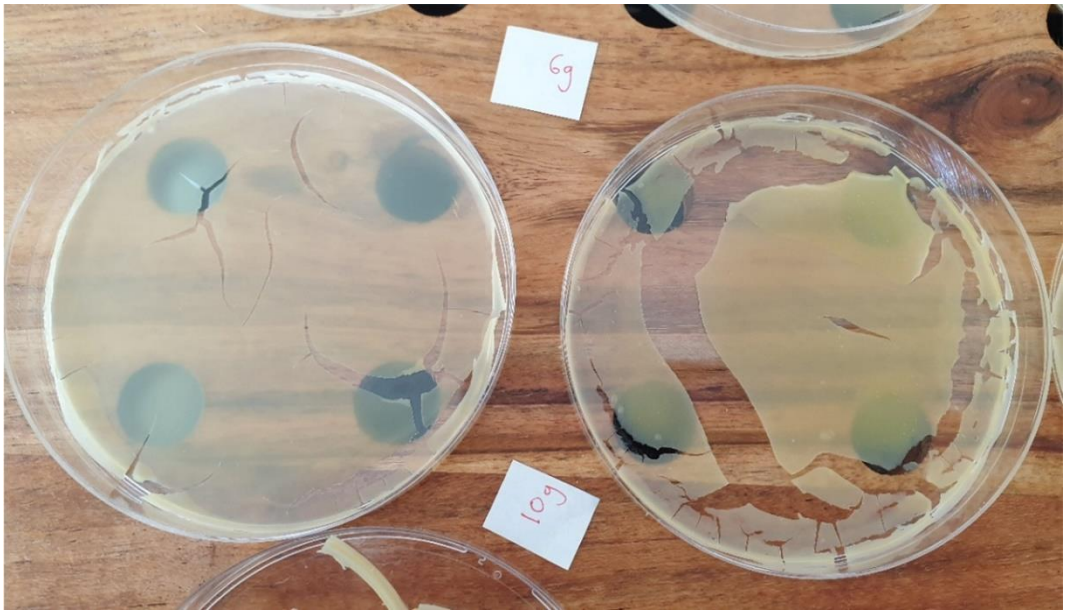

Figure 7. WPPC films made with 12% WPPC concentration after drying at 40 °C for 20 h (Trial 6).

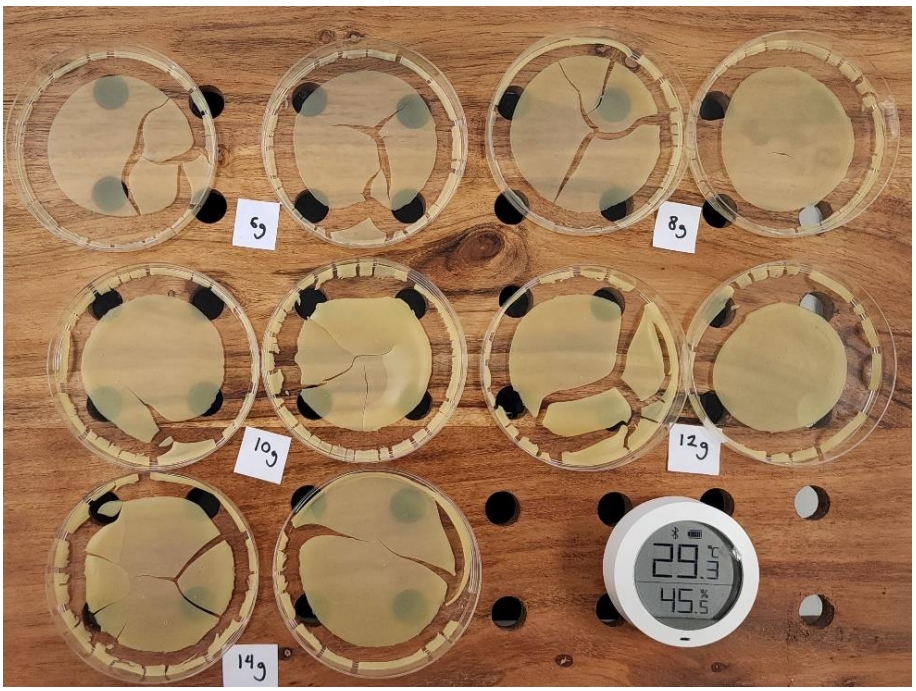

Figure 8. WPPC films made with 20% concentration after drying at 40 °C for 20 hrs (Trial 7). Films had different FFS dispensed. First row: 6 g and 8g; Second row: 10 g and 12 g; Third row: 14 g.

As whole films could be produced from a higher concentration of WPPC solution (16–20%), it is probably safe to assume that the amount of plasticiser in the formulation was sufficient. However, there was still a need to optimise the concentration of plasticiser for cost optimisation. Therefore, lower ratios of glycerol concentration were tested (**Trial 10**). Films made with a glycerol concentration lower than 1:2 WPPC:GLY cracked, so the glycerol concentration was kept at 1:2 for the film characterisation.

Trial 12 addresses the limitations found in the processing methodology. Issues during the processing revolve around the homogenising and drying mechanism. In films that were not homogenised well, the thickness and the unevenness expressed as a swirling pattern can be seen clearly (**Figure 9,** right). The homogenisation in the production was optimised using a homogeniser instead of the Thermomix. As a result, the effect of uneven mixing was minimised. Notice that the films in **Figure 10** lack the pattern.

In the drying mechanism, an uneven drying rate was an issue. The levelling table crafted had holes to allow sufficient airflow, but the air distribution was not enough due to the positioning of the food dehydrator. As seen in **Figure 11** (second row), the 10 g film in the middle was drying faster than the one on its left. As a result of this uneven heating, when the films had finished drying, some of the films that had more heat were thicker as darker colours can be seen in the films (**Figure 9**, left). The effect of uneven heating was minimised by re-arranging the film placement and re-directing the dehydrator fan, so it was not directly heating the films. The last

issue was with shrinking films, as observed in **Figure 10.** As mentioned above, protein shrink when dried. Furthermore, films containing lipids are also prone to shrinkage when dried at high temperatures (Pérez-Gago & Krochta, 2000). Based on this fact and information from the literature review, a probable explanation for the cracking and shrinking observed in the trials is that the amount of plasticiser used was not able to prevent extensive water loss. Because of that, the film shrinks during drying. The water loss was significant enough that the film became brittle with the decrease in plasticity. Further, because the fat decreases protein-to-protein interaction, the film cracked in an area where the exchange was the weakest. The shrinking was considered minor using the present formulation and processing technique, so it was not addressed further in this study but should be optimised in future studies.

It is important to mention that the films cast for the characterisation section had different total solid contents (**Table 1**). This is because the films were formulated based on the protein concentration and the assumption that proteins are the films' primary base material, which will directly impact its mechanical and barrier properties. The protein concentrations were calculated to be consistent between the WPPC, WPC and WPI powders to remove protein as a variable and investigate the effect of fat on the films. However, this protein standardisation resulted in differences in the total solids of the different formulations and could influence another aspect of the film. Furthermore, the WPC and WPI films were difficult to handle at higher protein concentrations. Therefore, FFS made with 10% WPC and WPI solutions were made to work around this issue. The amount of FFS dispensed was also adjusted to ensure films had the same amount of protein. The amount of FFS dispensed was determined by film thickness and ease of handling.

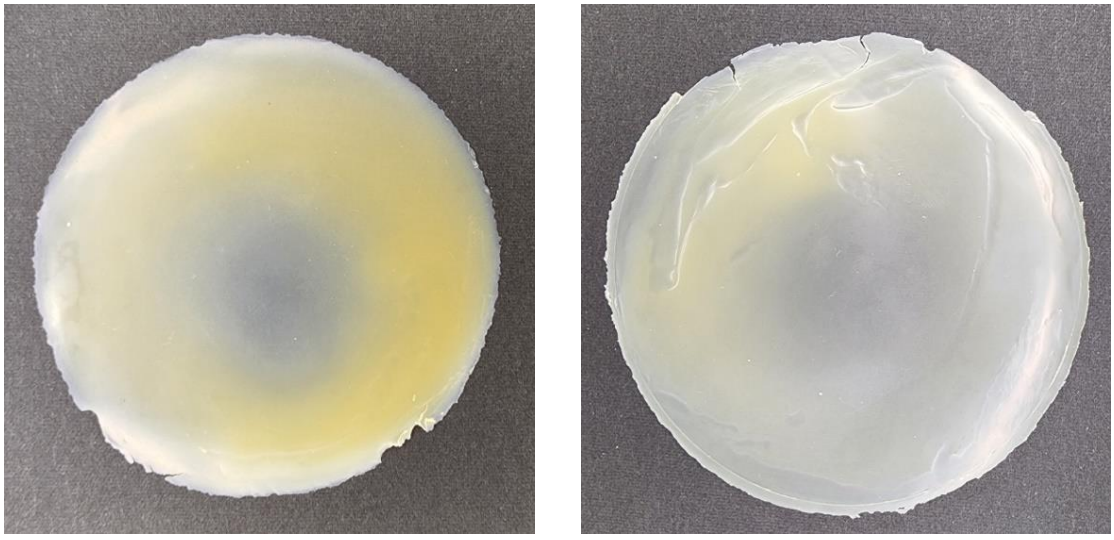

Figure 9. Appearance of WPPC film with uneven heating (left) and uneven mixing (right).

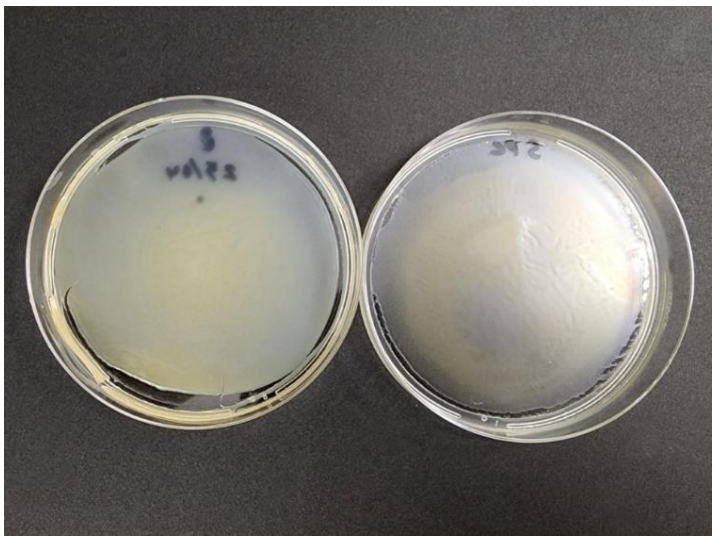

Figure 10. WPPC film (left) and WPC film (right) with shrinkage issue.

Figure 11. Uneven drying of the WPPC film made from 18% concentration (Trials 6 & 7). Films had different FFS dispensed. First row: 6 g and 8g; Second row: 10 g and 12 g; Third row: 14 g. Note: This image was taken prior during drying stage and was not dried.

4.3. Film characterisation

4.3.1. Film appearance

Visually, all of the dried films looked transparent when placed against a light object (**Figure 12**). WPPC and WPC film, however, may look opaque when placed against a dark object (**Figure 10**). The films had two different surface appearances depending on the orientation

during drying. The “smooth” and shiny surface can be seen on the side of the films facing down, while the “rough” surface can be seen on the films facing upwards during drying.

Looking closely at the film appearance, the surface of the WPPC and WPC films were affected by uneven heating and were a little darker in some parts of the film. Further, as mentioned in Section 4.2, the WPPC and WPC films still had unevenness and some shrinkage, as seen in **Figure 9** (left & right) and **Figure 10**. The swirling pattern in the characterised film was not noticeable unless closely observed and was attributed to phase separation of the lipid during drying. This observation was also present in films made by Papadaki et al. (2022). Some WPPC and WPC films also had small cracks or microbubbles (**Figure 13**, first row). The bubbles and microcracking were attributed to the study's limitation with the degassing mechanism. The microbubbles are only visible on the “rough” side, while the downside remains uniform. Cracks appear to go through the film. As this could impact the mechanical properties of the film, films displaying microbubbles were not used in the characterisation study. Meanwhile, WPI films had a spot pattern (**Figure 13**, second row). The spot pattern may be caused by lipid separation in the WPI film, but the pattern was different from what was observed in WPPC and WPC films. The spot pattern in the WPI films was not noticeable when the film was placed on a surface. The surface appearance of the WPI film was similar to what was captured by He et al. (2022).

Of greater importance than the visual appearance, Erdem et al. (2019) and Xu et al. (2021) focused on the film’s morphology when characterising their films. For example, in atomic force microscopy images taken by Erdem et al. (2019), the topography of the films facing down was smoother and more uniform, which would explain the shiny appearance. Unfortunately, the appearance and morphology of the films were not studied further due to time limitations.

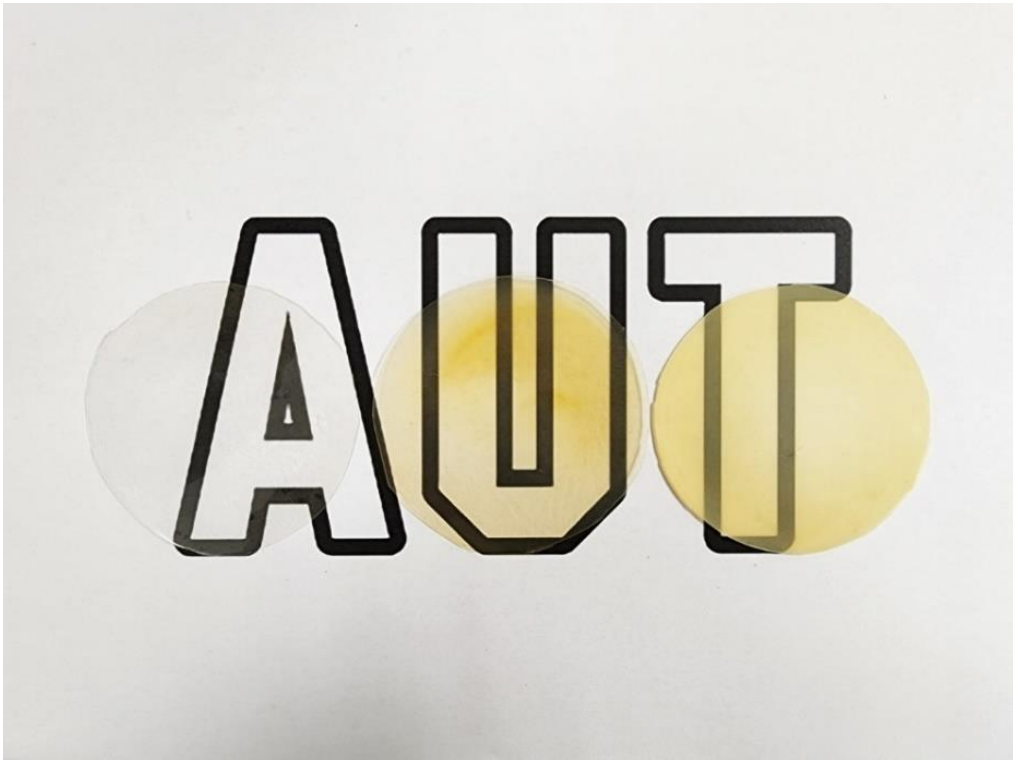

Figure 12. The appearance and transparency of WPI, WPC, and WPPC film (left, middle, and right, respectively).

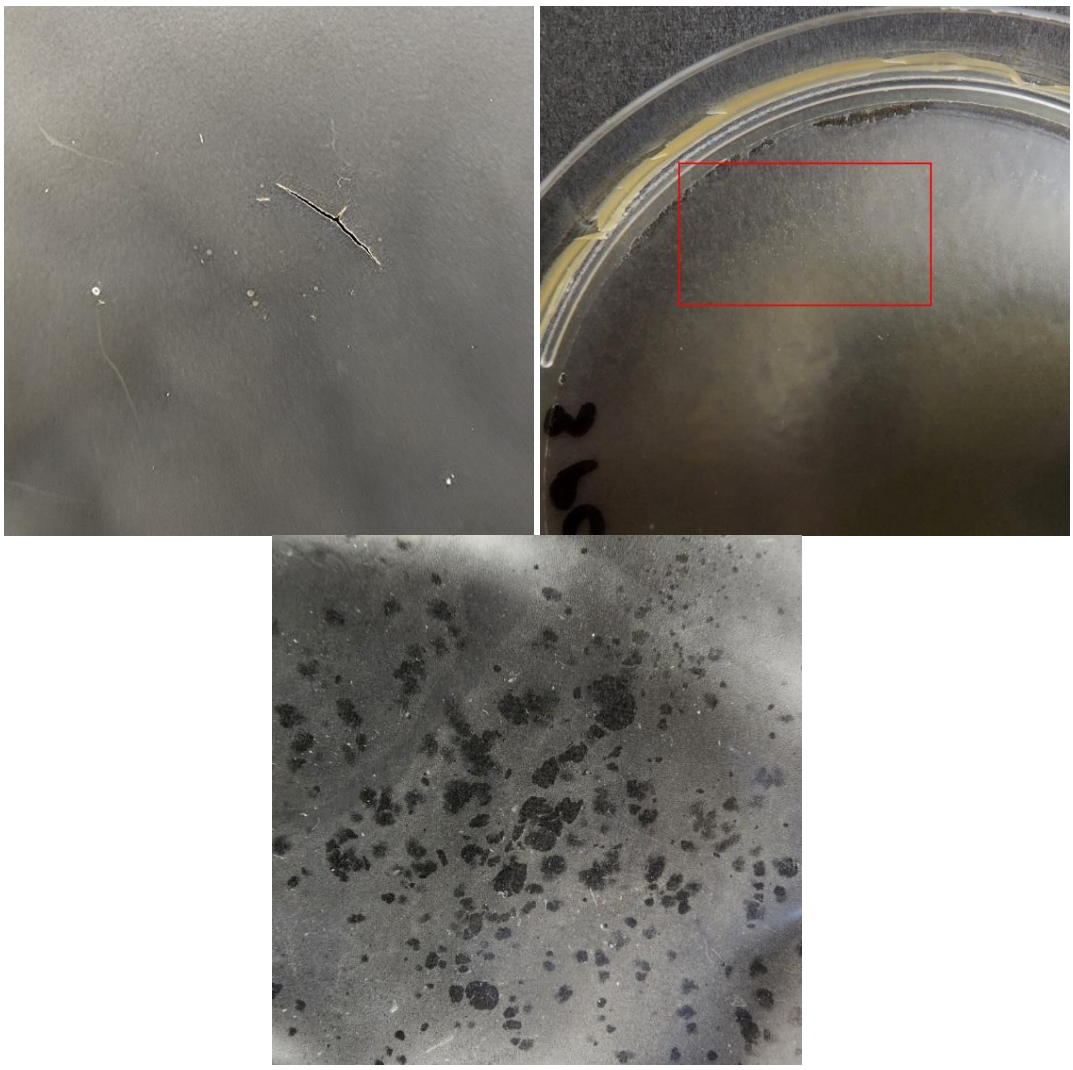

Figure 13. The close appearance of the whey films. First row: WPPC film with small cracks and microbubbles (left) and WPC film with microbubbles (right); Second row: WPI with some spotting pattern.

4.3.2. Colour

All the films are light in colour, with high L* values (**Table 3**). The L* values are consistent with the light transmission rates (**Figure 15**), where films with higher L* values are more transparent. Based on the ΔE values, the WPI films' values are not too different from the standard. Visually the WPPC and WPC films had a yellow colour (**Figure 12**), and the degree of yellow in these films was also represented clearly by the b* values (22.3 and 16.0, respectively). As a result, the WPPC and WPC films' ΔE values were large compared to the WPI ΔE value. The WPPC films' degree of yellowness was more green (a* = -0.13), while the WPC films' degree of yellow was more red (a* = 0.8). Based on statistical analysis, the degree of yellowness between these two films was significantly different ($P < 0.05$), but it was difficult to differentiate visually. However, part of the film affected by the uneven drying can be distinguished easily. The yellowness in WPPC and WPC comes from the carotenoid in milk fat (Chudy et al., 2020). The number of conjugated double bonds traps light in the yellow spectrum, and thus the

concentration of the fat is proportional to the yellow colour (Meléndez-Martínez et al., 2007). This explanation is coherent with the observations in this study. WPPC films with the highest fat content (**Table 2**) were more yellow than WPC and WPI films. Another probably explanation to the yellowness is probably due to maillard reaction when the lactose are heated (Bhat & Karim, 2014). However, this effect should be minimal as WPI film still appear transparent with minimal yellowness.

A side note on the colour experiments, the WPC film turned darker and more yellow after a few days in uncontrolled conditions. Xu et al. (2021) explained that the colour change was caused by the oxidation of -NH_2 in the WPC. Interestingly, the colour of WPPC and WPI films remained the same. From a consumer point of view, the film's colour after oxidation is probably undesirable. The study did not account for shelf-life; therefore, this observation was not investigated further in the current study but will be noted as a possibility for future studies. Furthermore, the physicochemical properties of the films may start to change with age due to degradation of the film. Colour change is only one of many aspects that may be affected, and, considering that only WPC film was affected, it is worth investigating in future studies.

Table 3. Colour parameters of WPPC, WPC, and WPI films.

	Colour parameters			
Samples	**L***	**a***	**b***	**ΔE**
WPPC	79.0 ± 0.06^{a}	-0.13 ± 0.06^{b}	22.3 ± 1.112^{a}	25.9 ± 1.04^{a}
WPC	81.6 ± 0.29^{b}	0.80 ± 0.10^{a}	16.0 ± 0.20^{b}	19.8 ± 0.28^{b}
WPI	84.9 ± 0.14^{c}	-0.23 ± 0.15^{c}	-0.23 ± 0.15^{c}	4.15 ± 0.73^{c}

L* value represents lightness, (0) = black, (100) = white; a* value represents red/greenness, (+) value = red, (-) value = green; b* value represents yellow/blueness, (+) value = yellow, (-) = blue; The small letters (a–c) indicate significant differences in the colour parameter between the films ($P < 0.05$).

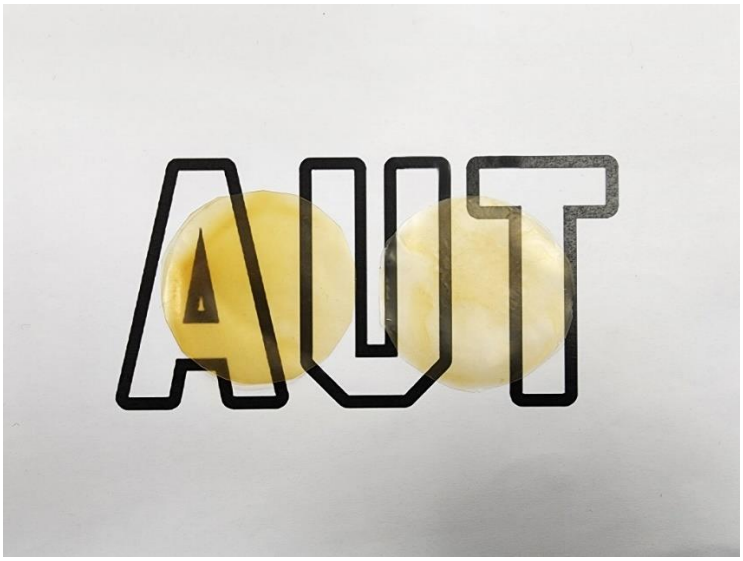

Figure 14. Oxidised WPC film (left) and normal WPC film (right).

3.3.3. Light transmission rate

The light transmission rate of the whey films is shown in **Figure 15**. In the lower UV range (200–300 nm), all films exhibited low transmission (>1%). However, the light transmission rate increased drastically in the higher UV range, between 280 and 350 nm. This observation was also reported by He et al. (2022) and Schmid et al. (2014b). For the WPI films, the change is more drastic (57.4%) compared to WPC and WPPC films (2.3% and 3.9%, respectively). WPPC and WPPC films were better at preventing UV light transmission. An explanation for this observation is that the fatty acid in WPC and WPPC absorb UV light at a proportional amount to their concentration (Forcato et al., 2005). While UV radiation can be used to preserve food, incorrectly applied or uncontrolled UV treatment may change the food composition and, therefore, may result in undesirable changes to the food (Csapó et al., 2019). At visible wavelengths (~380–750 nm), WPI had a good light transmission rate (>50%). On the contrary, WPC and WPPC film reached over 50% transmission at 500 nm and 600 nm, respectively. The light transmission rate peaked at around 700 nm (~77.5%) for both WPC and WPI films, while the WPPC films' light transmission rate was lower (~60.5%). All the films had good light transmission rates as the values reached above 50% within the visible light spectrum. Fernandes et al. (2020) stated that thick films may affect the film's light transmission rate. However, WPI films have similar light transmission between 600 and 1000 nm wavelengths. This observation was not coherent with what Fernandes et al. (2020) have observed. The film's colour can explain the difference in light transmission between the visible light spectrum. The yellow films (WPC and WPPC film) have the lowest light transmission at the violet–yellow spectrum (~380–600 nm). Considering the colour and transmission, the WPI film is probably preferable over WPPC and WPC films because consumers prefer colourless and transparent films to see the product inside the wrapping (Xu et al., 2021). Nonetheless, the application of the edible film for packaging that only serves as protection, e.g., chocolate wrappers, may work well with the films.

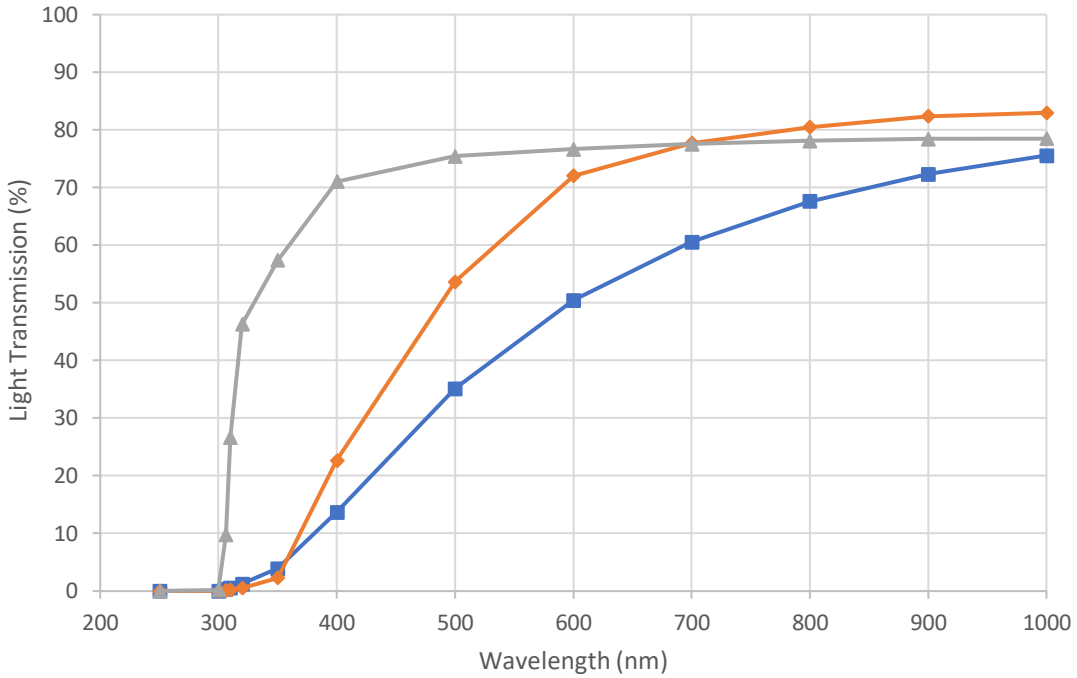

Figure 15. The light transmission rate of WPPC film (■), WPC film (◆), and WPI film (▲) in wavelengths 200–1000 nm.

4.3.4. Moisture content

WPPC had the highest moisture content at 35.9%. WPC and WPI both had a moisture content of around 29% (**Table 4**). The difference in moisture content is significant ($P < 0.05$). With the observed moisture content, microbial growth should be possible, yet, microbial growth was not noticeable after drying. The water activity of the film should be tested in future studies, which unfortunately could not be conducted due to time limitations. Considering that the films have the same protein and glycerol content, this difference in moisture content could be explained by other components that were not accounted for, i.e., fat, ash and any remaining lactose. Fat is generally hydrophobic and would not bind water, but the high phospholipid levels in the WPPC may contribute to moisture retention. As mentioned in the literature review, phospholipid has both hydrophilic and hydrophobic properties. The obtained result supports the hypothesis that hydrophilic functionality in the phospholipid may help retain water in the film. Unfortunately, this hypothesis cannot be confirmed as the defatted WPPC film experiment did not include characterisation of the defatted film. The effect of fat in WPPC films should be investigated in future studies. In regards to the moisture content in WPI films, Shaw et al. (2002) recorded that WPI films with the same whey to glycerol ratio have (2:1 WPI:GLY) higher moisture content of ~40%. WPI film produced by Seiwert et al. (2021) had 14.74% moisture content, but the formulation contained less glycerol (3:1 WPI:GLY). This observation supports the idea that moisture content increases are proportional to an increase in plasticiser concentration.

Table 4. Moisture content, swelling index, and water vapour permeability of WPPC, WPC, and WPI film.

Samples	Moisture content (%)	Swelling index (%)	WVP ($g.mm.hr^{-1}.m^{-2}.kPa^{-1}$)
WPPC	35.9 ± 0.34^{a}	22.0 ± 2.74^{b}	0.75 ± 0.01^{b}
WPC	29.5 ± 0.42^{b}	27.4 ± 2.13^{a}	0.80 ± 0.01^{b}
WPI	29.4 ± 0.24^{b}	30.5 ± 4.02^{a}	5.44 ± 0.01^{a}

The small letters (a–b) indicate significant differences in the colour parameter between the films ($P < 0.05$).

4.3.5. Swelling index

The swelling index of WPPC films was lower than the WPC and WPI films (**Table 4**) at 22.0%, 27.4, and 30.5%, respectively. The difference in swelling index between WPPC film and WPC and WPI films was statistically significant ($P < 0.05$). On the other hand, there was no significant difference between the swelling index between WPC and WPI film. The swelling index measures the films' water resistance. Thus, the recorded result suggests that the WPPC films are more water-resistant than the WPC and WPI films. However, interesting to note that the difference in the swelling index is approximately the same as the difference in moisture content. By adding the moisture content and swelling index percentage, all films would have approximately 60% moisture content. This could suggest that all films had the same overall water-binding capacity, but WPC and WPI films somehow did not retain the water during the drying process.

Comparing the film made in this study with WPI films made by Galus and Kadzińska (2016) (8% WPI, 2:1 WPI:GLY), the films had a higher swelling index compared to the WPI films observed in this study (51.6% and 30. 5%, respectively). Interestingly, WPI films produced by Gökkaya Erdem et al. (2019) (8% WPI, 1:1 WPI:GLY) had a similar swelling index to films made by Galus and Kadzińska (2016) despite having double the plasticiser concentration. WPC made by Kontogianni et al. (2021) (10% WPC, 2:1 WPC:GLY) had 8% swelling and films made by (Papadaki et al., 2022) (7% WPC, 2.5:1 WPC:GLY) had 39.5% swelling index. The differences in swelling index observed in the present and previous studies suggest the effect of formulation and processing techniques on the final film.

Regarding appearance and mechanical properties, the WPPC and WPC films turned opaque and became very brittle after the testing, as it was ripped when handled, indicating low mechanical resistance. However, despite the WPI films having the largest swelling index, the appearance of WPI films remained the same, and they were still sturdy after the testing. This observation is consistent with the recorded mechanical properties (**Table 5**) and the WVP of the films (**Table 4**).

4.3.6. Water vapour permeability (WVP)

WPPC and WPC films had lower WVP values compared to WPI films at 0.745, 0.795, and 5.44 g.mm.hr^{-1}.m^{-2}.kPa^{-1}, respectively (**Table 4**). The WPPC films had lower WVP values than the WPC films, but the difference in WVP values was not statistically significant ($P > 0.05$). This small difference could be attributed to the uniformity of the film thickness between the WPPC and WPC films. Shellhammer and Krochta (1997) also observed a decrease in WVP values when WPI films were formulated with milk fat fractions containing more lipid. Consequently, the WPI films Shellhammer and Krochta (1997) produced with 0% milk fat had a significantly lower WVP value at ~1.8 g.mm.hr^{-1}.m^{-2}.kPa^{-1} compared to this study at 5.44 g.mm.hr^{-1}.m^{-2}.kPa^{-1} . However, these films had lower plasticiser concentration than in this study, and a higher amount of plasticiser has been attributed to higher WVP values (Liaotrakoon & Raviyan, 2018; Shaw et al., 2002). In addition, the WVP value seems to decrease when the drying temperature increases. Pérez-Gago and Krochta (2000) reported WVP values of ~1.5, ~1.2, and ~1 g.mm.hr^{-1}.m^{-2}.kPa^{-1} for WPI films dried at 25 °C, 40 °C, and 80 °C (all had 40% RH), respectively. More importantly, Pérez-Gago and Krochta (2000) reported that the smooth side of the film has a lower WVP value when compared to the rough side, as the rough side was prone to phase separation during drying. This study did not investigate the differences between the rough and smooth sides of the WPPC, as the smooth side was always measured because it was considered that the smooth side would offer the best protection and thus should be facing outside.

4.3.7. Thickness

The thickness of the WPPC, WPC, and WPI films were 0.33, 0.34, and 0.24 mm (**Table 5**), respectively. The WPPC and WPC film samples had similar thicknesses, while the WPI films were thinner. Film thickness is affected by the materials type and the drying mechanism. Materials with poor solubility will precipitate and increase the film's thickness (Gounga et al., 2007; Xu et al., 2021). Based on the above explanation, the shrinkage observed in some WPPC films can also be explained. Regarding the differences in film thickness, four factors could contribute to these differences: (1) Due to solubility of the fat that caused the shrinking effect, (2) limitations caused by uneven drying, (3) the difference in unaccounted solids in the whey powders, and (4) the differences in the amount of total solids of the different FFS dispensed to create films with the same protein content.

The films' thickness made by previous studies also has variations. For example, WPI films produced by Gounga et al. (2007) (9%WPI, 2:1 WPI:GLY) and Fernandes et al. (2020) (10% WPI, 3:1 WPI:GLY) were 0.045 and 0.13 mm, respectively). In addition, the WPC film produced by Bahram et al. (2014) (8% WPC, 1:1 WPC: GLY)has a similar thickness to the WPC films in this study (0.31 mm). The WPC films made by Kontogianni et al. (2022) were thinner (0.19 mm)

despite having the same WPC concentration and having more FFS dispensed in the mould (which was also a Petri dish). Gounga et al. (2007) noted that thickness increases with an increase in glycerol (plasticiser) concentration. The differences in protein and glycerol concentration, formulation, amount of FFS dispensed, and type of moulds could explain the differences in thickness.

Table 5. Thickness and mechanical properties of WPPC, WPC, and WPI film.

Samples	Thickness (mm)	TS (MPa)	EAB (%)	E (MPa)
WPPC	0.33 ± 0.04^{a}	0.77 ± 0.16^{a}	6.10 ± 1.60^{b}	12.9 ± 2.05^{ab}
WPC	0.34 ± 0.07^{a}	1.94 ± 0.38^{b}	31.0 ± 6.80^{a}	6.69 ± 2.62^{b}
WPI	0.24 ± 0.04^{b}	3.77 ± 0.77^{c}	27.9 ± 5.07^{a}	14.3 ± 5.68^{a}

TS = Tensile strength, EAB = % Elongation at break, E = Young's modulus; The small letters (a–c) indicate significant differences in the colour parameter between the films ($P < 0.05$).

4.3.8. Mechanical properties

The mechanical properties of the WPPC films were inferior compared to the WPC and WPI films. The tensile strength for WPPC, WPC, and WPI films were 0.77, 1.94, and 3.77 MPa (**Table 5**), respectively. The tensile strength of each film was significantly different ($P < 0.05$). Tensile strength measures mechanical resistance due to cohesive forces between the chains, while elongation measures the film's plasticity (He et al., 2022). This suggests that WPI has the strongest crosslinking between the proteins for the three films. As for the elongation, the elongation at break for WPPC, WPC, and WPI films was 6.10, 31.0, and 27.9%, respectively. The EAB difference between WPC and WPI film was not significant ($P > 0.05$). However, it is clear that there is a significant difference ($P < 0.05$) between the EAB of WPPC films and WPC/WPI films. Shellhammer and Krochta (1997) explained that the increase in elongation was due to the plasticising of unsaturated and low molecular weight triglycerides in the milk fat. The Young's modulus of the films were 12.9, 6.69, and 14.3 MPa for WPPC, WPC, and WPI films, respectively. Based on these values, WPC films were more elastic than the WPPC and WPI films. Statistical analysis indicated that the difference in elasticity between WPC and WPPC film was not significant ($P > 0.05$). However, there was a significant difference the elasticity between the WPC and WPI films.

The mechanical properties were calculated based on the force applied in the stress area, considering the film's thickness and width. Even though WPI films were thinner, the tensile strength and elongation of the WPI films were larger than the WPPC films. Therefore, mechanical properties differences could be attributed to the films' formulation and processing. Di Pierro et al. (2018) noted that a higher concentration of plasticiser decreases the tensile

strength and Young's modulus while the elongation at break increases. However, as all the films have the same plasticiser concentration, it cannot be the factor causing the differences.

As protein concentration in all the films was consistent. The tensile strength and elongation weaknesses observed may be due to differences in the fat content. Fernández et al. (2007) and Shellhammer and Krochta (1997) reported that unsaturated fatty acid slightly reduced tensile strength and did not affect elongation. These findings are consistent with the observation in this study, as the tensile strength for the WPC film was lower than for the WPI film. On the contrary, WPC film also has a more significant elongation percentage than WPI film. Based on the above deliberation, the weakness observed in the WPPC film could have been due to the degree of crosslinking. As mentioned in Section 4.2, the two possible explanations for the degree of crosslinking were: (1) The WPPC powder extensive crosslinking combined with water loss made the film brittle and (2) The amount of fat in WPPC was interfering with the protein-to-protein interaction that the film becomes brittle.

5. Conclusion and future recommendations

5.1. Conclusion

This study formulated, optimised, characterised, and compared WPPC edible films with WPC and WPI films with the same protein concentration. Limitations with homogenisation, uneven drying, and bubbles prevented the creation of uniform films in the study. As a result, the thickness of the films was affected. However, edible films could be made with 16% (w/w) WPPC solution and glycerol at a 2:1 WPI:GLY ratio. In terms of its appearance and transparency, all films had good transparency and had above 50% transparency at 600 nm. WPPC and WPC films were also good at blocking the UV wavelength. The colour of the WPPC and WPC films was yellow, while the WPI films were colourless. Out of the three films, the WPI films had the best appearance. When comparing the mechanical properties of all the films, the WPPC films had significantly lower tensile strength and elongation at break than the WPC and WPI films (TS = 0.77, 1.94, and 3.77 MPa; EAB = 6.10, 31.0, and 27.9%, respectively). The WPPC films were very brittle and could not be used as edible films at the current stage. Interestingly, the WPPC film retained more water in the film matrix than the WPC and WPI films (moisture content = 35.9, 29.5, 29.4%, respectively). In terms of water barrier properties, the swelling index and WVP values for WPPC films were lower than WPC and WPI films (moisture content = 34.9, 29.-5, and 29.4%; swelling index = 22, 27.4, and 30.5%; WVP = 0.75, 0.80, and 5.44 $g.mm.hr^{-1}.m^{-2}.kPa^{-1}$, respectively). The WPPC films had greater water barrier properties than WPC and WPI films. The higher moisture content, lower swelling index and WVP suggested that the fat (phospholipid) and lactose may influence the interaction between the WPPC films and water. Further studies and improvements to the formulation are required to confirm the effect of phospholipids and their influence on barrier properties. The recommendations for future study are noted in Section 5.2. Unfortunately, this study could not fully characterise the extent of WPPC film's capabilities as an edible film due to the effect COVID-19 pandemic and the limitation of equipment.

5.2. Future Recommendations

The following are recommendations for future studies that may contribute to the further development of WPPC films:

- Investigate other WPPC's components on the effect of films such as minerals and lactose.
- Improvements with processing techniques: homogenisation, degassing, and drying mechanisms to obtain a more uniform film.
- Investigate the appearance and morphology of the dried WPPC film, such as under high resolution (AFM).

- Investigate the interaction between water and the WPPC film or phospholipid such as solubility, water activity, and phospholipid additions in WPI film.
- Investigate the degree of crosslinking in native WPPC film and dried WPPC film and how different thermal treatments affect the cross linking such as by using reducing or non-reducing SDS-PAGE.
- Investigate the shelf-life of the edible films
- Investigate the film's other barrier properties such as oxygen permeability.
- Investigate the effect of other additions and modifications to improve the mechanical properties of WPPC film such as pH adjustments and crosslinking.
- Investigate other ways to utilise WPPC as EPM, such as blending with other whey products and hydrolysing the WPPC before film production.
- Investigate the performance of WPPC film with different applications (coating).

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