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Evaluating Physicochemical Properties of Whey-Chia Seed Edible Films for Biodegradable Packaging

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ABSTRACT

The use of whey-chia seed edible films can help reduce environmental pollution while preserving the quality of food products. Films were produced using varying ratios of whey to chia seed (v/w) (whey-chia seed ratio of 1:0.5 (W1), 1:0.75 (W2), and 1:1 (W3)) through a completely randomized design with three treatments and six replications. The results showed significant differences (p<0.01) in elongation, tensile strength, moisture content, solubility, and color properties, except for crude fiber content (p>0.05). As the whey:chia seed ratio increased to 1:1, elongation increased up to 76.77%, while tensile strength decreased to 3.876 MPa, indicating an inverse relationship between these properties. The film with a whey:chia seed ratio of 1:0.5 showed 71.08% elongation but higher tensile strength (4.306 MPa) compared to the W3 treatment. The whey:chia seed ratio of 1:1 chia seed film also had the highest moisture content (49.52%), solubility (53.69%), and fiber content (15.67%). Increasing the ratio of chia seed resulted in a brighter and more transparent appearance. The microstructure of the film was continuous, compact, and homogeneous, without any irregularities such as cracks or air bubbles. The study concluded that chia seeds enhance the physicochemical and mechanical properties of whey-based edible films, with the optimal film produced at a 1:1 whey-chia seed ratio.

Keywords: biodegradable; chia seed; edible film; glycerol; whey

INTRODUCTION

In the last decade, plastic production has increased rapidly. Plastic provides advantages in maintaining packaged products, such as stability, freshness, and extending shelf life. The downside of plastic packaging is that, after the use, it accumulates residues because it is not biodegradable (Vega *et al.*, 2020), thus it becomes an environmental problem. Therefore, consumers today require biodegradable food packaging (Mellinas *et al.*, 2016). This has led to an increase in the production of innovative films derived from protein and polysaccharide polymers.

The emergence of natural polymer-based edible films as an eco-friendly substitute for plastics has created a potential solution to environmental pollution. Increasing attention has been given to the development of biodegradable packaging for the food industry, driven by concerns in the realms of sustainability and food safety (Fahrullah *et al.*, 2021; Maniglia *et al.*, 2015; Otoni *et al.*, 2017; Spotti *et al.*, 2016; Sukhija *et al.*, 2016). Polymers, including polysaccharides, proteins, lipids, and their combinations, are considered to be promising options for the production of packaging materials due to their biodegradability and abundance (Dick *et al.*, 2015).

The utilization of protein as a fundamental component in active packaging is an effective approach in the manufacturing process, as it serves to transport additives such as antioxidants and antimicrobial agents (Abdelhedi *et al.*, 2018; Nor Adilah *et al.*, 2018). Additionally, protein-based packaging exhibits barrier properties against water vapour (Cinelli *et al.*, 2014). A number of studies have been conducted on the use of whey protein in packaging applications, with encouraging results in terms of its ability to act as a barrier against moisture, oxygen, lipids, and aroma (Schmid, 2013). However, packaging developed from whey protein isolate has been found to exhibit poor water vapour barrier properties due to its hydrophilic nature (Azevedo *et al.*, 2015; Ramos *et al.*, 2012; Teixeira *et al.*, 2014).

The incorporation of polysaccharides derived from plant seeds with the capacity to form gels represents a highly promising avenue of research in the field of biodegradable packaging (Teixeira *et al.*, 2014). To date, the plant seed most commonly employed in the development of edible films is the chia seed (Cuomo *et al.*, 2020). The rationale behind the utilization of grain-based films in packaging development is their capacity to be metabolized within the human body in conjunction with foodstuffs (Aleksanyan, 2023). Chia seeds, in particular, can fulfill the requisite criteria for forming edible films. The functional properties of chia seed demonstrate that this polymer exhibits thickening properties, high viscosity in water, and produces favourable metabolic effects. Furthermore, chia seed has significant potential for utilization in the food industry, serving as a stabilizer, thickener, additive, and fat substitute (Chaves *et al.*, 2018; Zettel & Hitzmann, 2018; Chollakup *et al.*, 2020). This will allow them to work together to enhance functional characteristics and improve the material's flexibility, durability, thermal stability, and resistance to physical and environmental changes (Kandasamy *et al.*, 2021).

Polymers for producing edible films fall into two categories based on their source: hydrocolloids (polysaccharides and proteins) and lipids (fatty acids, waxes, resins, and composites) (Muñoz-Tebar *et al.*, 2021). One example of the hydrocolloids that can be used in the food sector are polysaccharides derived from plant seeds (Soukoulis *et al.*, 2018). Chia seeds are suitable raw materials for producing edible films due to their ability to be metabolized by the body into edible films (Cuomo *et al.*, 2020). Furthermore, chia seeds are characterized as polymers with properties that include thickening, high water viscosity, and the potential for favorable metabolic effects (Fernandes *et al.*, 2020).

The use of plasticizer in film making needs to be added to produce films to improve the flexibility and workability of the film (Dick *et al.*, 2015). The usual plasticizers used are polyols, specifically glycerol. The right type and concentration will produce mechanical properties and film permeability (Fahrullah *et al.*, 2022). These plasticizers are known to initiate various hydrogen bonds and interact with polymers by disrupting polymer bonds and creating space between polymer chains. In this research, investigating the synergistic effect of whey and chia seed added to improve the mechanical strength and stiffness of the films, as well as the film formation of whey and chia seed has not been explored. The aim of this investigation was to examine the effects of whey-chia seed on the physicochemical of edible films.

MATERIALS AND METHODS

This experiment used whey from bovine milk (Sigma-Aldrich, Germany), chia seed (Greenara, Indonesia), glycerol (Merck, Germany), NaOH, etanol pro analysis, distilled water, aluminium foil, and clean water.

Extraction of Chia Seed

Chia seeds were thoroughly washed with ethanol four times to eliminate extraneous material. Following this, the ethanol was removed from the seeds through a filtration process, followed by evaporation of the remaining solution in an oven set at 70 °C, then pulverization into smaller particles by using a blender. The resulting chia seed granules were subsequently macerated in pro-analysis ethanol (1:5) and soaked for three days. Afterward, the chia seed filtrate was filtered through the Whatman 41 filter paper. The chia seed filtrate was then put into a Heidolph Rotary Evaporators - Hei-VAP Value Digital G3 evaporation device to obtain a chia seed solution (Khazaei *et al.*, 2014).

Preparation of Whey-Chia Seed Film

Whey (1 g) and chia seeds (0.5; 0.75; and 1 mL) (w/v) were mixed in accordance with the treatment, followed by the addition of distilled water until a final volume of 15 mL was reached. The resulting whey and chia seed solution was supplemented with 30% plasticizer glycerol and heated on a hot plate at 90 ± 2 °C while being stirred using a magnetic stirrer at 250 rpm for 30 minutes. NaOH neutralizes the solution to reach pH 7–8. The film solution was subsequently transferred to a Petri dish and left to stand at room temperature for 24 hours. Finally, the completed edible film was packaged with wrapping paper before undergoing tests (Fahrullah *et al.*, 2021).

Elongation at the break. The elongation values of the edible film were assessed using a Universal Instrument Tensile Strength Meter. The film was cut into a 10x5 cm area before being stretched at a rate of 50 mm/minute.

Elongation (%) = L/L0 x 100%

where L represented the length of the film at a break in millimeters (mm) and L0 denoted the initial length in millimeters (mm).

Tensile strength. The edible films tensile strength was evaluated by cutting it into an 8x3 cm shape with a diameter of 1.5 cm, horizontally attaching it to the clamp, and measuring its highest tensile strength when it exhibited indications while being pulled (Wittaya *et al.*, 2013).

Moisture content. The porcelain cup was subjected to a thorough cleansing process, followed by drying in an oven for approximately one hour at a temperature of 105 ^{o}C. Subsequently, the cup was cooled in an applicator for approximately 10–20 minutes and weighed (C). Subsequently, a sample weighing between 0.5 and 1 gram (D) was weighed and placed into a porcelain cup. The cup and the sample were then subjected to drying in an oven at 105 °C for approximately 12 to 16 hours. Following this, the cup and the sample (E) were removed from the oven and cooled in an applicator for a duration of 10 to 20 minutes until a fixed weight was obtained (AOAC, 2005).

Moisture content (%) = [(C + D) - E] / D x 100%

Solubility. The percentage of film solubility is defined as the percentage of the film that has dissolved in water after a 24 hour soaking period. Film samples were cut into 3x3 cm pieces and placed in an aluminium cup that had been dried and weighed. The film sample was then placed in an oven at 100 °C for 30 minutes. The initial dry weight (W0) of the sample was recorded, and the sample was then soaked for 24 hours. Any sample that did not dissolve in the solution was removed and dried in an oven at 100 °C for 2 hours. The sample was then stored in a desiccator for 10 minutes. The weight of the sample after soaking was then measured again (W1). The percentage solubility of the sample in water was calculated using the equation (Gontard *et al.,* 1994):

S= (W0 - W1) / W0 x 100%

Crude fibre content. Calculation of crude fibre content using the Gravimetric method (AOAC, 2007).

Color properties. The measurement of colour properties using a digital colorimeter (T135, Taiwan) was carried out to determine colour (L, a*, and b*).

Microstructure film. The surface microstructure of the film was observed using the Scanning Electron Microscope (SEM JEOL JCM-7000, Japan). The film specimens were placed on cylindrical aluminum stubs using double-sided carbon adhesive tape and sputtered with a thin layer of gold. The test was conducted at 5 kV.

Energy Dispersive X-ray Spectroscopy (EDS). The edible film was cut into small pieces to fit into the SEM chamber, ensuring that the sample's surface was flat and free from contamination to obtain accurate results. Subsequently, the sample was positioned within the SEM chamber, where observations were conducted to obtain the surface morphology of the edible film. Subsequently, a specific region of the film's surface was selected for further analysis using EDS. The EDS measurement process commenced with the activation of the EDS detector on the SEM, followed by the focusing of the electron beam on the selected area. Subsequently, the EDS detector quantified the X-ray energy emitted from the sample as the electron beam interacted with the atoms in the material. The detected X-ray data were converted into a spectrum displaying energy peaks corresponding to specific elements in the sample. This spectrum was then subjected to further analysis to identify the elements present based on the position of the energy peaks, and element quantification was performed by comparing the intensity of the resulting peaks (Newbury & Ritchie, 2015).

Statistical Analysis

The study was conducted using a completely randomized design with six replications, employing an experimental approach. The treatments comprised distinct ratios of whey to chia seed (W1= 1:0.5; W2= 1:0.75; W3=1:1). The data were subjected to analysis of variance (ANOVA). Should the treatment prove to be statistically significant, it would be subjected to a Duncan Multiple Range Test (DMRT) using IBM SPSS Statistics 24.

RESULTS

Elongation at the Break

Elongation is the maximum amount of stretch a film can undergo before breaking. The physical property was achieved by gradually pulling the film until it reached its breaking point. Table 1 shows the effect of using a ratio of whey-chia seed on the elongation of the edible film. The analysis of variance revealed a significant effect ($p<0.01$) of the percentage of whey-chia seed on the elongation value of the edible film. The obtained average elongation value ranged from 71.08% to 76.77%, meeting the requirements of the Japanese Industrial Standard (JIS), which stipulates a minimum of 70%.

Tensile Strength

Tensile strength is a measure of the strength of a film. The test involves applying maximum tension until the film fractures. A higher tensile strength indicates greater resilience of the edible film against mechanical damage. The analysis of variance revealed a significant effect ($p<0.01$) of the percentage of whey-chia seed on the tensile strength value of the edible film (Table 1). The tensile strength values ranged from 3.876 to 4.306 MPa, meeting the Japanese Industrial Standard (JIS) requirement of at least 0.39 MPa.

Moisture Content

Moisture content is a critical parameter for edible films, affecting their mechanical properties, barrier properties, and overall performance. The analysis of variance revealed a significant effect ($p<0.01$) of the percentage of whey-chia seed on the moisture content of the edible film (Table 1). As the concentration of chia seeds increased, the water content also increased.

Solubility

Solubility is a crucial attribute of edible films, affecting their performance, application, and functionality. Edible films are designed to be either soluble or insoluble in specific environments, depending on their intended use. The analysis of variance revealed a significant effect ($p<0.01$) of the percentage of whey-chia seed on the solubility of the edible film (Table 1). The solubility of the films ranged from 52.23% to 53.91%, indicating low stability of the forming matrix.

Crude Fibre Content

The analysis of variance results suggested that the percentage of whey-chia seed did not have a significant effect on the crude fiber content of the edible film (Table 1). The study produced a crude fibre content ranging from 52.21% to 53.69%.

Color Properties

Table 2 presents the results of the film colour measurement, which was determined by three components: The L* component describes the brightness (luminance) of the colour on a scale of 0 to 100, where 100 represents the brightest colour. The a* component represents the proportion of green or red in the colour analyzed, while the b* component represents the proportion of blue or yellow color (Janik *et al.*, 2023; Stanisławska *et al.*, 2023). The results presented in Table 2 indicate that the brightness of the film increases with increasing chia seed concentration, resulting in a transparent appearance. The observed differences were statistically significant (p<0.01).

Table 1. Physicochemical properties of whey-chia seed edible films

Variables	Treatments		
	W1	W2	W3
Elongation at break (%)	71.08±2.67[a]	72.29±2.31[a]	76.77±1.50[b]
Tensile strength (Mpa)	4.306±0.26[a]	4.188±0.22[ab]	3.876±0.27[b]
Moisture content (%)	48.20±1.29[a]	48.40±1.32[a]	49.52±0.86[b]
Solubility (%)	52.21±0.49[a]	52.35±0.70[a]	53.69±0.77[b]
Crude fibre content (%)	15.34±0.46	15.57±1.14	15.67±0.53

Note: Means in the same column with different superscripts differ significantly (p<0.01). W1= Ratio whey:chia seed (1:0.5); W2= Ratio whey:chia seed (1:0.75); W3= Ratio whey:chia seed (1:1).

Microstructure Film

SEM (Scanning Electron Microscope) testing evaluates the homogeneity of the film, pores, cracks, and surface structure. It is used to assess the quality of the film. The aim of this SEM observation was to determine the cross-sectional and surface structures, as well as the homogeneity of the film solution mixture. Additionally, observing the microstructure would allow the study of the relationship between the properties present in the film. Figure 1 shows a cross-section of the surface struc-

Table 2. Colour measurement of whey-chia seed edible film with varying ratio

Treatments	Color Properties		
	L*	a*	b*
W1	80.65±0.49[a]	0.71±0.31[a]	23.49±0.31[a]
W2	81.85±0.65[b]	0.93±0.76[b]	23.93±0.12[b]
W3	82.44±0.49[b]	1.01±0.37[c]	23.99±0.45[b]

Note: Means in the same column with different superscripts differ significantly (p<0.01). W1= Ratio whey:chia seed (1:0.5); W2= Ratio whey:chia seed (1:0.75); W3= Ratio whey:chia seed (1:1).

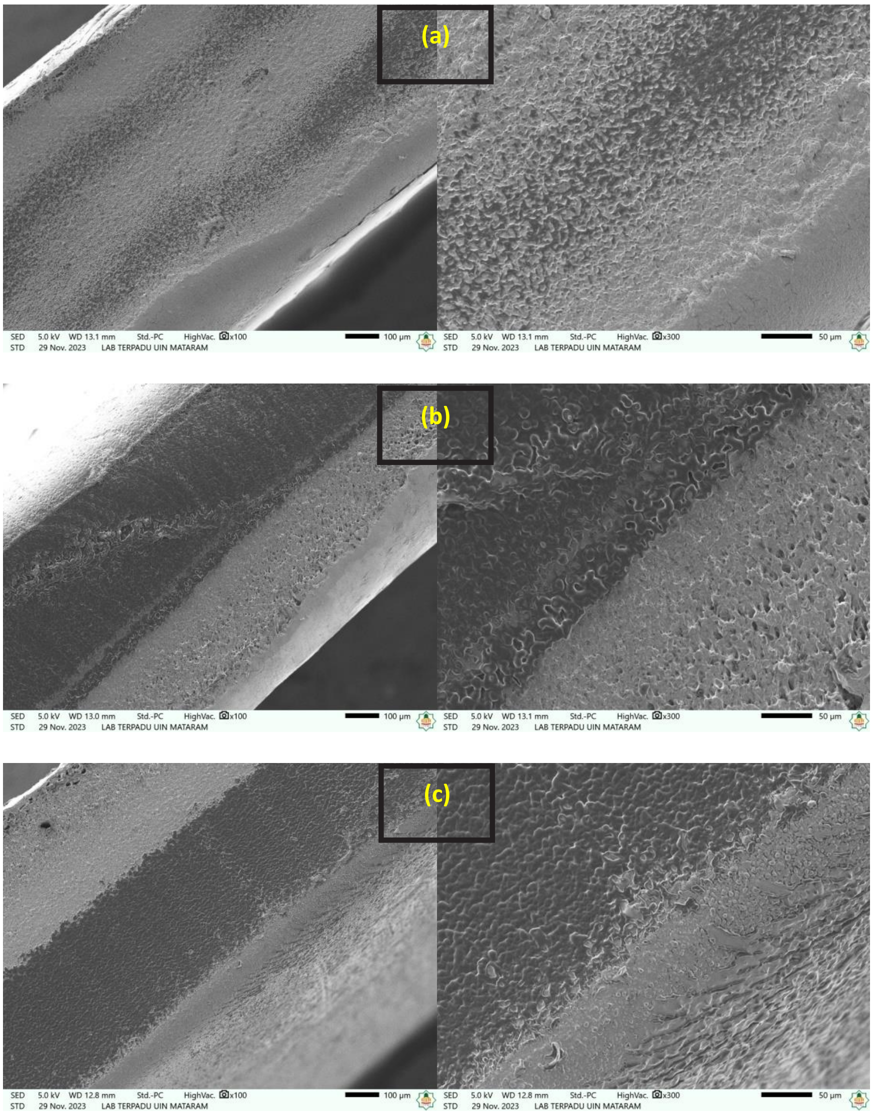

Figure 1. The microstructure of the edible film is composed of whey and chia seeds, with a ratio of (a) 1:0.5, (b) 1:0.75, and (c) 1:1. The images have been magnified to 100x (left) and 300x (right).

ture of a whey film with a higher concentration of chia seeds.

Energy Dispersive X-Ray Spectroscopy

Energy Dispersive X-Ray Spectroscopy (EDS) is a powerful analytical technique used to determine the elemental composition of materials. When applied to the study of edible films, EDS can provide valuable insights into the film's composition and potential functionality. The film elemental composition analysis results (Figure 2, 3 & 4, and Table 3) indicate the presence of several elements in the whey-chia seed film.

DISCUSSION

Elongation at the Break

Elongation was evaluated to characterize the amalgamation between whey and chia seed, which

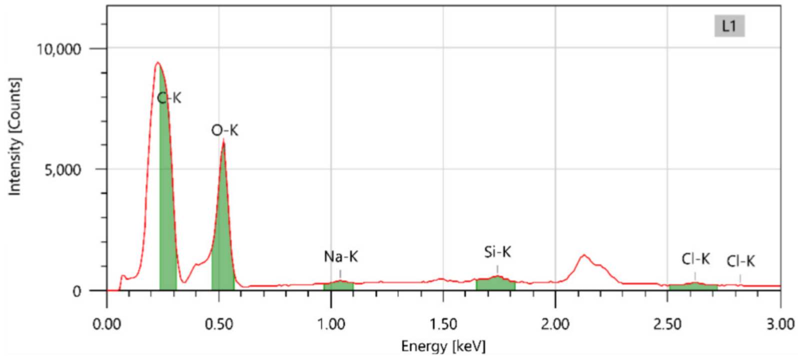

Figure 2. Energy dispersive X-ray spectroscopy analysis conducted on a whey-chia seed film with a 1:0.5 ratio.

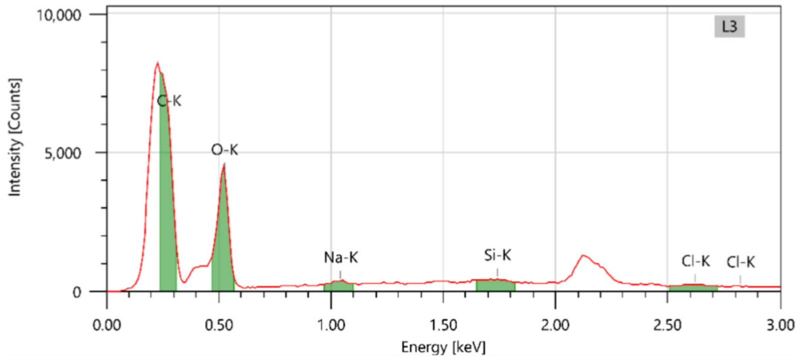

Figure 3. Energy dispersive X-ray spectroscopy analysis conducted on a whey-chia seed film with a 1:0,75 ratio

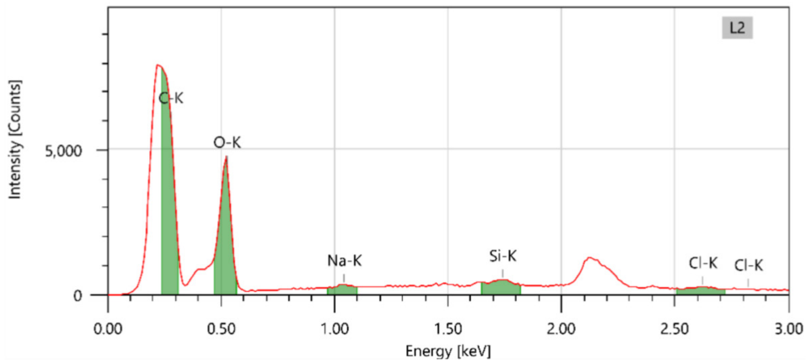

Figure 4. Energy dispersive X-ray spectroscopy analysis conducted on a whey-chia seed film with a 1:1 ratio.

Table 3. Elements of whey-chia seed edible film with varying ratio

Element	Treatments					
	W1		W2		W3	
	Mass (%)	Atom (%)	Mass (%)	Atom (%)	Mass (%)	Atom (%)
C	36.69±0.39	43.93±0.47	37.62±0.44	44.87±0.53	37.27±0.44	44.52±0.52
O	61.11±0.94	54.93±0.85	60.25±1.07	53.95±0.96	60.63±1.05	54.37±0.94
Na	0.81±0.10	0.51±0.06	1.31±0.14	0.82±0.09	0.84±0.12	0.53±0.07
Si	0.68±0.07	0.35±0.04	0.23±0.06	0.12±0.03	0.73±0.09	0.38±0.04
Cl	0.70±0.07	0.29±0.03	0.59±0.08	0.24±0.03	0.52±0.07	0.21±0.03

was a crucial parameter for comprehending the film's mechanical properties for food product applications. Analyzing this parameter will significantly aid in improving the understanding of the film's behavior (Muñoz-Tébar *et al.,* 2022). A high elongation value suggests that the film is more pliable when exposed to mechanical stress, including heat treatment that may dissociate the quaternary structure of the protein, denature the protein molecule, and cause intermolecular interactions that are absent in the initial protein form (Fahrullah *et al.,* 2021). The elongation value produced is directly affected by the concentration of chia seed used due to the gel created by the interaction between whey and chia seeds. The presence of chia seed as a polysaccharide is responsible for the formation and structure of the network, which may cause the aggregate formation of whey proteins. Films produced with a high ratio of polysaccharides are capable of greater elongation than those with a low amount (Muñoz-Tebar *et al.,* 2021).

The highest elongation value of the film was obtained with the addition of a glycerol plasticizer, which was 76.77%. This is in accordance with the research Dick *et al*. (2015), which resulted in an elongation value of chia seed-based edible film with the use of a glycerol plasticizer of 75%. Glycerol plasticizer is known to modify the functional properties of the film's constituent polymers by reducing intermolecular forces and increasing the mobility of polymer chains, thereby increasing the film's flexibility. The extent of hydrogen bonding between plasticizer molecules and matrix chains can determine the connection strength between the polymer and the plasticizer (Farhan & Hani, 2017). Glycerol can act as a plasticizer and absorb a significant amount of water. This property increases its ability to plasticize (Pak *et al.,* 2020). It can be inferred that the addition of more glycerol in the solution for film formation can enhance the flexibility of whey-chia seed film. However, this also reduces mechanical resistance, including tensile strength (Table 1).

Tensile Strength

The film's specific strength was evaluated by measuring its tensile strength on a scale ranging from the maximum achievable tensile strength to the point at which the film broke. Higher levels of tensile strength indicate greater mechanical resistance in edible films. The inclusion of plasticizers in the film solution results in a reduction of protein-protein interactions, an increase in polypeptide chain mobility, and a decrease in film resistance, leading to an increase in deformability (Silva *et al.,* 2020). Increasing chia seed concentration leads to lower tensile strength values, which are inversely proportional to the elongation value of the whey-chia seed edible film produced (Table 1). Adding chia seeds helps maintain the coherence and stability of whey-based edible film. The addition of polysaccharide-containing materials causes the stretching strength to increase, leading to increased stretching ability but decreased tensile strength (Timilsena *et al.,* 2015). The cross-linking between chia seed's hydrogen chains may contribute to the strength and integrity of whey protein films (Muñoz-Tebar *et al.,* 2021). Overall, the whey-chia seed films formulated with plasticizers demonstrated good tensile strength, indicating their potential to serve as edible packaging materials for a wide range of food products due to their flexibility and increased toughness (Dick *et al.,* 2016).

The film's tensile strength was enhanced by the addition of glycerol plasticizer. This enhancement can be attributed to glycerol's lower molecular weight when compared to sorbitol and PEG. The effectiveness of plasticizers in achieving the desired tensile strength is affected by their molecular weight. However, it has been found that the use of glycerol can weaken the film's resistance to mechanical stress (Fahrullah *et al.,* 2022) and reduce the stability of the solids dispersion system, resulting in poor physical properties, such as tensile strength, for edibles (Adamu *et al.,* 2017).

Moisture Content

When chia seed was used as the matrix, all films had slightly higher moisture content (average 36.6% to 41.9%) (Dick *et al.,* 2015). This suggests a high biodegradation capacity of the films (Capitani *et al.,* 2016). The observed outcome can be attributed to the polymer's high absorption properties (Steffolani *et al*., 2015) and the plasticizer's ability to form a network better retaining water within the film structure (Charles-Rodríguez *et al.,* 2020). Additionally, whey possesses hydroxyl properties that enable it to bind and absorb water in edible films (Mihalca *et al.,* 2021). Table 1 illustrates that an increase in the concentration of chia seed used resulted in a higher moisture content of the produced film; this can be attributed to the number of water molecules in the polymer chain and the loosening of the film matrix (Jouki *et al.,* 2014). The incorporation of chia seed in film formation allows for water absorption due to its significant water-soluble fibre content. As the concentration of chia seed increases, the possibility of water absorption by the whey polymer also increases. The glycerol plasticizer used in this study has hygroscopic properties that can cause it to attract water from the surrounding environment, thus contributing to the increase in film moisture content (Sanyang *et al.,* 2016).

Solubility

These results are consistent with previous studies that used chia seed as a matrix, which reported solubility in the range of 48%-80% (Capitani *et al.,* 2016). The study suggests that the polymer may be suitable for high-moisture foods, such as fruits, due to its high water solubility. This may be attributed to the improved interaction of its components, which are whey, chia seed, and plasticizer (Charles-Rodríguez *et al.,* 2020; Chiumarelli & Hubinger, 2014). The solubility of the resulting film increases with the concentration of chia seed used. This is due to the fact that changes in the ratio between chia seed and whey can affect the physical and chemical properties produced (Khodaman *et al.,* 2022). It is known that chia seeds contain fibre that can affect the hydrophilic properties of the film. Increasing the fibre content can increase the solubility of the film in water or other liquids (Fernandes *et al.,* 2020). Additionally, chia seeds have hydrophilic properties due to their fibre content and gel formation ability, which can affect film solubility and water absorption (Nehra *et al.,* 2023). The plasticizer used in this context tends to dissolve in water. Additionally, increasing its concentration leads to an increase in the solubility of the film. This relatively high solubility is due to the plasticizer's hydrophilic nature. An increase in plasticizer concentration can reduce the interactions between polymer molecules (Jaderi *et al.,* 2023). Increasing the empty space in the polymer matrix can affect water penetration into the film matrix, leading to an increase in water solubility (Edhirej *et al.,* 2017; Sanyang *et al.,* 2016). High solubility generally indicates lower water resistance. However, high solubility can be an advantage for certain applications such as packaging food as it indicates biodegradability.

Crude Fibre Content

Higher amounts of chia seeds resulted in increased crude fibre production due to the high fibre content of chia seeds (Capitani *et al.,* 2016). It is important to note that chia seeds contribute to the crude fibre content of edible films. Chia seeds are a rich source of fibre and can add nutritional value and functional properties when used in the production of edible films (Hrnčič *et al.,* 2020). The fibre content of chia seeds is 56.4 g/100 g of food, of which 53.45 g/100 g is insoluble fibre, and the rest is soluble fibre (Ding *et al.,* 2018). The preparation method of chia seeds, which involved maceration with ethanol solvent pro analysis, may have contributed to the results of total fibre measurements in this study. Chia seeds contain polysaccharides that can form hydrogen bonds with water, which affects the viscosity dispersion of the gel (Li *et al.,* 2023). Temperature can also affect the fibre content of the film as the constituent polymers become random and can potentially break the glycosidic bonds of the polysaccharides, thereby increasing the fibre content (Yi *et al.,* 2014). Similarly, for whey and plasticizer type applications, these polymers do not contain fibres.

Color Properties

The highest L* brightness was found in edible films produced using 1% chia seed ratio + glycerol, followed by a* and b* values. The incorporation of higher levels of whey protein tends to enhance the brightness (L*) of the resulting mixture. This is due to the fact that whey protein possesses a creamy hue, and the inclusion of chia seeds can elevate the a* towards redder hues. This is attributed to the pigments present in chia seeds, which can influence the red colour spectrum. Furthermore, the impact of chia seed on the b value is contingent upon the ratio and the processing methodology employed. The given references indicate that light absorption may occur at higher wavelengths and that transparency (Fahrullah *et al.,* 2021) and color are important parameters of edible films that can affect product acceptance and overall consumer perception. The yellowish color produced by the b* value was related to the composition of whey with chia seed. Dick *et al.* (2015) research on chia seed films with varying concentrations of plasticizers revealed that the resulting film had a slightly reddish and yellowish hue but remained transparent. The color formation (L*, a*, and b*) was mainly affected by the whey edible film composite and the use of plasticizers, which were influenced by the nature of the edible film formulation material. Additionally, the interaction between raw materials and the final result can be affected by edible film production methods such as mixing, heating, and drying.

Microstructure Film

A high ratio of whey to other ingredients will result in a film with a more uniform and denser microstructure. This is due to the tendency of whey molecules to form a strong and regular network. The addition of chia seeds can also act as a filler in the whey film matrix, thus affecting the cohesiveness and density of the film. The interaction between whey and chia seed occurs in two stages. Initially, chia seed fills the space within the whey matrix, increasing the thickness and reducing the transparency of the film. Subsequently, the active components in chia seed (mucilage) interact with whey molecules, influencing the film-making process. Such interactions can potentially affect the formation of hydrogen bonds and hydrophobic interactions within the whey network, which may lead to the emergence of diverse microstructures.

The image shows a cross-section of the film with varying concentrations of chia seed. The microstructure of the film affects its physical properties (Cofelice *et al.,* 2019). The surface cross-section structure in this study resulted in a continuous, compact, and homogeneous structure without any irregularities such as cracks or air bubbles. The microstructure of a material can be influenced by several factors, such as the homogenization method, composition, and structural arrangement of different components obtained at the end of the drying process (Fahrullah *et al.,* 2020; Fahrullah *et al.,* 2021). The concentration of chia seed was found to have no significant effect on the surface latitude, indicating that the film solution had a stable emulsion that could be maintained during the drying process (Hasheminya *et al.,* 2019). Glycerol is a plasticizer that affects the microstructure of the film by binding the molecules that make up the film, making it more flexible. The addition of glycerol results in a uniform morphology without remaining grains and improves the film's mechanical properties (Hassan *et al.,* 2018; Khodaman *et al.,* 2022).

Energy Dispersive X-Ray Spectroscopy

Energy Dispersive X-Ray Spectroscopy (EDS) is employed to analyze the elemental chemistry of materials (Hmmam *et al.,* 2023). Table 3 shows the mass and atomic unity percentages of several elements detected in this film, including carbon (C), oxygen (O), natrium (Na), silicon (Si), and chlorine (Cl). The EDS technique is used to detect the basic elemental composition (Hu *et al.*, 2022). The EDS spectrum displays prominent peaks for the elements carbon (C), oxygen (O), and natrium (Na), which are part of the whey and chia seed structures. The analysis of film elements demonstrates the outcome of the polymerization process in creating the polymer matrix. Combining whey, chia seed, and glycerol simultaneously ensures even distribution within the matrix. The X-ray patterns of all the complexes were very similar to each other, regardless of their composition or manufacturing method. The O element in the complexes exhibited higher mass and atomic percentage values than the other elements. While EDS may not be the optimal method for analyzing the ratio of whey and chia seed in foods, given its suitability for heavy element analysis, the technique can still provide valuable insights into the material and trace element content in the mixture (Newbury & Ritchie, 2015).

CONCLUSION

Increasing the chia seed ratio resulted in a notable increase in elongation, moisture content, solubility, crude fibre, and colour properties. Conversely, there was a discernible decrease in tensile strength. The combination of whey and chia seed at a ratio of 1:1 was found to be the most effective treatment, exhibiting the highest elongation (76.77%), tensile strength (3.876 MPa), moisture content (48.52%), solubility (53.69%) and crude fibre (15.67%) values. The findings of this research may facilitate the creation of packaging films with bespoke characteristics for a range of applications within the food industry.

CONFLICT OF INTEREST

The author has no conflict of interest with personal and other relationships related to this research.

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