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Edible Film Based on Whey-Chia Seed: Physical Characterization with Addition of Different Plasticizers

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Abstract: Edible film is a biodegradable packaging that can be an alternative as a food packaging material so as to reduce environmental damage and still have the function of maintaining the shelf life, quality and safety of food products. The aim of this research was to determine the effect of using different percentage of whey-chia seed and plasticizer type on the characteristics of films. This research used a completely randomized factorial design consisting of Factor A: percentage of whey-chia seed (A1= 1:0.5; A2= 1:0.75 and A3= 1:1) and Factor B: type of plasticizer (B1= sorbitol; B2= glycerol and B3= PEG). The interaction between of whey-chia seed and different types of plasticizer did not show a significant effect ($p>0.05$) on the characteristics of films, but the factor of whey-chia seed gave an effect on the thickness and gelation time of the films and the factor of different types of plasticizer too gave a significant effect ($p<0.01$) on the water vapor transmission rate (WVTR) value. The findings suggest that the incorporation of chia seed concentration with distinct plasticizers could enhance the physical properties of whey-based edible film. The use of percentage of whey-chia seed 1:1 with glycerol is the best treatment.

Keywords: Chia seed; Film; Plasticizer; Whey

Introduction

The development of edible packaging that has biodegradable properties to partially replace synthetic packaging has been intensified due to plastic waste disposal problems and impacts on the environmental pollution problems, one of which is from plastic waste which takes a very long time to decompose ranging from 10 to 1000 years (de Dicastillo et al., 2016). Therefore, the increasing consumer concern for the environmental regarding the effects of plastic packaging encourages research on packaging that has biodegradable properties made from renewable and environmentally friendly sources which are expected to be a substitute for conventional plastics (Han et al., 2018; Hosseini et al., 2015). The biodegradable packaging can be an alternative as a food packaging material so as to reduce environmental damage (Telussa et al., 2023) and still have the function of maintaining the shelf life, quality and safety of food products. Environmental concerns and food safety issues are driving increased interest in the development of biodegradable packaging for use in food packaging (Fahrullah et al., 2021; Fahrullah, 2021; Fahrullah et al., 2020a; Fahrullah et al., 2020b; Fahrullah & Ervandi, 2022; Maniglia et al., 2015; Maruddin et al., 2018; Otoni et al., 2017; Spotti et al., 2016; Sukhija et al., 2016). Biopolymer-based packaging materials are usually produced from proteins, polysaccharides and lipids or their mixtures in the form of edible films (Dick et al., 2015).

Whey protein-based edible films have been developed. Previous research by mixing other polymer materials (Fahrullah et al., 2022) has produced edible film characteristics such as flexibility, good tensile strength as a packaging material for food products. Several studies have been published on the use of whey protein for edible film applications and the results are very promising as a barrier against moisture, oxygen, lipids, aroma (Schmid, 2013). However, films developed from whey protein show poor water vapor barrier properties due to their hydrophilic nature (Azevedo et al., 2015; Teixeira et al., 2014). The addition of polysaccharides from plant seeds that can produce gels has been considered as one of the most promising in the

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development of edible packaging (Soukoulis et al., 2018). Plant seeds used so far in the development of edible films with essential oils are basil seeds (Hashemi & Khaneghah, 2017), chia seeds (Cuomo et al., 2020), lettuce seeds (Barzegar et al., 2020) and quince seeds (Jouki et al., 2014).

Chia seeds can be used in the food industry as foam stabilizers, emulsifiers, binders, suspending agents as well as adhesives as a result of their water holding capacity and viscosity (Salgado-Cruz et al., 2013). Chia seed gel consists of a branched matrix such as xylose, glucose and glucuronic acid (Timilsena et al., 2015) which can produce a gel in aqueous solution at low concentrations (Dick et al., 2015). Films from chia seeds are also biodegradable and can act as carriers of bioactive compounds such as antioxidants and antimicrobial agents (Hosseini et al., 2015; Sapper et al., 2018; Timilsena et al., 2015) to prevent microbial spoilage and food deterioration (Beikzadeh et al., 2020). Therefore, the gel obtained from chia seeds is a new source of polysaccharides that has a potential to produce interesting polymer blends as edible film manufacturing materials.

Problems that often occur in protein edible films are easy to break, hard texture and brittleness (Maruddin et al., 2018). To produce an efficient edible film, it must be optimized using plasticizers. In making edible films, plasticizers are needed to increase film flexibility, water vapor transmission rate (Farhan & Hani, 2017) and to prevent water loss, and can create space between polymer chains (Fahrullah, Eka Radiati, et al., 2020a). This study is crucial in transitioning from plastic to biodegradable packaging to offer a more sustainable alternative. Moreover, this could result in a functional food product due to whey protein's essential amino acids, which could provide consumers with added nutrients. Additionally, chia seeds are known to be a good source of fibre, promoting better overall health. Proper disposal of packaging materials would also minimize negative environmental impacts. So, the purpose of this research was to determine the effect of using different percentage of whey-chia seed and plasticizer types on the thickness value, gelation time, water vapor transmission rate and film microstructure.

Method

Extraction of chia seed

Chia seeds were first washed at least four times with ethanol to remove all foreign matter, after that is was crushed into smaller particles. The chia seed granules were then soaked (macerated) for 3 days using ethanol (pro analysis) and then it filtered using filter paper (Whatman 41) chia seed filtrate. The chia seed filtrate was then put into an evaporation device (Heidolph Rotary Evaporators-Hei-VAP Value Digital G3) to obtain a chia seed solution was obtained (Khazaei et al., 2014).

Preparation of Whey-Chia Seed Edible Film

Whey powder and chia seed (b/v) were mixed together according to the treatment and then distilled water was added until the solution reached a final volume of 15 mL. The whey + chia seed solution was added with 30% plasticizer and then it was heated at 90°C ± 2°C on a hot plate and stirred using a magnetic stirrer at 250 rpm for 30 minutes. The film solution was poured into a Petri dish and then allowed to stand at room temperature for 24 hours while calculating the gelation time. The finished edible film was packaged using wrapping paper before testing (Fahrullah et al., 2020a; Maruddin et al., 2018).

Thickness

Measurement of edible film thickness was carried out using a scruple micrometer model MDC-25M (Mitutoyo, MFG, Japan) with an accuracy of 0.0001 mm. It was calculated as the average of five different areas of films, namely 4 at the edges and 1 in the centre (Maruddin et al., 2018).

Gelation Time

Gelation time was measured by observing the length of gelation time in minutes.

Water Vapor Transmission Rate (WVTR)

Water vapor transmission rate was measured by placing the edible film into a desiccator containing 3 g of silica gel and then measuring every 24 hours for 5 days. WVTR is expressed in units of g/mm²/day using the formula in equation 1 (ASTM E 96 (1995), 1995).

$$WVTR = \frac{n}{t \times A} \quad (1)$$

Note:
n : change of weight (g)
t : time (days)
A : the surface area of the edible film (mm²)

Microstructure Edible Film

The microstructures of edible films were tested using an electron microscope of SEM JEOL JCM-7000. The edible film was prepared with the size of 0.5×0.5 cm, and then it coated with carbon and gold. It was then placed on the SEM device for microstructural observations.

Statistical Analysis

This research used a Completely Randomized Design Factorial with 6 replications. Factor A consisted of whey-chia seed precentage (A1= 1:0.5; A2= 1:0.75 and A3= 1:1) and Factor B consisted of different plasticizer types (B1= sorbitol; B2= glycerol and B3= PEG). The data obtained were analyzed using Analysis of Variance (ANOVA). If the treatment gave a difference then Duncan Multiple Range Test (DMRT) was conducted.

Research Scheme

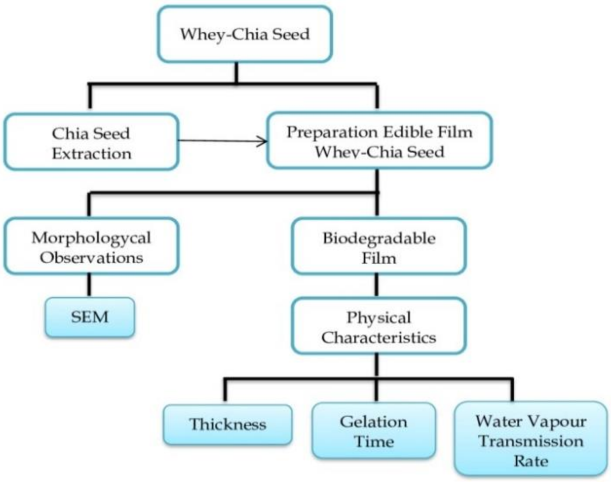

Figure 1. Research scheme

Result and Discussion

Thickness

The analysis of variance showed that the interaction between the percentage of whey-chia seed and different types of plasticizer did not have a significant effect ($p>0.05$) on film thickness. This is thought to be due to the difference in chia seed concentration which is not that large and the plasticizer concentrations used are all 30%. Fahrullah et al. (2020b) stated that the thickness of edible film is determined by the content of total solids, surface area and volume of film solution.

Table 1. Effect of Whey-chia Seed with Different Plasticizer Types on Film Thickness (mm)

Whey-chia seed	Plasticizer Sorbitol	Glycerol	PEG	Average
1 : 0.5	0.0294 ± 0.0001	0.0293 ± 0.0001	0.0293 ± 0.0000	0.0293^{a}
1 : 0.75	0.0301 ± 0.0001	0.0302 ± 0.0001	0.0300 ± 0.0006	0.0301^{b}
1 : 1	0.0308 ± 0.0005	0.0309 ± 0.0003	0.0310 ± 0.0002	0.0309^{c}
Average	0.0301	0.0301	0.0301	

Notes: Superscript ^{abc} in the same column gives a significant effect ($p<0.01$) between treatments.

The results of factor the percentage of whey-chia seed showed a significant effect ($p<0.01$) on the thickness of films (Table 1). The higher the concentration of whey-chia seed, so the film thickness too higher. The thickness values with the addition of whey-chia seed concentration were 0.0293 mm, 0.0301 mm and 0.0309 mm respectively. This increasing occurr due to the addition of an amount of polymer concentration, where there is a relationship between film thickness and the concentration of polymer added to the filmogenic solution (Capitani et al., 2016; Dick et al., 2015; Hauser et al., 2016). The thickness determines the properties of the film itself, It has been observed that the main factors modifying the thickness of films are the amount of plasticizer concentration added to the film solution (Dick et al., 2015) and the amount of layers superimposed on films (Dick et al., 2016; Salazar et al., 2020). Kaewprachu et al. (2016) states that increasing the total amount of solution will increase the total solids in the solution so that the number of polymers that form the edible film matrix is increasing.

The addition of different plasticizer types did not significantly affect ($p>0.05$) to the thickness of films. The overall average thickness was 0.0301 mm (Table 1). Similar results where the addition of glycerol plasticizer concentration did not significantly affect to film thickness were reported by Dick et al. (2015) who used chia seed sap. In this research, the concentration of plasticizer was 30% by weight of whey-chia seed. A high concentration of plasticizer can absorb more moisture resulting in an increase in thickness due to the swelling process. However, the use of different plasticizers did not give significant results, because the concentrations used were all the same.

Gelation Time

The analysis of variance showed that the interaction between the percentage of whey-chia seed with different types of plasticizer did not give a significant effect ($p>0.05$) on the length of film gelation. This is thought to be due to the concentration of plasticizer used using the same concentration and this plasticizer does not contribute significantly to the formation of gel on whey-chia seed film. The results of the addition of whey-chia seed percentage showed a significant effect ($p<0.01$) on the length of film gelation. The higher the concentration of chia seed so the faster the gelatination process of the resulting film. The average length of film gelation produced ranged from 13.94 until 17.53 minutes. This is because chia seeds have the ability to form a gel layer. It was formed after the chia seed was hydrated with water (Safari et al., 2016). Chia seed is a hydrocolloid with hydroxyl groups that can dissolve in water and are able to form a gel to form a thick solution (Adawiyah et al., 2022). The amount of hydroxyl groups found in hydrocolloids causes it dissolve more easily and be able to form gels (Munir et al., 2017). The results using different types of plasticizer did not significantly affect (p>0.05) to the length of film gelation. The use of plasticizer is only intended to reduce the stiffness of the polymer so as to obtain an elastic and flexible coating.

Table 2. Effect of Whey-chia Seed with Different Plasticizer Types on Film Gelation Time (Minute)

Whey-chia seed	Plasticizer			Average
	Sorbitol	Gliserol	PEG	
1 : 0.5	18.04 ± 5.62	16.50 ± 5.93	18.04 ± 5.62	17.53[b]
1 : 0.75	14.66 ± 5.82	14.64 ± 4.55	14.62 ± 2.59	14.64[b]
1 : 1	12.63 ± 3.02	9.34 ± 2.14	9.17 ± 2.60	10.38[a]
Average	15.11	13.49	13.94	

Notes: Superscript [ab] in the same column gives a significant effect (p<0.01) between treatments.

Water Vapor Transmission Rate (WVTR)

The analysis of variance showed that the interaction between the percentage of whey-chia seed and different types of plasticizer did not have a significant effect (p>0.05) on WVTR, but the average WVTR value produced in ranged from 5 until 6 g/mm2/day which still meets the Japanese Industrial Standard (JIS, 1975) which is a maximum of 7 g/mm2/day. This WVTR plays a role in the development of edible films to regulate water transfer between coating and external environment, prevent water loss as well as a determinant in food spoilage, therefore a low WVTR value is highly desirable. (Charles-Rodríguez et al., 2020; Kurt & Kahyaoglu, 2014; Sandoval et al., 2019).

Table 3. Effect of whey-chia seed with different plasticizer types on water vapor transmission rate (g/mm^2/day)

Whey-Chia Seed	Plasticizer			Average
	Sorbitol	Gliserol	PEG	
1 : 0.5	5.69 ± 0.72	6.64 ± 0.33	6.09 ± 0.51	6.14
1 : 0.75	5.55 ± 0.94	6.23 ± 0.41	6.69 ± 0.60	6.16
1 : 1	5.69 ± 0.68	6.64 ± 0.51	5.69 ± 0.51	6.01
Average	5.64[a]	6.50[b]	6.16[a]	

Notes: Superscript [ab] in the same column gives a significant effect (p<0.01) between treatments

The results of using the percentage of whey-chia seed showed no significant effect (p>0.05) on the WVTR value of films. The low WVTR value is produced by the 1:1 concentration, this is due to the interaction between the proteins contained in whey and the lipids contained in chia seed which will cause more hydrophobic zones that prevent the diffusion of water vapor through the film (Muñoz-Tebar et al., 2021). Table 3 shows that the higher percentage of whey-chia seed (1:1) shows a lower WVTR value, which is related to the thickness of films (Table 1). This is in accordance with Dick et al. (2015) which states that the difference in WVTR values produced can be caused by the source of hydrocolloids, the proportion of film solution and the thickness of the film used.

The results of using different types of plasticizer showed a significant effect (p<0.01) on the WVTR value of the film. Plasticizer characteristics including shape, structure and size generally affect the WVTR of edible films (Kaewprachu et al., 2018). WVTR is directly influenced by polymer chain mobility and edible film thickness as well as type and amount of plasticizer used (Mihalca et al., 2021). The highest WVTR value is produced by addition of glycerol plasticizer type, which is 6.50 g/mm^2/day. The results of this research are almost same as the research of Fahrullah et al. (2023) on addition of different types of plasticizers, where glycerol produces the highest WVTR value, this is because glycerol can reduce the intermolecular forces in the polymer region and increase the release of water by the polymer (Fahrullah et al., 2023), besides that the hydrophilic and hydroscopic properties of glycerol easily attract swater molecules that can form complex bonds so that more water will pass through the film layer (Nuanmano et al., 2015). This happens because of the small molecular size of glycerol which can reduce the free volume between polymers, making it easier for water molecules to move (Al-Hilifi et al., 2023). The hydrophilic nature of glycerol is what induces the absorption of water molecules so as to increase the WVTR value of films (Kaewprachu et al., 2018). The lowest WVTR value is produced by the use of sorbitol, which is 5.64 g/mm^2/day, this is because sorbitol has a lower ability to bind a water compared to glycerol and PEG. Sorbitol has a larger molecular size compared to glycerol, this size will increase the free volume between chains thus affecting the transfer of water molecules (Juliani et al., 2022).

Film Microstructure

Observation of the microstructure of whey films with addition of chia seed and different types of plasticizers using a Scanning Electron Microscope (SEM) tool which aims to display the constituent particles of materials contained in whey-chia seed films. Observation of film microstructure is a very important element to determine film characteristics (Fahrullah & Ervandi, 2021). The research that has been done produces microstructures in Figures 1, 2 and 3 and 1000 times magnification.

Figure 1 shows the microstructure of whey-chia seed film with a percentage of 1:0.5 each with sorbitol, glycerol and PEG plasticizers. The microstructure photo in Figure 1(a) produced using SEM shows that the polymer added is not perfectly fused (homogeneous), it can be seen that the whey-chia seed polymer only gathers at a certain point and does not spread evenly. Figure 1(b) shows the presence of cracks on the surface of films, this result is the same as research (Fahrullah et al., 2020a) where the surface structure with the addition of glycerol produces cracks in films. These cracks occur because during the process of making the film solution using a heating temperature of 90°C and air bubbles that are inserted during film formation. Cofelice et al. (2019) stated that the microstructure of films reveals the structural arrangement of components that can affect the mechanical properties of films. Figure 1(c) shows particles with a granular shape, which can be attributed to water loss through evaporation resulting in insoluble particles deposited on the film surface.

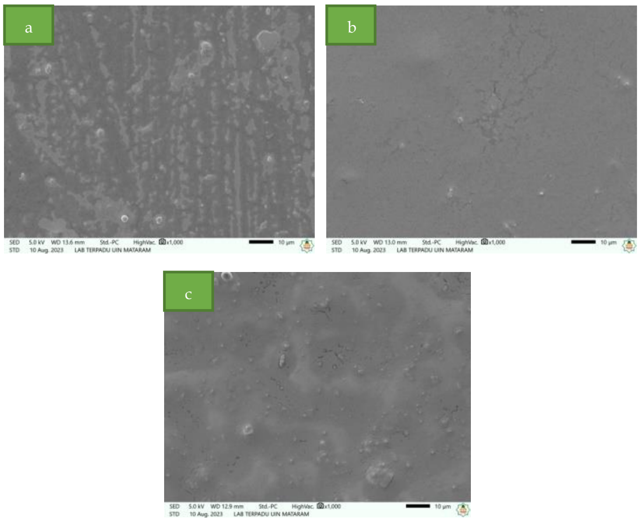

Figure 1. Microstructure of whey-chia seed film with a percentage of 1:0.5 using plasticizer types (a) sorbitol; (b) glycerol and (c) PEG

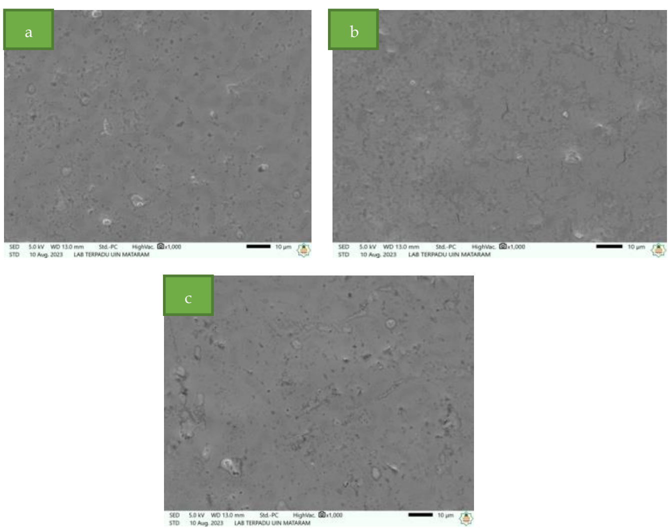

Figure 2. Microstructure of whey-chia seed film with a percentage of 1:0.75 using plasticizer types (a) sorbitol; (b) glycerol and (c) PEG

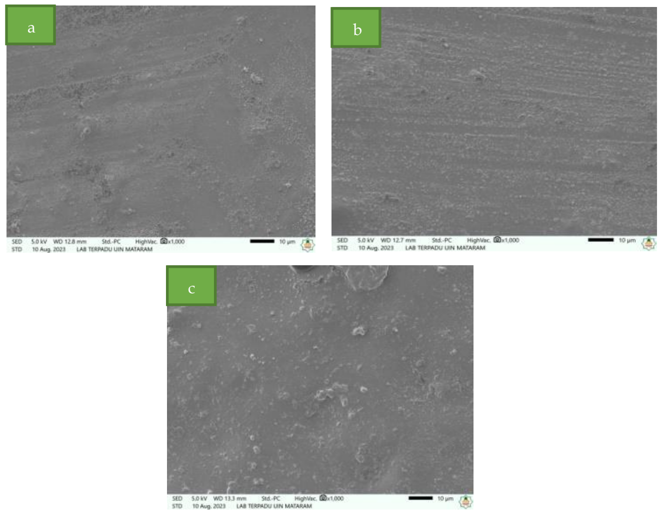

Figure 3. Microstructure of whey-chia seed film with a percentage of 1:1 using plasticizer types (a) sorbitol; (b) glycerol and (c) PEG

Figure 2 shows the microstructure of whey-chia seed films with a percentage of 1:0.75 with different plasticizer types. The microstructure photograph shows a flat surface for all plasticizer types, but there are some granules as the chia seed concentration increases and indicates that the film solution has a stable emulsion system that can be maintained during the drying process (Hasheminya et al., 2019). Figure 3 shows the microstructure of whey-chia seed film at a percentage of 1:1 with different types of plasticizer. The microstructure photograph shows that the addition of sorbitol and glycerol plasticizers produces a flat surface on whey-chia seed film compared to PEG plasticizer, this is because the molecular weight of sorbitol and glycerol is smaller than PEG. Saberi et al. (2017) stated that the molecular weight of plasticizer can affect the microstructure of films, the smaller the molecular weight of a plasticizer sothe film matrix will become more compact and homogeneous. Glycerol as a plasticizer makes the film more flexible by bonding the molecules that make up the film (Elmi et al., 2017). Saberi et al. (2017) found results similar to previous research indicating that the molecular weight of the plasticiser impacts the microstructure of the film. A lower molecular weight results in a more compact and homogeneous film matrix. The molecular weight of glycerol is measured at 92.09 g/mol. Additionally, Hassan et al. (2018) reported that the addition of polyol (glycerol) produces a uniform morphology without any remaining granules. Technical abbreviations will be explained when first introduced. The addition of glycerol as a plasticizer can enhance the film's flexibility, thereby improving its mechanical properties. As demonstrated in Figure 1b, 2b, and 3b, the resulting microstructure appears smoother. This observation aligns with the findings of Purnavita et al. (2020) study, wherein the incorporation of glycerol led to a more uniform and smoother film surface when combined with glucomannan and palm starch ratio. This aligns with the properties of glycerol as a plasticizer, which enhances the film's flexibility by elongating the bonds linking the film's molecules (Kamsiati et al., 2017).

Conclusion

The utilization of varying chia seed concentrations in conjunction with diverse plasticisers holds significant potential for enhancing the physical properties of whey-based edible films. The percentage of whey chia seed 1:1 with glycerol plasticizer is the best treatment.

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Author Contributions
Conceptualization, F.F, D.K. and A.N.; design experiment, F.F.; conducting research, F.F, D.K. and A.N.; interpretation of data, F.F. and A.N.; writing—original draft preparation, F.F, D.K. and A.N.; writing—review and editing, F.F, and A.N. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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