Jurnal Biologi Tropis

Original Research Paper

Modification of Protein-Based Edible Film Characteristics with Different Glycerol Concentrations: A Study on Thickness, Gelation, and Microstructure

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Article History
Received : October 10th, 2024
Revised : October 30th, 2024
Accepted : November 05th, 2024

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Abstract: A thin layer of material added directly to food products that comes from consumable sources is called an edible film. Proteins can be used to create edible films with barrier qualities against oxygen, moisture, and smell, making them appropriate for use as packaging materials that improve product appearance and have preservation effects. Finding out how different glycerol concentrations affect the thickness, gelation duration, and microstructure of protein-based films is the aim of this investigation. A completely randomized design with three treatments and three replications was used in this investigation. The treatments comprised distinct glycerol concentrations, designated as P1 (35%), P2 (40%), and P3 (45%). The protein-based films exhibited thickness values of 0.108–0.113 mm, gelation times of 16.00–20.67 minutes, and a uniform microstructure. At higher concentrations, such as 45%, glycerol resulted in increased porosity and aggregation within the polymer matrix, which in turn led to a reduction in the homogeneity and mechanical strength of the films. A glycerol concentration of 35% proved to be the most effective treatment for the production of a whey-gelatin-based edible film, resulting in a smooth surface and an even distribution of glycerol within the protein matrix. This approach effectively reduced the occurrence of cracks or irregularities.

Keywords: Edible film, gelatin, glycerol, whey.

Introduction

The environmental consequences of plastic waste disposal are a growing cause for concern. The decomposition of plastic waste can take up to 1000 years (de Dicastillo et al., 2016), making it a significant contributor to environmental contamination, particularly in marine and terrestrial ecosystems. This has resulted in heightened consumer awareness of environmental issues and a corresponding drive to identify more sustainable solutions. One promising avenue is the development of edible packaging products. These biodegradable alternatives could supplant synthetic plastic packaging while mitigating its detrimental environmental impact (Han et al., 2018; Hosseini et al., 2015).

The discrepancy between the optimal and the actual state of affairs emerges when the existing plastic packaging, despite its efficacy in safeguarding the safety and quality of food products, is exceedingly challenging to decompose and causes environmental degradation. The optimal packaging solution would be biodegradable, environmentally friendly, and still serve the primary functions of extending product shelf life, maintaining quality, and ensuring food safety (Mellinas et al., 2016). Consequently, research into the creation of biodegradable packaging derived from renewable resources, particularly biopolymers, is becoming increasingly prevalent. Such materials are deemed capable of producing environmentally friendly packaging that is suitable for use in food packaging applications. (Fahrullah et al., 2021; Maniglia et al., 2015; Otoni et al., 2017).

Whey protein is one of the most extensively investigated materials for use in edible films. These proteins have significant potential for the production of edible films with barrier properties against oxygen, aroma, and aroma (Schmid, 2013). However, the primary challenge associated with its utilisation is its hydrophilic nature, which results in whey films exhibiting low resistance to water vapour transmission (Azevedo et al., 2015; Ribeiro-Santos et al., 2018; Teixeira et al., 2014). Therefore, more innovation is needed to improve the films' mechanical and physical qualities, specifically by adding additional ingredients such polysaccharides. (Fahrullah et al., 2021; Fahrullah et al., 2023b; Fahrullah & Ervandi, 2022; Soukoulis et al., 2018).

In order to produce an optimal edible film, the use of plasticizers is necessary. The aforementioned plasticizers serve to enhance flexibility, refine the surface, and curtail the WVTR of the resulting film (Farhan & Hani, 2017). One of the most commonly utilised plasticizers is glycerol, which has been demonstrated to enhance the flexibility and physical quality of whey-based edible films (Fahrullah et al., 2022; Fahrullah et al., 2023c; Fahrullah et al., 2020a). Glycerol has the capacity to create space between polymer chains, enhance the structural integrity of the film, and augment its moisture resistance (Acquah et al., 2020; Pérez et al., 2016).

This research offers a novel contribution to the development of whey-based edible packaging, incorporating additional polysaccharide and glycerol components to produce films with enhanced functional and environmentally friendly properties. This study examines the role of glycerol as a plasticizer to enhance flexibility, with a particular focus on the impact of glycerol concentration on thickness, gelation time and microstructure stability. This area has not been extensively explored in previous studies, which have primarily investigated the influence of glycerol on tensile strength or elongation (Fahrullah et al., 2022; Fahrullah et al., 2023a; Pak et al., 2020). The objective of this study was to ascertain the impact of glycerol concentration on the thickness, gelation time and microstructure of protein-based edible films.

Material and Methods

Tools and materials

The following tools were employed in this research: a hot plate stirrer, a magnetic stirrer, an Erlenmeyer flask, a measuring cup, a measuring tube, a thermometer, a desiccator, and a Petri dish. The materials employed in this research are as follows: whey protein, gelatin, distilled water, silica gel and glycerol.

Preparation of edible film

Whey powder and gelatin powder were combined in a 1:1 ratio to create the edible film solution, which was then diluted with water until it had a volume of 10 milliliters. After heating the solution to 90°C with a hot plate stirrer, it was agitated for 30 minutes at 250 rpm with a magnetic stirrer. The plasticizer was added at concentrations of 35%, 40%, and 45% at the 25th minute. After that, the heated film solution was transferred to a petri dish and cooked for two days at 50°C in an oven. Before the testing procedure started, it was then kept in a room at room temperature for a whole day. (Modification of (Fahrullah et al., 2020b).

Thickness

The thickness was determined by means of a screw micrometer, with the mean thickness calculated by averaging the measurements taken from five distinct regions of the film, including four edges and one centre. (Maruddin et al., 2018; Sabil et al., 2021).

Gelation Time

The gelation time of an edible film is determined by observing the length of time required for the gelation process to occur, which is typically expressed in seconds.

Microstructure

The JEOL JCM-7000 SEM electron microscope was used to examine the film's microstructure. After preparing edible films of 0.5 × 0.5 cm, they were coated with gold and carbon. The sample was then put on the SEM apparatus so that the microstructure could be seen.

Data analysis

This study employed a completely randomized design, utilising distinct glycerol

concentration treatments. The treatments were designated as P1 (35%), P2 (40%), and P3 (45%). Analysis of variance (ANOVA) was performed on the data, and if significant differences were found, the Duncan multiple range test (DMRT) was used to assess the data.

Results and Discussion

Thickness

The thickness values of protein-based edible films made with different glycerol concentrations are shown in Figure 1. The results of the variance analysis demonstrated that the incorporation of varying concentrations of glycerol into the thickness measurement did not yield a statistically significant impact ($p>0.05$) on the film thickness value. However, it was observed that as the concentration of glycerol increased, the resulting film exhibited a corresponding increase in thickness. This phenomenon can be attributed to the specific composition of the materials utilized in the production of these films (de Jesus et al., 2020; Sanyang et al., 2016).

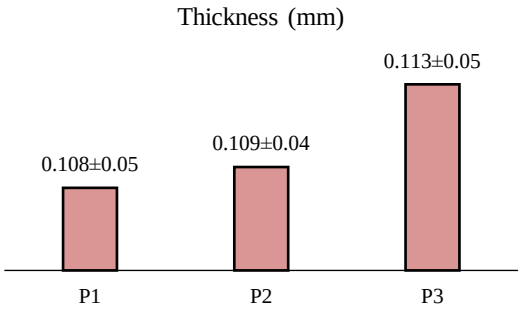

Figure 1. The thickness of the protein film with different glycerol concentration

The incorporation of whey protein, for instance, has been observed to result in an increase in film thickness. The thickness of the film is contingent upon the solids content of the film-forming solution. The components of the film-forming solution influence the alignment and solidification of the molecules during the drying process of the film on the Petri dish, thereby resulting in variations in the thickness of the final film (Ayunita & Fahrullah, 2024; Fahrullah et al., 2021, 2024; Fahrullah et al ., 2023c; Fitrah & Fahrullah, 2024). Films have been shown to form thicker when the total solids content of the film solution increases (Capitani et al., 2016; Dick et al., 2016).

The reason for this is because glycerol contributes to the network's development in the film. Furthermore, this glycerol can enter into the cavity of the film matrix, which results in thickening (Rusli et al., 2017; Sanyang et al., 2016). The thickness of the edible film is a crucial factor, as it influences the barrier and mechanical properties of the film. The addition of glycerol as a plasticiser resulted in the lowest film thickness compared to other plasticisers (Fahrullah et al., 2020a; Fahrullah et al., 2020b). It is established that glycerol has the lowest molecular weight and total solids content, which results in a lower film thickness. However, at the same concentration, the difference is not statistically significant. Conversely, a thicker film will impede the transmission of gases during respiration, which will result in the accumulation of ethanol and the development of off-flavours in the foodstuff. Therefore, it is essential to ensure that the film thickness is appropriate in order to maintain optimal food quality.

Gelation time

The gelation time of protein-based edible films made with different glycerol concentrations are shown in Figure 1. The results of the analysis of variance demonstrated that the utilisation of varying glycerol concentrations did not exert a notable influence on the gelation time of the edible film. The gelation time test results indicated that the gelation time at a 35% glycerol concentration (P1) was 20.67. At a glycerol concentration of 40% (P2), the gelation time was reduced to 16 minutes, which was the lowest observed gelation time among the three treatments. At a glycerol concentration of 45% (P3), the gelation time exhibited a further increase, reaching 19.33 minutes. These findings suggest that the gelation time is subject to fluctuations that are not significantly influenced by an increase in glycerol concentration.

The use of glycerol as a plasticiser is of significant benefit in the production of edible films, as it increases the flexibility and reduces the stiffness of the polymer network. The incorporation of glycerol typically results in a reduction in intermolecular tension, thereby facilitating a more relaxed configuration of polymer chains and enhanced molecular

mobility. This enhanced molecular mobility should facilitate a more rapid gel or film formation process. Nevertheless, the present study revealed that an increase in glycerol concentration from 35% to 45% did not result in a notable alteration in gelation time. This may be attributed to the intricate interactions between glycerol, whey protein and gelatin that constitute the polymer network. The hydrophobic and hydrophilic characteristics of these materials can influence the overall gelation behavior (Sanyang et al., 2016).

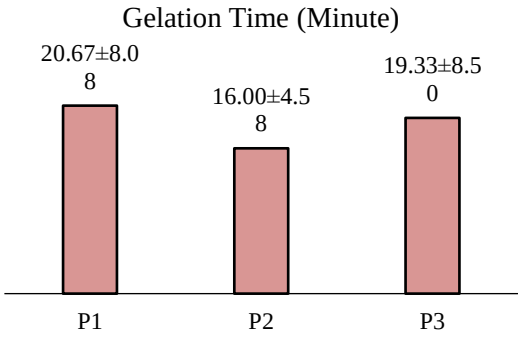

Figure 2. The gelation time of the protein film with different glycerol concentration

Furthermore, the stabilization of the protein-protein matrix in the whey and gelatin mixture may also exert an influence on the aforementioned results. Whey proteins, particularly β-lactoglobulin, exhibit the capacity to form a gel under specific conditions through thermal denaturation. In contrast, gelatin displays a distinct gelling behaviour, whereby its structure is influenced by temperature and concentration. It is possible that higher glycerol levels may result in a more complex balance between molecular interactions within the polymer matrix, which could in turn affect gel formation dynamics in a way that is not significantly affected by changes in glycerol concentration (Bonilla & Sobral, 2019).

The impact of glycerol on the protein-polymer system is frequently contingent upon the ratio of the plasticizer to the remaining film components. The findings of this study indicate that a balanced ratio of whey and gelatin (1:1) can facilitate a more stable equilibrium within the polymer matrix, thereby preventing significant alterations in the gelation process when different concentrations of glycerol are employed. Other studies have demonstrated that an increase in glycerol concentration in protein-polymer edible films can influence other physical properties, including thickness, transparency and mechanical strength. However, this does not always result in a notable impact on the initial physical structure formation process, such as gelation (Chen et al., 2019; Hosseini et al., 2016).

Although the gelation duration results demonstrated no statistically significant difference, the variation of glycerol concentration within the range of 35% to 45% may potentially impact other properties of the edible film, including tensile strength, elongation, and water vapour permeability. It is possible that glycerol may play a more significant role in increasing the elasticity of edible films in other physical property tests. (Rusli et al., 2017; Sitompul & Zubaidah, 2017).

Microstructure

The purpose of examining the microstructure of protein film with using a scanning electron microscope is to reveal or display the material's constituent particles within the whey film.The value of microstructure observation lies in the ability to examine the interrelationship between material properties, structures, and defects. The composition of the material and the appropriate processing method are the determining factors in the characteristics of the edible film.

Figure 3. The microstructure of the protein film with 35% glycerol

The addition of 35% glycerol to the whey-gelatin edible film (Figure 3) resulted in a relatively smooth surface, although some small dots or voids indicative of non-uniformity were

observed. This phenomenon is a common occurrence in films that have been plasticized with plasticizers such as glycerol, which can create spaces between polymer chains. The addition of glycerol serves to weaken the interactions between protein and gelatin molecules, thereby increasing the distance between chains and enhancing the flexibility of the film.

Glycerol plays a pivotal role in enhancing the flexibility of edible films by ensuring an even distribution within the whey protein and gelatin matrix. At a concentration of 35%, glycerol tends to distribute evenly, resulting in a surface devoid of cracks or breaks that are often observed in films lacking plasticizers. However, at higher concentrations, the excessive plasticizing effect can enhance hydration and softness, leading to micro-deformation of the film, as evidenced by the figure, which depicts surface variations.

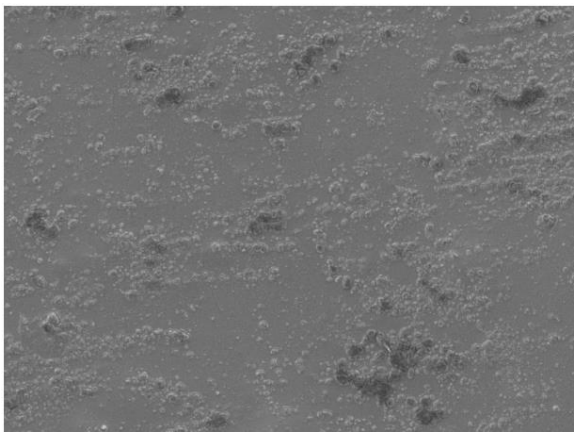

Figure 4. The microstructure of the protein film with 40% glycerol

Figure 4 illustrates that the surface of the whey-gelatin edible film with a 40% glycerol concentration exhibits a porous and heterogeneous texture. This suggests that the incorporation of glycerol at this concentration results in an uneven distribution within the protein matrix. The formation of pores on the film surface can be attributed to an incomplete interaction between glycerol, whey, and gelatin, where the plasticizer is not adequately dispersed within the polymer chains. Previous research by Lorevice et al. (2016) demonstrated that an increase in glycerol concentration frequently results in the formation of separate phases within the film structure. This, in turn, reduces the homogeneity of the film and leads to the formation of air bubbles or pores on the surface.

The interaction between the whey, gelatin, and glycerol at a concentration of 40% can result in segregation or phase separation, whereby the glycerol is not fully incorporated into the protein matrix. This is corroborated by the presence of granules or agglomerates in the SEM images, which may be indicative of inadequate incorporation of the glycerol with the polymer chains. This phenomenon can result in a reduction in the cohesiveness of the film, which in turn leads to a reduction in mechanical stability. As demonstrated by Said & Sarbon (2022), high concentrations of glycerol can lead to segregation due to its disruptive effect on the hydrophobic interactions between protein chains, resulting in the formation of non-uniform structures.

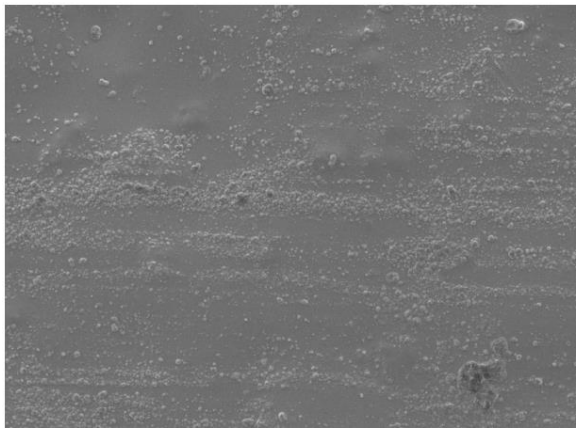

Figure 5. The microstructure of the protein film with 45% glycerol

The observed increase in porosity in the SEM images at a 45% glycerol concentration can be attributed to the plasticizing effect of glycerol, which creates space between the polymer chains of whey protein and gelatin. The formation of a porous structure indicates that the film undergoes significant internal expansion, which is a consequence of the uneven distribution of glycerol. This increase in porosity, in addition to enhancing flexibility, also diminishes film density, thereby producing a more relaxed film. As demonstrated by the research of Suderman et al. (2018), augmenting the concentration of a plasticizer within a biopolymer matrix can precipitate the formation of a hollow structure, resulting from the generation of space between chains. This phenomenon leads to a reduction in

mechanical strength, while concurrently imparting a softer quality to the film.

At higher concentrations, such as 45%, glycerol tends to form aggregates or lumps due to the limited ability of the protein matrix to disperse the glycerol evenly. In the SEM images, the presence of darker or lighter areas can be observed, which indicate the agglomeration of glycerol. This phenomenon also contributes to the heterogeneity of the film. The formation of these agglomerates indicates that the whey-gelatin protein polymer chains are unable to maintain their cohesive structure, resulting in a reduction in the structural integrity of the film. The findings of Zhang et al. (2019) are in alignment with this conclusion, indicating that at elevated plasticizer concentrations, the interaction between the plasticizer and polymer is diminished, culminating in the formation of non-uniform and mechanically degraded films.

Conclusion

The incorporation of glycerol as a plasticizer into whey-gelatin edible films did not result in a notable alteration in film thickness or gelation time. However, it was observed that the film thickness exhibited an increase in correlation with the glycerol concentration. Additionally, the complex interaction between the components, namely whey, gelatin and glycerol, did not exert a direct influence on the duration of gel formation. Nevertheless, this study revealed that an increase in glycerol concentration resulted in alterations to the film microstructure. At higher concentrations, such as 45%, glycerol resulted in an increase in porosity and aggregation within the polymer matrix, which in turn led to a reduction in the homogeneity and mechanical strength of the film. Additionally, increased porosity enhances the flexibility of the film, yet concurrently diminishes its barrier properties against water vapour and gases, which is a crucial consideration in food packaging applications.

Acknowledgement

The author would like to express gratitude to LPPM, University of Mataram, for providing financial support for this research project through the Research Percepatan Lektor Kepala programme, with the grant number 1269/UN18.L1/PP/2024.

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