DRF-444
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RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume Issue : ()

Effect of Glycerol on Physical, Mechanical and Color Properties of Chicken Intestine Gelatin Films

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ABSTRACT

Background: The vast majority of the polymers for plastic food packaging used today are synthetic. The increasing use of synthetic plastic packaging films causes severe ecological problems. Hence, there is a thin layer of ecologically beneficial and consumable packaging material found to replace plastic called as edible film. The main ingredient to make edible film is gelatin. Edible film made from chicken intestines gelatin has not been carried out. Gelatin films offer a strong defense against light, air and scent transfer, but they can be too fragile as packaging material. Glycerol can be used as a plasticizer to solve these weak. This work aims to establish the best glycerol concentration on the physical, mechanical and color properties of edible films.

Methods: This research applied a completely randomized design, involving five glycerol concentrations (10%, 20%, 30%, 40%, 50%) and three replications. The data collected were further analyzed using the variance test and the Tukey test was used to find out the differences between the treatments provided.

Result: According to the findings, glycerol concentration treatments had a significant effect ($P<0.05$) on moisture content, thickness, water vapor permeability, solubility, tensile strength, elongation, Young's modulus, L* and transparency. While a*, b*, ΔE and WI had no significant effect ($P>0.05$). In this case, the best chicken intestine gelatin edible film was made of 10% glycerol concentration.

Key words: Chicken intestine gelatin, Edible film, Glycerol.

INTRODUCTION

The majority of the polymers used today are synthetic. Because synthetic plastic food container films are toxic and non-biodegradable, their growing use is causing serious ecological difficulties. Therefore, bio-based polymer materials that are safe, renewable and environmentally friendly are urgently necessary (Yeddes *et al.,* 2020). Studies using bio-polymers still need to be improved to replace petroleum-based packaging materials (Youssef and El-Sayed, 2018). The most commonly used biopolymers are gelatin, pectin and chitosan because of their good mechanical and biological characteristics (Ramos *et al.,* 2016).

In this case, gelatin comes from collagen hydrolyzed under regulated conditions, forming a protein with high molecular weight (MW) (Ali *et al.,* 2018). Every part of the body contains collagen, yet it is most plentiful in the ligaments, tendons, bones and skin (Bou-Gharios *et al.,* 2020). A lot of sources have been gaining more attention to be developed into gelatin. One of them is poultry industry by-products. The characteristics of gelatin derived from poultry species are very similar to gelatin derived from mammals in the case of the secondary structure, molecular weight and amino acids (Abedinia *et al.,* 2018). Gelatin produced from chicken intestines can be used for food because it has a water content of 5.49%, ash content of 3.80%, pH of 5.5 and gel strength of 157.48 g bloom (Gumilar and Pratama, 2018), this quality meets the standards for edible gelatin, namely pH of 3.8 - 5.5, gel strength of 50 - 300 bloom and gelatin is included in the category of generally recognized as safe (GRAS) food ingredients (GMIA, 2012).

Gelatin has been applied in many industries numerously such as film-based packaging for food, pharmaceuticals, biomedical, cosmetic and biomaterials because of its distinct functional characteristics (Ali *et al.* 2018). Some research on film-based packaging for food, such as for pork sausage (Liu *et al.,* 2007) and chicken meat slices (Bhattacharya and Kandeepan, 2017). Studies reported that films made from chicken gelatin have been published such as films from chicken skin (Loo and Sarbon, 2020) and chicken feet (Lee *et al.,* 2015). Nevertheless, to our current awareness, an investigation has not been carried out on the film made of gelatin from chicken intestines.

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How to cite this article: Gumilar, J., Pratama, A., Wulandari, E., Tavares, L. and Maharani, R. (2024). Effect of Glycerol on Physical, Mechanical and Color Properties of Chicken Intestine Gelatin Films. Asian Journal of Dairy and Food Research. 1-8. doi: 10.18805/ajdfr.DRF-444.

Submitted: 07-08-2024 **Accepted:** 08-10-2024 **Online:** 17-12-2024

Gelatin films offer a strong defense against light, air and scent transfer, but they can be too fragile as packaging material (Gomez-Guillen *et al.,* 2011). A plasticizer is required for such materials to provide them plasticity and increased functionality, common plasticizers include glycerol (Jyothsna and Nair, 2022). The improved qualities occurred through the addition of plasticizers include increased toughness and flexibility as well as a decreased glass transition temperature and interaction within protein-protein chain. Furthermore, the addition also causes the biopolymer matrix to be more pliable and extensible (Bergo *et al.,* 2013). Plasticizers, however, typically reduce both the moisture barrier qualities and the film strength. They should thus be added in the proper quantities. Based on the polymer's stiffness, the addition of plasticizers can be carried out in the film-forming solution at a dry percentage of around 10% to 60% (Tongdeesoontorn and Rawdkuen, 2019). For this reason, the current study set out to assess how the difference in plasticizer concentrations affected the mechanical, physicaland color properties of casting-produced chicken intestinal gelatin (CIG) films as a cutting-edge substitute for the current gelatin film.

MATERIALS AND METHODS

The experiment was conducted from September 2022-December 2023 at the Animal Product Processing Technology Laboratory, Department of Animal Product Technology, Faculty of Animal Husbandry, Universitas Padjadjaran, Indonesia.

Materials used were chicken's intestines purchased from the local market (Bandung, Indonesia), chloride acid (Merck, Germany), glycerol (Merck, Germany, MW: 92.09 g/mol), sodium benzoate (Merck, Germany).

Gelatin and film preparation

Chicken's intestine gelatin (CIG) preparation was done by using the Gumilar and Pratama (2018) methods (Fig 1). Film preparation used the casting process that was done by Abedinia *et al.* (2018) method with a little change in different concentrations of glycerol-based on CIG weight (4 g) (Fig 2) and the edible film ready for testing can be seen in Fig 3.

Physical properties

Moisture based on the AOAC technique (2006). A micrometer (Mitutoyo, Japan) was used to measure film thickness with an accuracy of 0.001 mm (Nur Hazirah *et al.,* 2016). To determine water vapor permeability (WVP), a plastic cup was sealed with film and silica gel was placed within and then weighed together. The cup was put in a desiccator containing distilled water for eight hours and the cup's ultimate weight was noted every hour. WVP was calculated with the following formula:

$$\text{WVP (g mm/m}^2\text{.h.kPa)} = \frac{w \times x}{A \times t \times \Delta P}$$

Where,

W= Increased cup's weight (g).

X= Film thickness (mm).

A= Film surface area exposed to the permeant (m^2).

t= Time of gain (h).

P= Partial pressure of atmosphere with silica gel and pure water (2800 Pa at 24°C) (Nur Hazirah *et al.,* 2016).

Solubility analysis is especially crucial for biodegradable films (Chandla *et al.* 2020). The film pieces were cut and then baked for 24 hours at 70°C before being weighed. After that, films were submerged in distilled water and sodium benzoate 0.01% (w/w) in a closed tube for a whole day. Film fragments and solution were filtered, then rinsed with 10 ml of distilled waterand dried in an oven for twenty-four hours at 70°C. Film solubility was computed based on the formula (Tongdeesoontorn and Rawdkuen, 2019):

Film solubility (%) =

$$\frac{\text{Initial dried weight - Final dried weight}}{\text{Initial dried weight}} \times 100$$

Mechanical properties

Texture Analyzer (Lloyd Instruments, Hampshire, UK) was used to assess the film's tensile strength, elongation at the breakand Young's Modulus (Loo and Sarbon, 2020). The film was divided into 10 mm by 70 mm pieces. Furthermore, 20 mm and 0.5 mm/s were chosen as the starting grasp distance and drawing rate, respectively. In addition, there is a 25 N weighing sensor. The tensile strength and elongation at break were computed using the formula:

$$\text{Tensile strength (MPa)} = \frac{F_{max}}{TW}$$

Where,

Fmax = Maximum load (N).

T = Film thickness.

W = Film width.

$$\text{Elongation at break (\%)} = \frac{L - L_o}{L_o} \quad 100\%$$

Where,

L = Film elongation (mm) during the rupture.

L_o = Initial grip length (mm) of the sample.

$$\text{Young's modulus (MPa)} = \frac{\text{Stres (MPa)}}{\text{Strain}}$$

Color properties

The film's color was assessed using a color meter derived from BYK Gardner, USA. In this case, the measurement units are L for lightness, a for red or green colorand b for yellow or blue color. Film samples standard plate were (L = 91.33, a = 0.24 and b = 0.87). The following formula was applied to determine the color difference ("E) and whiteness index (WI) (Yeddes *et al.,* 2020):

$$\Delta E = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2}$$

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

The film's transparency was determined using UV-Vis spectrophotometer (Shimadzu, Japan) at 600 nm wavelength. The following formula was applied to calculate it (Soo and Sarbon, 2018):

$$\text{Transparency} = \frac{\text{Log T}}{x}$$

Where,
T = Absorbance at 600 nm.
x = Film thickness (mm).

Statistical analysis

The data of each five treatments with different glycerol concentrations were compared using a one-way analysis of variance (ANOVA). In addition, significant differences that might occurred were assessed through Tukey's with a significance of 0.05 ($p<0.05$). The investigation for each treatment was carried out three times and the results were reported as mean ± standard deviation. SPSS version 26 (SPSS Inc, USA) was further applied for data analysis.

RESULTS AND DISCUSSION

Physical properties

Physical properties can be seen in Table 1. The moisture content in this research was 15.58±0.64% (T1) - 19.22±0.49% (T4). This moisture content was similar to the edible film from gelatin, namely 16.46 ± 0.19% (Li *et al.*, 2020), but lower than the results of previous research, specifically 22.08 ± 0.09% (Karim *et al.,* 2020). Tukey's test demonstrated that T1, T2 and T3 were significantly different lower ($P<0.05$) than T4. The results of this study indicate that when glycerol concentration rises, the film's moisture also rises. This can occur because glycerol as a plasticizer can increase the coherence between molecules so that the hydrocolloid's water-binding capacity will rise. Glycerol has hydroxyl groups which have a strong affinity with water molecules, allowing films that have a high glycerol content to hold more water in their matrix to form hydrogen bonds (Abedinia *et al.,* 2018).

The findings discovered that the thickness of CIG edible films was between 0.13±0.00mm (T1) to 0.17±0.01 mm (T5). These results were lower than previous research findings obtained by Baskara's *et al.* (2012) research, 0.20 - 0.25 mm. Meanwhile, the results were greater than those of Nilsuwan *et al.* (2021) study, these were 0.043 - 0.044 mm.

Tukey's test showed that T4 and T5 were significantly higher ($P<0.05$) than T1 and T2. The different thickness in edible film increases its total solids and polymers during drying process. According to Ratna *et al.* (2023), the edible film matrix is made up of more polymers when there are more solids in the solution; so, the higher the concentration of glycerol, the thicker the film is.

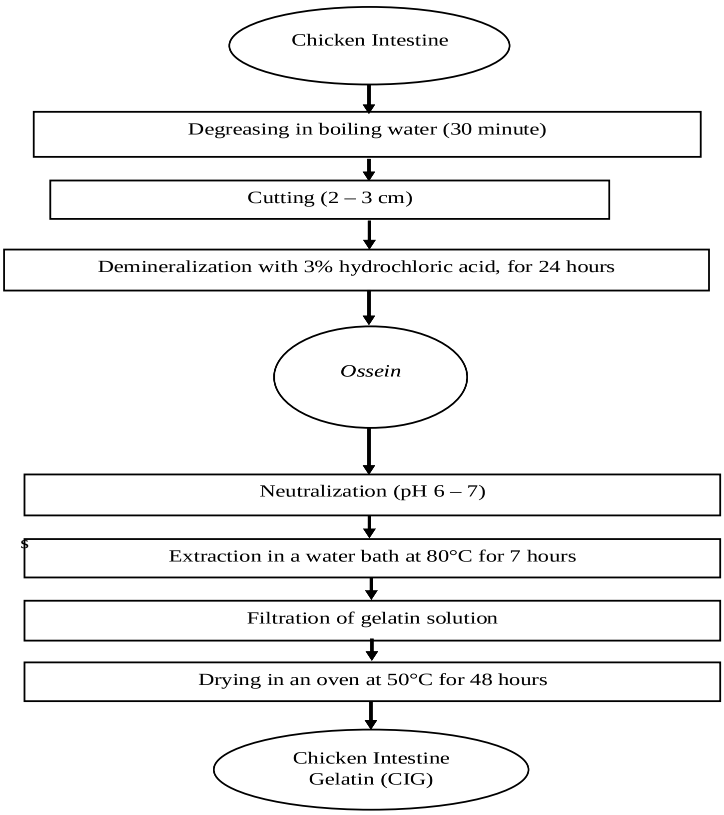

Fig 1: Gelatin preparation flow chart.

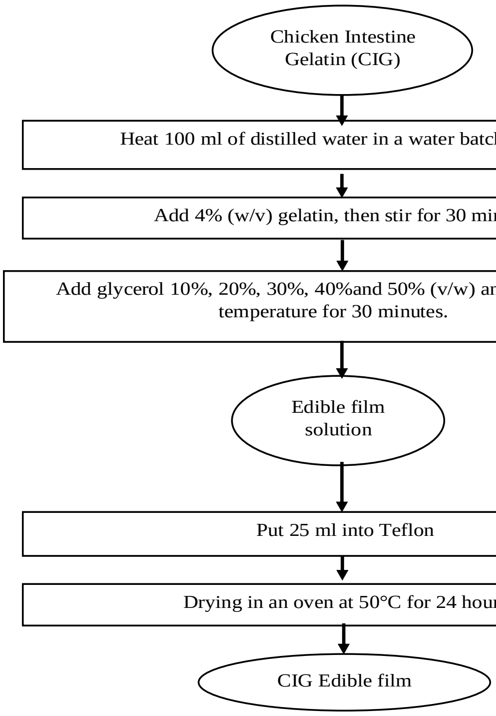

Fig 2: Edible film preparation flow chart.

Fig 3: CIG edible film ready for testing.

Table 1: Physical properties of edible film made from chicken intestine gelatin.

Parameters	Glycerol treatments				
	10% (T1)	20% (T2)	30% (T3)	40% (T4)	50% (T5)
Moisture (%)	15.58±0.64^{a}	15.87±0.89^{ab}	16.76±0.74^{ab}	19.22±0.49^{c}	17.70±0.67^{bc}
Thickness (mm)	0.13±0.00^{a}	0.15±0.01^{ab}	0.16±0.00^{bc}	0.17±0.01^{c}	0.17±0.01^{c}
WVP (g mm-m^{2}.h.kPa)	4.83±0.81^{a}	4.75±0.79^{a}	4.72±0.35^{a}	6.64±0.42^{b}	5.39±0.32^{ab}
Solubility (%)	12.93±0.44^{a}	16.65±0.95^{a}	20.91±1.61^{b}	57.55±2.47^{c}	65.34±1.66^{d}

$^{a-d}$Different superscripts within the same line indicate significant differences among treatments ($p<0.05$).

The CIG edible film has an average water vapor permeability that ranges from 4.72±0.35 g mm/m^{2}h kPa (T3) to 6.64±0.42 g mm/m^{2}h kPa (T4). In general, the findings obtained by the current research were higher than the previous research findings in the range of 2.90 - 4.49 g mm/m^{2}h kPa (Jusoh *et al.,* 2022). Tukey's test indicated that T4 was substantially different higher ($P<0.05$) from T1, T2 and T3. In this case, the characteristics of films are influenced by their hydrophilic/hydrophobic properties of the plasticizer used. Moreover, the introduction of plasticizers like glycerol modifies the structure of protein interactions by rupturing intra and intermolecular hydrogen bonds, creating greater space that can improve oxygen permeability (Cardoso *et al.,* 2016).

The CIG edible film solubilities are between 12.93±0.44% (T1) - 65.34±1.66% (T5). This result is lower than the research results of Loo and Sarbon (2020) of 83 ± 3% - 94±2%. According to the results of Tukey's test, T5 showed a significant difference (P<0.05) higher from the other treatments. The higher the addition of glycerol causes more molecular interactions in the polymer chain. This is because glycerol, a plasticizer used instead of sorbitol, has a lower molecular weight, interacts with the polymer chains more readilyand increasing their attraction for water (Tong *et al.,* 2013).

Mechanical properties

Mechanical properties can observed in Table 2. The CIG edible film's tensile strength with various glycerol concentrations ranging from 0.52±0.0 MPa (T5) to 6.65±0.36 MPa (T1). Previous research conducted by Said and Sarbon (2020) obtained similar results, which were between 0.235 to 8.708 MPa and higher than the edible film from chicken skin gelatin researched by Nazmi *et al.* (2017) namely 0.98±0.14 MPa.

The Tukey test showed that T1 was significantly different (P<0.05) higher from the other treatments. Increasing the concentration of glycerol lowers the value of the tensile strength of edible films because, as a plasticizer, it has hydrophilic properties that cause flexibility in edible films by forming cavities that can obstruct the intermolecular forces of attraction (Wulandari *et al.,* 2017).

The elongation values varied from 4.23±0.10% (T1) to 18.84±1.07% (T5). The findings of this investigation were further in accordance with the study conducted by Lee *et al.* (2015) which used edible films from pectin green grass jelly using glycerol as plasticizers with elongation percentages of 13.7% - 18.4%, but lower than the results of studies on making edible films from gelatin split cowhide with elongation percentage of 55.92% - 74.44% (Wulandari *et al.,* 2017).

When compared to the other treatments, the T4 and T5 were different higher from the other treatments considerably (P<0.05). The stretch factor of the edible film increases with the amount of glycerol used as a plasticizer. Film elongation can be caused by the plasticization effect originating from glycerol which tends to increase chain entanglement and increase the distance between polymer chains (Said and Sarbon, 2020).

The Young's Modulus value ranged from 0.29±0.02 (T5) to 15.54±0.41 (T1). This value was higher than the results of Loo and Sarbon (2020) in the film-making of the chicken skin and tapioca flour produced a maximum Young's Modulus value of 3.3 MPa but lower than the research results of Syahida *et al.* (2020). Additionally, the T1 was substantially different (P<0.05) higher from the other treatments. The use of glycerol affected the mechanical quality of CIG edible film. The mechanical qualities of packaging films have a direct impact on their quality and ability to maintain the packaged product's integrity (Dyankova and Solak, 2023).

Color and transparency

Color and transparency can be seen in Table 3. The average lightness (L*) shows that the value of L* ranges from 76.65±1.47 (T1) to 79.66±0.63 (T3), this value is still within the range of research results by Getachew *et al.* (2021) namely 53.19 - 89.29, but higher than the research

Table 2: Mechanical properties of edible film made from chicken intestine gelatin.

Parameters	Glycerol Treatments				
	10% (T1)	20% (T2)	30% (T3)	40% (T4)	50% (T5)
Tensile strength (MPa)	6.65±0.36^{a}	2.93±0.63^{b}	1.09±0.11^{c}	0.57±0.06^{c}	0.52±0.0^{c}
Elongation (%)	4.23±0.10^{a}	5.17±0.25^{a}	12.67±0.68^{b}	17.16±1.10^{c}	18.84±1.07^{c}
Young's modulus (MPa)	15.54±0.41^{a}	6.02±0.36^{b}	0.86±0.04^{c}	0.32±0.02^{cd}	0.29±0.02^{d}

$^{a-d}$Different superscripts within the same line indicate significant differences among treatments (p < 0.05).

Table 3: Color and transparency properties of edible film made from chicken intestine gelatin.

Parameters	Glycerol Treatments				
	10% (T1)	20% (T2)	30% (T3)	40% (T4)	50% (T5)
L*	76.65±1.47^{a}	76.62±0.68^{a}	79.66±0.63^{b}	78.26±1.27^{a}	77.35±0.43^{a}
a*	3.59±0.13^{a}	3.54±0.46^{a}	3.87±0.86^{a}	3.96±0.65^{a}	4.06±0.19^{a}
b*	27.77±2.05^{a}	26.84±1.34^{a}	27.25±4.22^{a}	26.73±2.87^{a}	26.17±2.38^{a}
ΔE	30.83±2.38^{a}	30.03±1.48^{a}	29.10±4.06^{a}	29.24±2.82^{a}	29.17±2.03^{a}
WI	63.50±2.37^{a}	64.22±1.44^{a}	65.72±3.62^{a}	65.29±2.60^{a}	65.13±1.76^{a}
Transparency	1.92±0.05^{ab}	1.96±0.19^{ab}	2.13±0.28^{b}	1.62±0.04^{a}	1.63±0.09^{a}

$^{a-b}$Different superscripts within the same line indicate significant differences among treatments (p<0.05). L* (Lightness), a* (red-green color), b* (yellow-blue color), ÄE (total color difference), WI (whiteness index), absorbance at 600 nm.

results of Santos *et al.* (2022) of 58.45 - 72.02 and lower than the research results from Salimiraad *et al.* (2022) of 86.78-89.51. Redness (a*) is marked with red to brown or darker red. The value of a* ranging from 3.54±0.46 (T2) to 4.06±0.19 (T5) indicates that the edible film is reddish. These results are in line with research conducted by Maruddin *et al.* (2017) that films which use glycerol as a plasticizer have a reddish color, this a* value is also similar to Neves *et al.* (2019) around 3.88 - 4.80. The b* value ranges from 26.17±2.38 (T5) to 27.77±2.05 (T1) indicating that the edible film is yellowish, this value agrees with Amjadi *et al.* (2020) namely 21.40-27.32. Total color difference (ΔE) and WI were calculated based on delta L*, a*and b* color differences. ΔE represents the distance of a line between the sample and the standard. The results showed that the delta E value ranged from 29.10±4.06 (T3) to 30.83±2.38 (T1), this value inline with Getachew *et al.* (2021), namely 11.47-36.95. The degree to which a surface resembles the characteristics of a perfect reflecting diffuser at equal intensities in all directions is measured by the whiteness index. The results showed that the whiteness index for the treatment ranged from 63.50±2.37 (T1) to 65.72±3.62 (T3). The analysis of variance results has shown that treatment with various concentrations of glycerol did not have a significant effect (P>0.05) on various color parameters. This occurs because glycerol is a clear liquid so if added to edible film it will not change color (Akili *et al.,* 2012).

Transparency is a crucial property of edible film gelatin that affects its potential application (Liu *et al.,* 2023). The test results showed that the transparency of the CIG edible film ranged from 1.62±0.04 (T4) to 2.13±0.28 (T3). This value is in line with the results of the study Nilsuwan *et al.* (2021), but higher than the results of the study carried out by Abedinia *et al.* (2018) and lower than the results of Soo and Sarbon's, (2018) study. The T4 and T5 were considerably (P<0.05) lower than the T3, according to the Tukey test findings. The study's findings supported the assertion made by Warkoyo *et al.* (2014) that when an active ingredient's concentration rises, the film's thickness tends to grow as well, decreasing the film's clarity and lowering its transparency.

CONCLUSION

The study provides us with scientific information on developing edible film from chicken intestine gelatin using glycerol as a plasticizer, the use of various concentrations of glycerol as a plasticizer produces edible films with different qualities. Using 10% glycerol gives the best quality of edible film from chicken intestine gelatin in terms of moisture content, thickness, water vapor permeability, tensile strength and Young's modulus.

ACKNOWLEDGEMENT

I expressed gratitude to the Rector who supported this research with the Universitas Padjadjaran research grant. I also expressed gratitude to Imas Nuraeni, Alfi Hanafi Alpiyah and M. Fajar Ramadhan who have helped the research activities.

Conflict of interest

The authors declared no potential conflict of interest in this research.

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