Research Article

Edible Film from Whey: The Impact of the Addition of Rosella Extract (*Hibiscus sabdariffa L*)

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Abstract | Valorisation of whey as an edible film has been extensively studied. This study aimed to evaluate the moisture content, physical properties of color, antioxidant activity, and sensory properties of edible films made from whey with the addition of antioxidants from Rosella calyx extract (*Hibiscus sabdariffa L*). The edible film was prepared with the addition of four different levels of rosella extracts (0, 0.2, 0.4, and 0.6%). The addition of rosella calyx extract led to a significant difference (P<0.05) in color (redness), antioxidant activity, and color sensory attributes, but did not affect the moisture content, aroma, and flavor (P>0.05). The greater the amount of rosella extract added, the higher the a* value of the color attribute (redness), which gives a deeper red color to the whey edible film. Increasing the concentration of rosella added to the whey edible film also increased antioxidant activity. The use of rosella extract can increase the antioxidant properties (79.60%) of edible films whey and consumer acceptance of color attributes (3.84-like) with the best treatment at an additional concentration of 0.6%.

Keywords | Antioxidant, Edible Film, *Hibiscus sabdariffa L*, Sensory Properties, Whey

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INTRODUCTION

Whey is a byproduct of cheese production, generated from the curd filtering process. Every year, over 24,000,000 tons of cheese are made worldwide, resulting in approximately 21,600,000 tons of cheese whey. Approximately 90% of the volume utilized in cheese making is turned to whey, which preserves 55% of the nutrients found in milk (Lopes et al., 2019). Whey utilization is only around 54% reused in food systems, 41% as feed or fertilizer, and the rest for non-food systems (Ganju & Gogate, 2017). Whey can cause negative effects on the environment. Whey's biochemical oxygen demand (BOD) may vary from 30,000 to 50,000 g/g, although the exact number is determined by milk waste (Almeida et al., 2008). Whey is made up of ranging from 0.5 to 0.7% minerals, 0.8 to 1% total protein, 0.5 to 6% dry matter, and up to 0.8% lactic acid (Tsakali et al., 2010). Therefore, valorization of whey can be expected to reduce its negative impact on the environment.

The utilization of whey as biodegradable packaging has been extensively investigated. Biodegradable packaging can be broken down naturally by microorganisms, such as fungi, bacteria, and algae, into environmentally friendly compounds (Greene, 2007). An example of environmentally friendly biodegradable packaging is an edible film. It is possible to preserve the quality of food ingredients while simultaneously extending their shelf life by using edible films. Edible films have no impact on the organoleptic qualities or nutritional composition of ingredients (Embuscado and Huber, 2009; Ganiari et al., 2017). Using Whey protein as an edible film improves the oxygen barrier properties (Cinelli et al., 2014) and has excellent aroma and oil barrier properties (Kumar et al., 2022). Moreover, whey protein can preserve the biodegradability of the resulting edible films (Filipini et al., 2021). As a film base material, whey protein can transport food additives such as citric acid, allowing the resulting film to serve additional purposes, such as inhibiting enzymatic browning reactions in various food products (Azevedo et al., 2017). The addition of 1% carboxymethyl cellulose and 3% glycerol to whey-based edible films affects the moisture, density, and dissolving time of the edible film coating (Juliyarsi et al., 2011).

Antioxidant compounds can be added to make edible films, which can increase their functional value. The addition of natural antioxidant compounds to edible films has been widely carried out in various studies, such as the use of bee extracts (Velásquez et al., 2022), fermented soy protein (Zareie et al., 2020), tea polyphenols (Dou et al., 2018), and fireweed extract (Kowalczyk et al., 2021). Many polymer films with added antioxidants provide much better food preservation properties than polymer films without the addition of antioxidant compounds (Bayer et al., 2022). Edible films containing antioxidant compounds can increase shelf life and maintain the nutritional value of food products by protecting them from oxidative damage, microbiology, and discoloration (López et al., 2017; Keshari et al., 2022). Juliyarsi et al. (2020) stated that edible film whey added with 0.6% turmeric extract significantly affected antioxidant levels by up to 73%.

One source of antioxidants that can be used in food products is herbal plants, which have many health benefits. Natural antioxidants can be obtained by extracting natural ingredients or by chemical reactions during processing. Rosella is an herbal plant that is used as a health drink for the treatment of degenerative diseases. Rosella plants contain polyphenols, such as hydroxybenzoic acids, hydroxycinnamic acids, flavanols, flavonols, and anthocyanins (Singh et al., 2021). The main antioxidant flavonoid compound in rosella plants is the anthocyanin group, which is a natural water-soluble pigment. Rosella flowers contain anthocyanins (0.5–3.5 mg/g) when extracted with ethanol (Christian and Jackson, 2009). Therefore, this study aimed to investigate the impact of rosella extract as a source of antioxidants in biodegradable biofilm made from whey. It was hypothesized that the addition of different levels of rosella extracts will impact the quality of whey biofilm, including the moisture content, color, antioxidant activity, and sensory properties of edible film whey.

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MATERIALS AND METHODS

Materials

The materials used in this study were whey derived from industrial cheese by product from Lassy Dairy Farm in the Lasi Tuo area of Agam Regency and rosella calyx (*Hibiscus sabdariffa L*) originating from the city of Padang.

Extraction of rosella calyx (*Hibiscus sabdariffa L*)

The extraction of rosella calyx was performed as described by Zhang et al. (2021) with some modifications. 1000 grams of fresh rosella calyx were cut into small pieces. Subsequently, it was baked in an oven at 50°C for 1 x 24 hours. The final moisture content of rosella calyx was 15.25%. The sample was then blended to obtain a powder and sieved using a 44 mesh sieve. The samples were stored in closed, dry containers. Rosella powder (250 g) was macerated with 70% ethanol so that all the powder could be wetted and submerged in a maceration container. The rosella powder was then closed and left for 3 × 24 h in a place protected from light at room temperature (29°C), while stirring using a magnetic stirrer. The maceration results were then filtered using a vacuum filter using Whatman no. 1 filter paper and concentrated by utilizing a rotary evaporator set to 50°C for 30 min to obtain a paste extract. The extract results were stored in dark bottles and at 4°C.

Production of Edible Film Whey

Edible film whey were prepared using a method as described by Juliyarsi et al. (2011). Whey and ethanol (96%) were combined in a volume of 65 mL at a ratio of 1:3, and the mixture was then heated for 30 min at 55°C. During heating, 1% carboxymethyl cellulose (CMC) and 3% glycerol of the total ingredients were added to the mixture. Extract of rosella was added following the treatment, 0% (control), 0.2%, 0.4%, and 0.6%, and stirring was carried out until it was evenly mixed. The rosella extract solution was then poured into a 20 × 20 cm, and 3 mm-thick glass plate mold. Finally, the edible film was dried at 50°C for 24 h, cooled for 6 h at room temperature, and removed from the mold.

Moisture content and color of edible film whey

The gravimetric method according to AOAC (2005) was used to determine the moisture content of edible films whey containing roselle extract. Color analysis was performed using the method described by Mir et al. (2023) using a digital colorimeter (T135). White standards were used to calibrate the digital colorimeter, which resulted in the following calibration values: L= 94.76, a= -0.795, and b= 2.200. The parameter tested for color was the a* (redness) value of the edible films.

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Antioxidant Activity

Antioxidant activity was analyzed as described by Huang et al. (2006) using the DPPH assay method. A sample of 1 mL was mixed with a methanol solution of 1 mL containing 80 ppm of diphenylpicryl-hydroxyl (DPPH). The mixture was stirred and left in the dark for 30 min. Measurements were performed using a spectrophotometer (Shimadzu UV-1800) with an absorbance reading of 517 nm. Methanol was used as blank. Antioxidant activity was calculated as follows:

Scavenging activity (%) = $[(A - A_{sample}) / A] \times 100$

A = the absorbance of blank without tes sample, A_{sample} = absorption of test sample

Sensory Properties

Sensory assessment was carried out in the Department of Livestock Products Technology Laboratory, Faculty of Animal Science, Universitas Andalas. Consumer sensory evaluation was carried out in accordance with Lawless and Heymann, (2010) with certain adjustments. The sensory test required 25 semi-trained panelists to assess color, flavor, and aroma attributes on a five-point hedonic scale of 1 (strongly dislike) to 5 (strongly like).

Data analysis

This study used a randomized block design (RBD) with four different treatments of rosella calyx extract. Each treatment used 5 replications and the results were provided as mean ± standard deviations. Data obtained from the study were analyzed statistically by analysis of variance and further tested using Duncan's Multiple Range Test (DMRT) if there were differences between the treatments using SPSS software (version 16.0; SPSS Inc., USA).

RESULTS AND DISCUSSION

Moisture content and color of edible film whey

The average moisture content and color values of edible films whey with various concentrations of rosella extract are shown in Table 1. Statistical analysis showed that adding rosella extract at various concentrations had no significant effect on the moisture content (P>0.05) of the edible films.

Table 1: Average moisture content and color (a*) of edible film whey

Treatment	Moisture content (%)	Colour (a*)
0	23.13	-2.27^{d}
0.20%	22.98	3.81^{c}
0.40%	23.79	8.64^{b}
0.60%	23.90	12.20^{a}

Note: Superscripts letters in the column show significantly different (P<0.05)

The addition of rosella extract to edible film whey provided a moisture content ranging from 22.13 to 23.90%. The addition of up to 0.6% rosella extract had no effect on the moisture content of the edible film compared with the control (without the addition of rosella extract). This may be related to the process of extracting rosella, which was performed in this study using the drying process of fresh rosella. The rosella extract added to the edible film is a thick extract that has undergone evaporation during the concentration process, so that the moisture content in the rosella extract does not affect the moisture content of the resulting edible film. Fresh rosella calyx is known to have a moisture content of 89.4%, whereas rosella calyx that have been oven-dried at 50°C for 12 h have a much lower moisture content of 8.9% (Juhari et al., 2021). In addition, Manjula et al. (2022) stated that extraction of rosella flowers using ethanol solvents has a much lower level of water activity (A_w) than extraction using water solvents.

The edible film whey in this study had a lower moisture content than the edible film whey made using turmeric extract by Juliyarsi et al. (2020), which had a moisture content ranging from 32.17 to 35.29%, but much higher than the moisture content of edible film whey with various concentrations of glycerol, which had a moisture content ranging from 2.12-5.07% (Aritonang and Melia, 2014). A higher moisture content in the film indicates a higher molecular mobility owing to the plasticizing properties of the water molecules. An increase in moisture content can cause a decrease in intermolecular bonds and an increase in the plasticization reaction of glycerol, as water molecules act as plasticizers. Thus, the moisture content affects the sealing property of the edible film by facilitating the formation of strong bonds between surface adhesive molecules (Suh et al., 2020). However, the high moisture content of the edible film affects the resistance of the resulting film and the packaged product. A higher water content can reduce the tensile strength and increase the water vapor permeability of the edible film (Alvarado et al., 2015).

Regarding the color parameter, there was a significant difference (P<0.05) in the color parameter (redness) of the edible film whey produced by adding various concentrations of rosella extract. In this study, the color parameter tested was the a* value (redness) of the edible film whey, because rosella flowers contain the primary anthocyanin pigment that can produce various hues of red (Cissé et al., 2012). Table 1 shows the edible film whey treated with roselle extract at 0 to 0.6% ranging from -2.27 to 12.20 in a* (redness) value. The highest mean value of color (redness) was measured in the sample with 0.6% rosella extract, which was significantly different from that of the other treatments (P<0.05). The average value of the color (redness) increased with increasing concentration of rosella

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extract in the edible film whey. These findings align with those of Mendes et al. (2022) where an increase in the concentration of rosella flower extract increased the redness of the resulting edible film.

Dehankar et al. (2023) stated that a positive a* value means a reddish color, whereas a negative a* value means a greenish color. The control sample (without rosella extract) showed a negative a* value. This was due to the edible film whey sample did not get a red color from rosella flowers, and the color produced only came from the whey as raw material. According to Blazic et al. (2017), the color of whey originates from the riboflavin compound found in milk. The riboflavin pigment gives the whey a greenish-yellow color. The highest concentration of rosella had the darkest color edible film than the other treatments (Figure 1). According to Huang et al. (2021), a greater concentration of rosella anthocyanin extract results in a deeper red color of the resulting film because of the higher anthocyanin content. The addition of rosella extract to edible film whey, which has a purplish red color, can be applied to sausage casings and has a similar color to that of sausages in general so that it can replace the color of the casing (artificial casing) in addition to providing other benefits from the use of edible films.

Figure 1: Color of the edible film, (a) 0 % addition of rosella extract, (b) 0.2 % addition of rosella extract, (c) 0.4 % addition of rosella extract, (d) 0.6 % addition of rosella extract.

Antioxidant activity of edible film whey

The antioxidant activity of the edible film whey is presented in Table 2. DPPH radical scavenging activity was performed to evaluate the antioxidant activity of edible film whey with the addition of roselle extract at various concentrations. According to the results of the statistical analysis, the addition of rosella extract to edible films whey resulted in a significant difference (P<0.05) in the level of antioxidant activity.

Table 2: Average antioxidant activity of edible film whey

Treatment	Antioxidant activity (%)
0%	49.40^{c}
0.2%	62.60^{b}
0.4%	71.80^{ab}
0.6%	79.60^{a}

Note: Superscripts letters in the column show significantly different (P<0.05)

The results showed that the average antioxidant activity ranged from 49.40 to 79.60%. The edible film without rosella extract (control) had the lowest antioxidant activity of 49.40%, whereas the edible film with the addition of 0.2, 0.4, and 0.6% rosella extract had antioxidant activities of 62.60, 71.80, and 79.60%, respectively. The antioxidant activity of the control sample originates from whey used in making edible films. According to Panesar et al. (2007), whey is the remaining milk serum after curd separation and is the result of the coagulation of milk proteins with acids or proteolytic enzymes. Many previous studies have reported that whey is one of the main contributors of antioxidants in milk, besides casein (Alenisan et al., 2017; Pihlanto, 2006; Zulueta et al., 2009). This also caused the level of antioxidant activity in the control sample to have the lowest average value when compared to other treatments because the source of antioxidants only comes from whey raw material.

This study found that the antioxidant activity of edible films increased as the quantity of rosella extract increased. The increase in antioxidant activity in this study showed that more rosella extract provided greater anthocyanin content and acted as an antioxidant (Abidoye et al., 2022; Sáyago-Ayerdi et al., 2007; Steenkamp et al., 2004). Rosella extracts are known to have a DPPH radical scavenging activity value of 87.9% (Mohd-Esa et al., 2010). Rosella contains several antioxidant compounds, such as ascorbic acid, anthocyanin, β-carotene, and lycopene (Wong et al., 2002). This was also reported by Zhai et al. (2017) who stated that rosella flower contain anthocyanins that can reach 9.51 mg/g.

Sensory Properties

The sensory properties results are shown in a spider web diagram (Figure 2) for the color, flavor, and aroma attributes tested on 25 semi-trained panelists. Regarding to the color attribute, the color sensory value of the edible film whey ranged from 3.36 (rather liked) to 3.84 (liked) with the addition of 0.6% rosella extract resulted in the highest color value in the edible film whey. The addition of rosella extract had no significant effect (P>0.05) between control, 0.4 and 0.6% addition of rosella extract, respectively. However, there was a significant difference with treatment 0.2% addition of roselle extract. The panelists tended to prefer a more intense red color on the edible with the addition of 0.6% rosella extract (3.84).

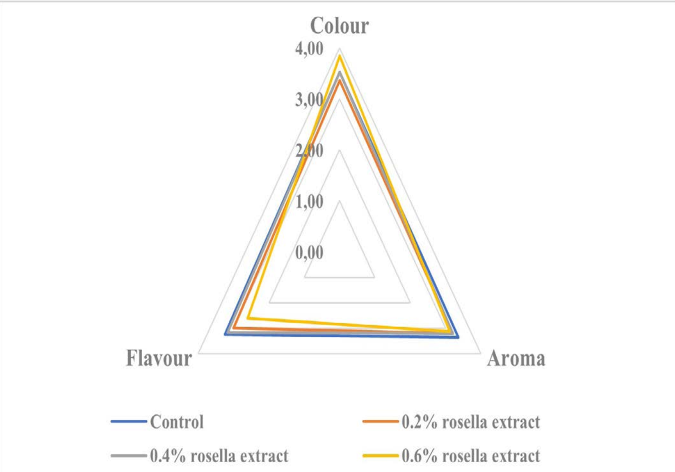

Figure 2: Spider web diagram of the sensory evaluation

Color is one of the important factors and is the main attraction of the product. The color of edible films is affected by the color of the primary ingredients, which is rosella and whey. According to Rawdkuen et al. (2020), the adding anthocyanins to gelatin films increases their reddish color, which can provide a good visual appearance for food packaging. In addition, the red color of anthocyanins in edible films can reduce transparency and increase UV light barrier properties that can prevent food oxidation (Alizadeh-Sani et al., 2021).

In terms of flavor and aroma attributes, the addition of rosella extract at various concentrations to the edible film did not have a significant effect (P>0.05). The flavor value of edible films whey ranged from 2.60 (rather like) to 3.24 (rather like) and for aroma ranged from 3.12 (rather like) to 3.36 (rather like). These results are in accordance with the results of research by Elsayed et al. (2022) that the use of edible film whey did not make a difference in the sensory properties of flavor, aroma, texture, and panelist acceptance of the product when it had not been stored. The edible films exhibit different sensory properties when the product is stored. Sun et al. (2023), also reported that edible film added with curcumin, phycocyanin, or modified lycopene colorant not only changed the color of the display, but also provided different preferences and sensory characteristics in tactile and visual aspects.

CONCLUSION

The addition of rosella extract (*Hibiscus sabdariffa* L) significantly affected (P<0.05) the color (redness), antioxidant activity, and color attributes of the sensory but did not affect (P>0.05) the moisture content, aroma, and flavor sensory attributes of the edible film whey. The addition of rosella extract can increase antioxidant properties and consumer acceptance of color attributes in edible film whey. Adding 0.6% rosella extract was the best treatment to produce edible film whey in terms of antioxidant activity parameters (79.60%) and acceptance of color attributes of 3.84 (likes). However, the effectiveness of using whey edible film for its application in products is still unknown. Further studies on the application of whey edible films to products are important to determine the function of edible films in maintaining quality and extending shelf life in its application to various types of food.

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CONFLICT OF INTEREST

All authors have no conflicts of interest and agree to submit this manuscript to the journal.

NOVELTY STATEMENT

Valorization of whey as a by-product of cheese production as an edible film with the addition of roselle (*Hibiscus sabdariffa L*) extract as a source of antioxidants

AUTHORS CONTRIBUTION

All authors were involved in conducting experimental trials, statistical analysis, and writing and editing of the manuscript.

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