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Preparation and physicochemical characterisation of modified cassava starch-based edible films fortified with beetroot extract

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Abstract

The use of plastic-based food packaging can harm the environment, thus requiring solutions that are more environmentally friendly, such as cassava starch-based edible films. Beetroot extract has been used as an antioxidant source and an active pH-detecting agent to produce smart packaging films, so that give additional properties of functional food. The effects of modified (cross-linked acylation) and unmodified starch-based edible films have been investigated. The present work further investigated the effects of beetroot extract amounts (0, 0.25, 0.5, 0.75, and 1.0 g) and various pH levels (4, 7, and 9) on edible film properties. Three major stages were performed, including (i) cassava starch modification, (ii) edible film modification and fortification, and (iii) characterisations of mechanical and physical properties, antioxidant activity, and functional groups. The results indicated that modified cassava starch-based edible films with cross-linking acylation, where tensile strength ranged from 1.37 to 1.9 MPa and elongation ranged from 70 to 92%, were better than edible films made from unmodified starch, where tensile strength ranged from 0.16 to 0.59 MPa and elongation ranged from 11.30 to 15%. The modified cassava starch-based edible films were more compact and homogeneous. Moreover, the addition of beetroot extract changed the film colour due to antioxidative activity up to 57%. The present work demonstrated an important first step in the development of smart edible films to enhance their usefulness for preserving and monitoring food quality.

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Introduction

Plastic-based food packaging is commonly used to maintain food quality, and increase food shelf life. The demand for food packaging made from petroleum-based plastics increases by 8% annually. Only 5% of the total waste generated is recycled (Mostafavi and Zaeim, 2020), while the remaining 95% is in open landfills, polluting the environment as they do not decompose naturally. For this reason, environmentally friendly packaging materials, such as edible films, are necessary.

Edible films are thin and fragile layers used as food packaging made from biomacromolecules, thus requiring plasticisers. As food wrappers, edible films provide external and passive protection without affecting the taste and nutrients of the food (Viana *et al*., 2018). They are made of polysaccharides, proteins, and lipids. Polysaccharides are considered unique for their low prices, ease of processing, and good functional properties. They can form hydrogen bonds and complex molecular networks when fabricated into polymers (Kong *et al*., 2022).

Cassava starch is polysaccharides potentially processable into edible films for their contents: amylose and amylopectin. This starch also has high molecular weights and low impurities (fat, protein, and ash) (Colivet and Carvalho, 2016). However, edible films made from this material have several disadvantages, such as poor mechanical properties and non-resistance to moisture (Liu *et al*., 1999). Therefore, the modification of the active hydroxyl groups on the surface of cassava starch is required to obtain better physical and chemical properties. Such modification can be conducted using cross-linking and stearic acid acylation, which will affect the hydrophilic and hydrophobic sides of the starch, leading them to bind to each other. The characteristics of cross-linked acylation starch are shown through the interaction of amylopectin. However, it is very

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difficult to provide direct evidence of where the modification occurs. Cross-linking acylation can increase shear stability, which can be determined by the tensile strength of the film; film swelling can be determined by the penetration of water into its surface (Liu *et al*., 1999).

Recently, cassava starch-based edible films have been processed into smart films that can detect food quality degradation, and identify changes in food acidity levels (Esfahani *et al*., 2022). Higher levels of acidity indicate the growth of microorganisms that can lead to food spoilage. Smart packaging films can be produced by adding an active pH-detecting agent such as natural pigments from fruits or vegetables. The changes in the film's colour can help food consumers immediately notice that food quality is going to degrade. Therefore, with smart films, people can protect and maintain food quality, prolong its shelf life, and ensure its safety (Chen *et al*., 2022; Esfahani *et al*., 2022).

Asia has a diverse range of native plants, some of which have medicinal properties or are being used as food preservative/flavouring. The incorporation of natural additives from plant extracts or essential oils into edible films can considerably improve the edible films' antioxidative and antimicrobial activities (Kong *et al*., 2022). Some studies have investigated the effect of adding various plant extracts to edible films, such as seed gum-grass pea, pomegranate peel, and moringa leaf (Ebrahimi *et al*., 2016; Esfahani *et al*., 2022; Rahmawati *et al*., 2024).

Anthocyanin and betacyanin are natural colour pigments that can assess food quality, and have anti-inflammatory and anti-carcinogenic components that are beneficial to human health (Roy and Rhim, 2021). They are found in several natural sources, such as beetroot. Given the unique properties of beetroot pigments, their incorporation into edible films offers a promising approach to developing smart packaging materials with enhanced functionality. Smart edible films that are added with beetroot extract experience colour change, and have antioxidative activity induced by anthocyanin and betalain as colouring agents. Beetroot is commonly found in North Sumatra, especially in Berastagi Regency, and other regions throughout Indonesia. Beets can be eaten raw, boiled, steamed, fermented, or roasted. The plant is also used as a natural colouring agent as it contains betalain, which is responsible for the red colour of the plant (Amila and Sembiring, 2021; Ghanbar Soleiman Abadi *et al*., 2024). Furthermore, smart edible films enriched with betacyanin can detect the rottenness of food.

The physical characteristics of edible films should be enhanced to increase their applications in food quality monitoring and preservation. Studies on edible films using modified cassava starch have been conducted (Bergo *et al*., 2010; Colivet and Carvalho, 2016; Dai *et al*., 2019; Ulyarti *et al*., 2020; Naghdi *et al*., 2021; Vargas *et al*., 2021; Colivet *et al*., 2022; Esfahani *et al*., 2022). They reported that modified cassava starch-based edible films had better mechanical properties (tensile strength, elongation, and water vapour transmission rate) compared to unmodified cassava starch (native starch)-based edible films. The mechanical properties of edible films were found to increase with the application of modified cassava starch into the films using a cross-linking method (Colivet and Carvalho, 2016).

Previous research demonstrated that edible films containing beetroot pulp extract displayed changes in colour and pH (Abadi *et al*., 2024). One of the key advantages of this method is the use of beetroot peel extract, which is a natural and cost-effective source of both pH indicators and preservatives. Beetroot peel, as an agricultural by-product, is available abundantly. It is an eco-friendly and inexpensive alternative to synthetic preservatives or colouring agents commonly used in food packaging. The addition of beet pulp extract can increase water permeability of the edible film due to the presence of anthocyanin that acts as an antioxidant in increasing the permeability. Edible films containing large amounts of betalain exhibit different colours and pH levels, ranging from red (acidic) to yellow (alkaline). Here are the colours and their pH levels: cherry red (pH = 3 - 4), bright red (pH = 5 - 6), orange (pH = 7 - 8), brown (pH = 9), and yellow (pH = 10) (Abadi *et al*., 2024).

However, edible films produced by prior works did not have additional smart functions, such as pH sensory properties, to enhance the shelf life of the protected products. Smart edible films are a profound turning point in the packaging sector, providing a sustainable alternative to plastic packaging while addressing environmental issues through biodegradability and lesser waste. Incorporating natural compounds such as beetroot extract not only improves food shelf life but also provides users with an appealing and intuitive way to check food freshness, thereby enhancing both food safety and quality. This study will give an additional functional value for food. The present work investigated the production of smart edible films with modified cassava starch. Smart edible films can be produced by incorporating beetroot extract, which contains betalain as an antioxidant and an active pH-detecting agent. The present work also investigated the effect of pH levels during the edible film production.

Materials and methods

Materials

Cassava (*Amylum manihot*) starch was purchased from Agung Jaya Inc. (Yogyakarta, Indonesia). Distilled water and high-purity glycerol (> 85%) were obtained from CV. Indrasari (Semarang, Indonesia). The beetroot extract was provided by Herbiology (Bogor, Indonesia). Sodium alginate, stearic acid, hydrochloric acid solutions (> 32%), sodium carbonate powders, and solid caustic soda were purchased from Merck Chemicals and Life Sciences (Jakarta, Indonesia).

Preparation of modified cassava starch

Preparation for modified cassava starch started by mixing 300 g of cassava starch with sodium tripolyphosphate (5%, w/v). The mixture was dissolved in 600 mL of distilled water. Then, 1.8 g of caustic soda and 9 g of sodium carbonate were added to the solution, and stirred at room temperature for 24 h. The acylation process of the suspension was carried out by adding stearic acid (4%, v/v). The pH was set to 6.5 using 3 mol/L of hydrochloric acid solutions. This solution was stirred at 600 rpm for 1 h at room temperature to completely dissolve the stearic acid in the mixture. Finally, the suspension was filtered through a filter paper, and dried at 45°C for approximately 12 h to evaporate all the moisture in the starch. The drying process at high temperature caused the modified cassava starch structure to melt and re-associate into a new structure (Yan and Zhengbiao, 2010).

Edible film formation

Eight edible films were produced, each by mixing 3 g of cassava starch with 0.5 g of alginate, which were then dissolved in 100 mL of distilled water. The pH levels were set using acidic and alkaline solutions, namely 1 mol/L of hydrochloric acid solution and 1 mol/L of caustic soda, respectively, according to the pH variables (4, 7, and 9), and then 2 mL of glycerol was added with the stirring process at 70°C for 90 min. After the heating process, the solutions were left to cool to room temperature. Beetroot extracts (0, 0.25, 0.5, 0.75, and 1 g) were added to the edible film solutions. After stirring, the solutions were poured into glass moulds, and dried in an oven at 45°C for 120 min, and left to dry at room temperature for 2 d (Bergo *et al*., 2010).

Film surface micrograph

The morphological properties of the edible modified cassava starch-based films were determined using scanning electron microscopy (SEM) at 20.0 kV excitation voltage (Philips XL series 30, Netherlands) operated at 15.0 kV acceleration. The films were mounted on bronze stubs after immersion in liquid nitrogen for cryofracturing, and the samples were stained with gold coating (Gutiérrez *et al*., 2015).

Mechanical properties

The mechanical properties (tensile strength, elongation at break, and film thickness) of the edible modified cassava starch-based films were measured following the standard procedures. Tensile strength and elongation analyses were performed following the ASTM D412-98 procedure. A mechanical universal testing machine (Lamy Rheology TX-700 Texture Analyser, France) was operated at a load of 50 N at a speed of 1.0 mm/sec on a film size of 15 × 15 × 0.2 cm. The film thickness was measured using a screw micrometre (LISM micrometre, France) with a precision of 0.01 mm. Measurements were conducted at five different points (n = 5) on each edible film.

Water vapour transmission rate (WVTr)

Water vapour transmission analysis of the edible modified cassava starch-based films was performed following the ASTM E96/E96M-16 procedure. A 30-mL cup or 5-cm diameter glass was filled with 10 g of silica gel, and the film was glued to the glass. The mass of the initial glass was then calculated. Next, the glass was placed in a condition-controlled desiccator at 50 - 55% relative humidity at 27 - 28°C (for condition adjustments, the RH on the glass should always be lower than that outside the glass). The glass was weighed every 24 h for 7 d to determine the water vapour transmission. The slopes (the change in mass at each time interval) were calculated to determine the WVTr value using Eq. 1.

$$WVTr = \frac{slope}{area} \cdot \frac{1\ day}{24\ h} \left(\frac{g}{m^2.h}\right) \quad (Eq. 1)$$

Colour measurement

Colour properties of edible modified cassava starch-based films were examined using a portable colorimeter (PCE-CSM 5, USA). The CIELAB colour coordinates *L*, *a*, and *b* were measured (geometry 8°/d. Ø 8 mm, the light source D65), and the ISO for whiteness and yellowness indices were calculated using colour measurement software. The instrument was calibrated using white ceramic (L = 97.17; a^* = -0.3; b^* = 0.56). All colours of the films were measured three times (n = 3) at different locations (Zhelyazkov *et al*., 2022).

Antioxidative activity (%AA)

Antioxidative activity of the edible modified cassava starch-based films was evaluated *in vitro* using the DPPH assay. DPPH (1,1-diphenyl-2-picrylhydrazyl) was prepared according to Karabagias *et al*. (2018). After preparation, antioxidant activity analysis was conducted according to Esfahani *et al*. (2022). All sample solutions (before the films had dried) were mixed with a solution containing 2 mL of ethanol and 0.2 mM of the DPPH radical. The mixture was stirred and incubated in a dark room at room temperature, and then centrifuged. The absorbance was recorded using a spectrophotometer (GBC Cintra 101, Australia) at 517 nm (Karabagias *et al*., 2018; Esfahani *et al*., 2022). The DPPH free radical scavenging activity was calculated using Eq. 2:

$$\%AA = \frac{A_0 - A_t}{A_0} \times 100 \quad (Eq. 2)$$

where, A_0 = initial absorbance of the DPPH solution, and A_t = absorbance of the remaining DPPH after the reaction with the film sample at the steady state. Each analysis was performed in triplicate (n = 3) (Karabagias *et al*., 2018).

Functional group analysis

The functional groups of the edible modified cassava starch-based films were determined using Fourier transform infrared spectroscopy (FTIR; Thermo Scientific Diamond Nicolet IS 5, USA). The samples were analysed using 128 scans at an infrared spectrum frequency range of 400 to 4000 cm^{-1} and a resolution of 2 cm^{-1}. The results were analysed as a diffractogram of the relationship between wave numbers and intensity (Tongdeesoontorn *et al*., 2021).

Statistical analysis

All experiments were conducted in triplicate. The data were subjected to One-way analysis of variance (ANOVA) with SPSS (IBM SPSS Statistics 25 (IBM, Germany)). The Tukey's test was employed to evaluate the main differences when the main effects or interaction means were statistically significant ($p \leq 0.05$). Means and standard deviations were calculated using Microsoft Excel (Microsoft Office 2019, USA).

Results and discussion

Modified and unmodified cassava starch-based edible films were successfully produced in the present work. The modification of cassava starch was performed using the cross-linking acylation method. These edible films were then characterised to obtain physical (morphology), mechanical (tensile strength, elongation at break, and WVTr), and chemical (functional groups) properties. The effect of pH and beetroot extract addition on the mechanical and antioxidant properties of edible films was also investigated.

Film surface micrograph

The morphological properties of modified and unmodified cassava starch-based edible films in varying amounts of 0 and 1 g of beetroot extract are shown in Figure 1. Variations of beetroot extract (0 and 1 g) were used to determine the optimum interaction between the particles at minimum and maximum concentrations.

The results showed that the modified cassava starch-based edible film (Figure 1c) had compact and homogeneous internal structures compared to the unmodified cassava starch-based edible film (Figure 1a). Li *et al*. (2022) reported that modifying starch alters the granular morphology after the modifying process. The alteration of the granular morphology can be attributed to the disruption of inter- and intra-molecular hydrogen bonds caused by the replacement of hydroxyl groups by acylate groups (Lin and Chou, 2004; Li *et al*., 2022). The alteration of the granular size in modified starch facilitates the gelatinisation and the formation process into a compact film matrix structure (Ulyarti *et al*., 2020). The rougher surface of modified starch-based edible film could have been due to the cross-linking acylation process, which reduces the surface energy of the modified starch. Its surface energy is related to the polar group, as cassava starch has glucose molecules containing three hydroxyl groups. A study conducted by Colivet *et al.* (2022) showed that modified starch based-edible films had lower energy surface due to the replacement of the hydroxyl with acyl groups. This led to the localisation of the acyl groups on the surface, leading to the formation of films with irregular structures (Colivet and Carvalho, 2016).

The presence of beetroot extract altered the structures of both modified and unmodified starch-based films. At 10 μm SEM magnification, the molecular distances of the unmodified starch-based film (Figure 1b) increased, and the voids in the matrix between the particles became wider. This is because the starch and betacyanin molecules carry negative charges, thus repelling each other (Naghdi *et al.*, 2021). The surface of the modified starch-based edible film (Figure 1d) had a heterogeneous and rough surface structure with white agglomerates. Another study found the same result, which indicated a better film structure in the presence of beetroot extract (Gutiérrez *et al.*, 2015). Beetroot extract can influence the internal structure of the film by interacting with the starch molecular network. When incorporated, anthocyanin can form bonds with starch molecules, which affect the crystallinity and morphology of the starch matrix. This can lead to improved mechanical properties, such as increased tensile strength and elasticity, as well as a change in moisture barrier properties. The antioxidant properties of the beetroot extract may also play a role in preventing oxidative degradation of the film, further enhancing the stability and durability of the packaging (Tshamisane and Adeyemi, 2025). This structure formation was caused by insoluble particles, which may be associated with fibrous material present in the beetroot extract (Gutiérrez *et al.*, 2015). Beetroot extract contains approximately 46.01 g of insoluble fibrous particles per 100 g of beetroot extract (Iahnke *et al.*, 2016). These insoluble fibrous particles can reduce the tackiness of the film (Kramer, 2009; Iahnke *et al.*, 2016).

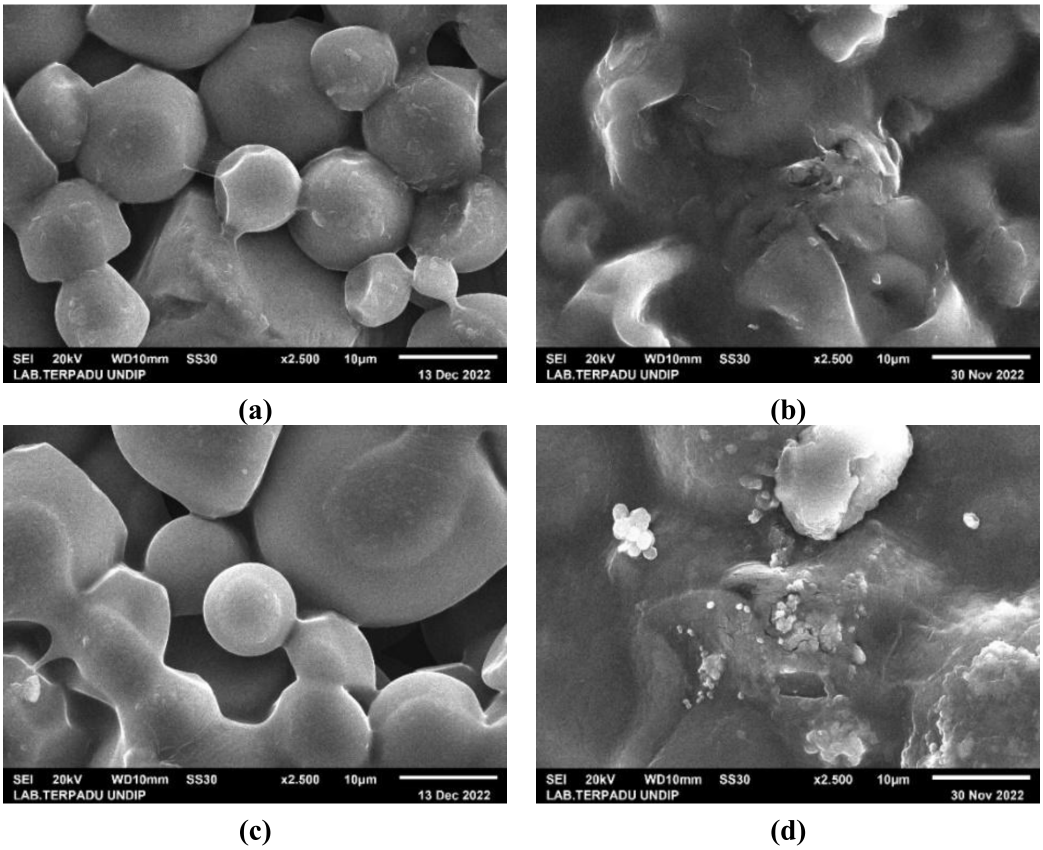


Figure 1. SEMs of **(a)** unmodified starch-based edible film in the absence of beetroot extract; **(b)** unmodified starch-based edible film in the presence of beetroot extract; **(c)** modified starch-based edible film in the absence of beetroot extract; and **(d)** modified starch-based edible film in the presence of beetroot extract.

Mechanical properties of films

The tensile strength properties of edible films prepared using starch and various beetroot extract are depicted in Figures 2a and 2b, respectively. The tensile strength values of the modified cassava starch-based edible film were higher than those of the unmodified edible film, not significant ($p > 0.05$). This could have been due to modified cassava starch's granule size, which has a denser and stronger matrix (Ulyarti *et al*., 2020). In addition, the cross-linked modified cassava starch had strong interaction with the particles in the film, thereby increasing the tensile strength (Dai *et al*., 2019).

Figure 2b shows that modified edible films in the presence of beetroot extract had significant differences ($p < 0.05$) with higher tensile strength values compared to those without the addition of beetroot extract, across all pH variations. This could have been due to the intermolecular hydrogen bond interaction between the hydroxyl groups of the beetroot extract and the modified cassava starch molecules. Additionally, it could have also been due to the increasing matrix strength in the presence of beetroot antioxidants (Vargas *et al*., 2021).

Other experimental data showed that the tensile strength values of modified starch-based edible films without beetroot extract addition increased as the pH increased from 4 to 9. Acidic conditions can lead to cassava starch degradation, and rearrange the film starch chains. As the pH-level increases and the conditions become alkaline, cassava starch with negatively charged amylopectin becomes stronger. This phenomenon is caused by anions binding from the alkaline solution, thus making the film stronger (Chatpapamon *et al*., 2019). However, modified starch-based edible films in the presence of beetroot extract had fluctuated tensile strength values. This could have been due to the effect of pH (other than neutral conditions) on betalain stability. At pH values of 4 and 9, betalain oxidises into simple compounds; the film structure becomes compact, thus increasing the film strength (Manchali *et al*., 2012). Incorporating modified cassava starch into the cross-linking process could improve the mechanical properties of the edible films significantly. A study by Colivet *et al*. (2022) investigated the effect of incorporating cross-linked cassava starch with watermelon seed oil emulsion on film properties. Regarding tensile properties, the tensile strength of the film without watermelon seed oil emulsion was found to be 9.5 MPa. However, when 0.4 g/100 g of emulsion was added, the tensile strength decreased to 4.2 MPa. This finding indicated that while cross-linking could increase the strength of the film, the addition of certain emulsions could reduce tensile strength (Colivet *et al*., 2022).

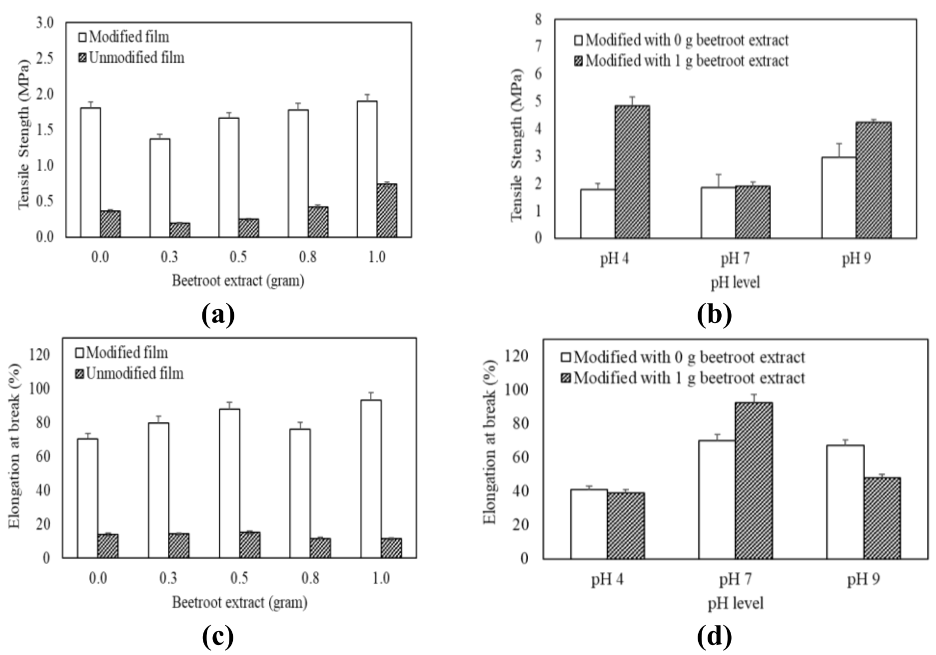


Figure 2. (a) Tensile strength values of modified and unmodified starch-based edible films in varying beetroot extract amounts; **(b)** tensile strength values of modified starch-based edible films in the presence and absence of beetroot extract at varying pH levels; **(c)** elongation values of modified and unmodified starch-based edible films; and **(d)** elongation values of modified starch-based edible films in the presence and absence of beetroot extract amounts at varying pH levels.

The elongation values of the modified and unmodified starch-based edible films are shown in Figure 2c. The results showed that the modified cassava starch-based edible film with significant differences ($p < 0.05$) had higher elongation, compared to the unmodified cassava starch-based edible film. This could have been due to intermolecular interactions from cross-linking modification (Dai *et al*., 2019). The elongation values of the modified starch-based edible film in the presence and absence of beetroot extract at varying pH levels are depicted in Figure 2d. The elongation values of the modified film increased significantly ($p < 0.05$) as the pH increased from 4 to 7, but did not change significantly when the pH was 9. This phenomenon was caused by the interaction of the polyphenolic bonds from the beetroot extract with the polymer matrix, which strengthened the film structure at pH 7 through covalent and non-covalent bonds. These results were consistent with previous work (Chaari *et al*., 2022). However, the elongation values of edible film in the presence of beetroot extract at pH levels of 4 and 9 were lower than those of the edible film without the addition of beetroot extract. The betalain contents in beetroot extract were stable at a pH level of 7, but degraded and formed simple compounds under acidic (pH 4) and alkaline (pH 9) conditions (Manchali *et al*., 2012). This made the film at pH 7 more compact. However, this is not applicable when the pH condition changes as the movement of molecules is limited, and the elongation decreases (Manchali *et al*., 2012). The decrease in elongation values could also have been due to the strengthening of the matrix, which was caused by the increase in the stiffness of the film (Vargas *et al*., 2021; Chaari *et al*. 2022). The elongation values of all variations met the Japanese Industrial Standards (JIS) standards (≥ 70%) (Yulistiani *et al*., 2020). Elongation values increased from 19.8% for films without emulsion to 48.5% for films with 0.4 g/100 g of emulsion, indicating an increase in flexibility. The elastic modulus decreased from 589.5 MPa for the film without emulsion to 192.4 MPa for the film with 0.4 g/100 g emulsion, indicating an increase in elasticity. These results suggested that cassava starch cross-linking improved the mechanical properties of edible films, thus making them suitable for various food packaging applications. However, certain ingredients, such as emulsions, could affect these properties, and should therefore be carefully optimised based on the required film properties (Colivet *et al*., 2022).

The WVTr was also investigated in the present work. It is a parameter that indicates the ability of a film to protect food from moisture. High WVTr values indicate that air and humidity can penetrate the edible film layer more easily. It can also be influenced by many factors, such as the integrity of the film matrix, hydrophilic or hydrophobic properties, intermolecular interaction of water with the film's constituent materials, and several other factors (Ebrahimi *et al*., 2016).

Figure 3a depicts the WVTr values of modified and unmodified starch-based edible films in varying amounts of beetroot extract. The WVTr values of the modified starch-based edible film were significantly ($p < 0.05$) lower than those of the unmodified film. This could have been due to cross-linking acylation modification, which caused the hydroxyl groups of native cassava starch to be substituted by the hydrophobic ester group (Dai *et al*., 2019). The lower WVTr values indicated that the film had good moisture-protective properties.

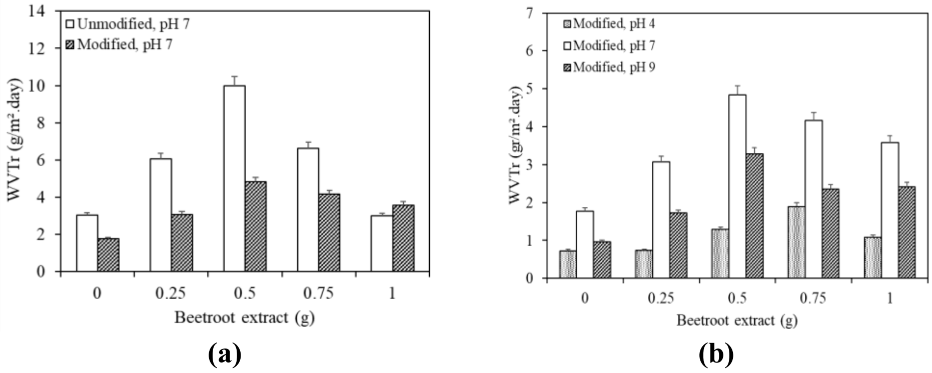


Figure 3. (a) WVTr values of modified and unmodified starch-based edible films; and **(b)** WVTr values of modified starch-based edible films in varying beetroot extract amounts.

Figure 3a also shows the significant difference ($p < 0.05$) in the WVTr values of edible films until a specific threshold. Betacyanin and hydroxyl groups of cassava starch underwent intermolecular interactions that decreased the compact matrix structure of the film. The hydroxyl groups in the film led to an increase in the interaction between the water and the film structure, making it easier for water or air to penetrate the film layer. However, further addition of beetroot extract to the edible film decreased the WVTr values due to hydrogen bond formation from starch and betacyanin, which constructed tortuous paths, and inhibited the passage of water and air in the film matrix (Naghdi *et al*., 2021).

The effect of pH and the addition of beetroot extract on the WVTr values of the modified starch-based edible film is depicted in Figure 3b. The films with pH levels of 4 and 7 showed the lowest and highest WVTr values, respectively. This could have been due to starch degradation under acidic conditions, and the strengthening of intermolecular interactions under alkaline conditions (Chatpapamon *et al*., 2019). In addition, starch is hydrophilic at a pH of 7, so it is more easily penetrated by water and air. The Figure also indicates that adding 0.75 and 1 g of beetroot extract decreases the WVTr values.

Colour properties of films

The colour of edible films can affect consumer acceptance. The ingredients in edible film production are the main factor determining the film's appearance.

Table 1 shows the colour properties and the appearance of modified and unmodified starch-based edible films in varying pH levels and beetroot extract amounts. The letters *L*, *a*, and *b* are indicators used to determine colour values. *L* indicates lightness from black (0) to white (100), and *a* and *b* indicate chromaticity. The negative value of *a* indicates greenness, and the positive value of *a* indicates redness. The negative value of *b* indicates blueness and positive *b* value indicates yellowness. Typically, betalain contained in beetroot extract changes the physical appearance of the edible film, which makes the film red and darker. This indicates positive values from *L* and *a* (Esfahani *et al*., 2022).

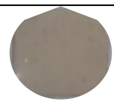
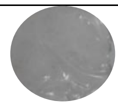
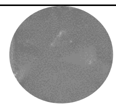
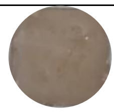
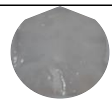
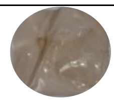
Table 1 also shows that the modified and unmodified starch-based edible films had different colour values, as represented by *L*, *a*, and *b* values. The unmodified starch edible films had brighter colours, compared to the modified ones, as shown by the *L* value. A higher value of *L* means that the film is becoming whiter. The *L* value in the unmodified cassava starch film was higher than that in the modified cassava starch film. The unmodified film was more white because the molecular density affected the structure matrix, which was more sprawled, so the light could penetrate the film surface easily. Unmodified cassava starch films had sprawl density due to the weak intermolecular interaction between unmodified cassava starch and plasticisers, which formed light-penetrable matrix (Dai *et al*., 2019). However, the modification of cassava starch film through the cross-linking method could increase the interaction between the cassava starch molecule and plasticiser (Ulyarti *et al*., 2020).

Furthermore, Table 1 shows that different pH levels affect the colour appearance of edible films. The colour of the films also indicated the existence of antioxidant agents, as reflected by the changes in *L*, *a*, and *b* values. The film without beetroot extract showed a significant colour change at certain pH levels due to changes in the size of the cassava starch molecules during the gelatinisation process. Such a phenomenon causes the film structure to become more compact, and had darker colour (Chatpapamon *et al*., 2019). The film with beetroot extract had the highest *L*, *a*, and *b* values at pH 7, and this could have been due to the betalain stability (Table 1). Betalain is stable under neutral conditions (pH 6 - 7). However, it can be easily oxidised by the change in pH levels (Elbandy and Abdelfadeil, 2008).

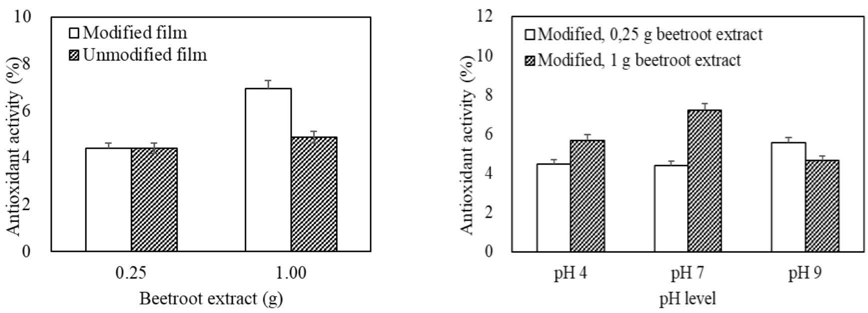
Antioxidant activity and functional groups of films

Free radicals can reduce the packaged food's nutritional quality and safety, and avoid spoilage. Therefore, antioxidants are substantial as additional constituents of edible films. In the present work, a functional group investigation was also conducted to obtain complete information on antioxidant existence and role. Figure 4 shows the antioxidant properties of modified and unmodified-based edible films at varying pH levels and beetroot extract amounts. The addition of beetroot extract increased the antioxidant activity of modified and unmodified edible films (Figure 4). This was due to the high antioxidant content of phenolic (53,700 mg/g) (Borjan *et al*., 2022), polyphenolic (1,276 mg/kg) (Kavalcová *et al*., 2015), and betalain (17.24 mg/g) (Sawicki *et al*.,

Table 1. Colour properties of modified and unmodified based-edible films at varying pH levels and beetroot extract amounts.

Starch	pH	Beetroot extract (g)	*L*	*a*	*b*	Film appearance
Modified	4	0	87.43 ± 0.50^{e}	0.43 ± 0.32^{a}	2.10 ± 0.39^{a}	
		1	79.58 ± 0.56^{c}	7.99 ± 0.64^{d}	9.23 ± 0.48^{b}	
	7	0	82.97 ± 0.56^{dC}	1.76 ± 0.35^{bB}	3.12 ± 0.33^{b,A}	
		1	65.84 ± 0.75^{aA}	25.55 ± 0.58^{fD}	19.20 ± 0.4^{d,C}	
	9	0	82.19 ± 0.45^{d}	2.01 ± 0.31^{c}	7.01 ± 0.34^{b}	
		1	70.37 ± 0.54^{b}	17.82 + 0.30^{e}	15.40 ± 0.4^{c}	
Unmodified	7	0	86.37 ± 0.61^{D}	0.90 ± 0.12^{A}	2.49 ± 0.57^{A}	
		1	68.07 ± 0.56^{B}	4.96 ± 0.76^{C}	9.763 ± 0.56^{C}	

Number of trials = 3. Values are mean values ± standard deviations. Means followed by different lowercase superscripts within similar column are significantly different ($p < 0.05$) for modified cassava starch in varying pH levels. Means followed by different uppercase superscripts within similar column are significantly different ($p < 0.05$) between modified and unmodified starch (pH = 7).



(a) **(b)**

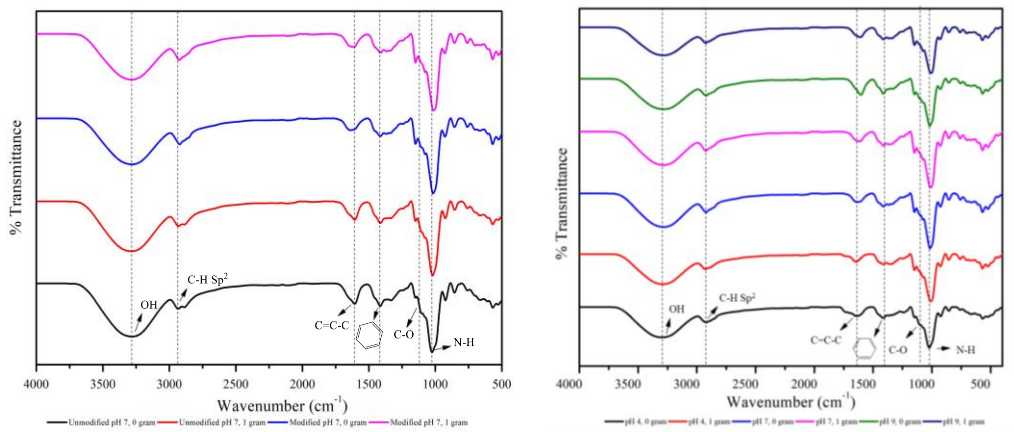
Figure 4. (a) Antioxidant activity of modified and unmodified starch-based edible films with varying beetroot extract amounts; and **(b)** antioxidant activity of modified starch-based edible films with varying pH levels and beetroot extract amounts.

2016) in beetroot extract. Furthermore, betalain is present in betacyanin and betaxanthin forms, which also act as pigments, imparting a red colour to fruits and roots. As demonstrated in the present work, it also influenced the colour appearance of the edible film (Table 1).

Figure 4a also shows that the antioxidant activity of the modified starch-based edible film was significantly higher than that of the unmodified film ($p < 0.05$). In addition, the variations of beetroot extract used to determine the antioxidant activity in the film were 0 and 1 g, since the optimum effect appeared either in the minimum or the maximum concentrations. Starch modification was carried out through a cross-linking acylation process. This process used stearic acid - as a modifying agent - through rapid gelatinisation and a more even granular distribution of starch. Furthermore, it can affect the stability of betacyanin, a type of betalain found in beetroot extract, by reducing racemisation and improving colour stability due to the molecular build-up of acyl residues (Cai *et al*., 2001). Betaine, another type of betalain, can re-assemble and form a more complex betacyanin (Polturak and Aharoni 2019). This phenomenon is an effect of a modification that provides stability to betalain; thus, the antioxidant value of the film with modified starch was higher than that of the film with unmodified starch.

The present work used beetroot extract as a natural indicator of food freshness through pH changes. Figure 4b shows that the antioxidative activity of the modified film at a pH of 7 was also significantly higher than that at other pH levels ($p < 0.05$). Betalain is only stable in neutral pH conditions (pH 6 - 7) but not under acidic and alkaline conditions (Elbandy and Abdelfadeil, 2008). Betalain can be condensed and hydrolysed under acidic and alkaline conditions (Manchali *et al*., 2012). Therefore, the antioxidative activities of edible films at pH 4 and 9 were lower than those at pH 7. Changes in the antioxidative activities of edible films are also indicated by colour changes, as shown in Table 1. Following these results, betalain contained in beetroot extract can also be used as a natural indicator of food spoilage through pH changes in packaged food (active pH-detecting agent). The beetroot extract can change the colour of the film from whitish red (neutral pH) to pale white (more acidic pH) or dark brown (more alkaline pH). Therefore, consumers can immediately identify a decrease in food quality.

The FTIR spectra of modified and unmodified starch-based edible films at varying pH levels and beetroot extract amounts are shown in Figures 5a and 5b. All films had similar spectra patterns. The polysaccharide spectrum was observed at 3200 - 3400, 2800 - 2900, and 1500 - 1650 cm^{-1}; corresponding with hydroxyl (O-H), C-H sp^2, and aromatic groups (C=C-C), respectively (Naghdi *et al*., 2021). Figure 5a shows the FTIR spectra of modified and unmodified starch-based edible films. A different peak was also observed at around 1700 cm^{-1}, where the modified starch-based edible film had sharper peak, compared to the unmodified film's peak. The FTIR spectra indicated that O-H bands were weakening, which essentially contributed to the hydrophilicity properties of the material. O-H groups in starch serve to increase the interaction with water, thus increasing the hydrophilicity properties of starch. However, when cross-linking occurs and the O-H groups decrease, the ability of starch to interact with water decreases, leading to a change in properties to become more hydrophobic. This decrease in O-H groups indicates reduced polarity on the starch surface, which makes it more water-repellent, and increases the hydrophobic properties. After plasma treatment, which causes cross-linking, starch films tend to change from hydrophilic to more hydrophobic due to the loss of O-H groups that play a role in interaction with water (Otálora González *et al*., 2022). In addition, within this wave range, the interaction between the C-O bond stretching shows the carboxylic ester group of stearic acid (Sondari *et al*., 2018). The presence of an ester group indicates the presence of stearic acid as a modifying agent. Figure 5b depicts the FTIR spectra of modified starch-based edible films at varying pH levels and beetroot extract amounts. Among these spectra, some differences were observed in the range of 1500 - 1650 cm^{-1}, indicating the aromatic group chains in starch was affected by the increase in pH levels. Starch in the form of amylopectin, which has an aromatic group, is affected by anionic molecules (Rajha *et al*., 2014). FTIR spectra also peaked at around 1115 and 1020 cm^{-1}, representing the C-O bond and amine group (N-H). Key functional groups such as hydroxyl (-OH), carbonyl (C=O), and amide (N-H) are indicative of critical interactions like hydrogen bonding or covalent linkages within the polymer matrix. For instance, hydroxyl stretching, commonly observed in polysaccharide or protein-based films,



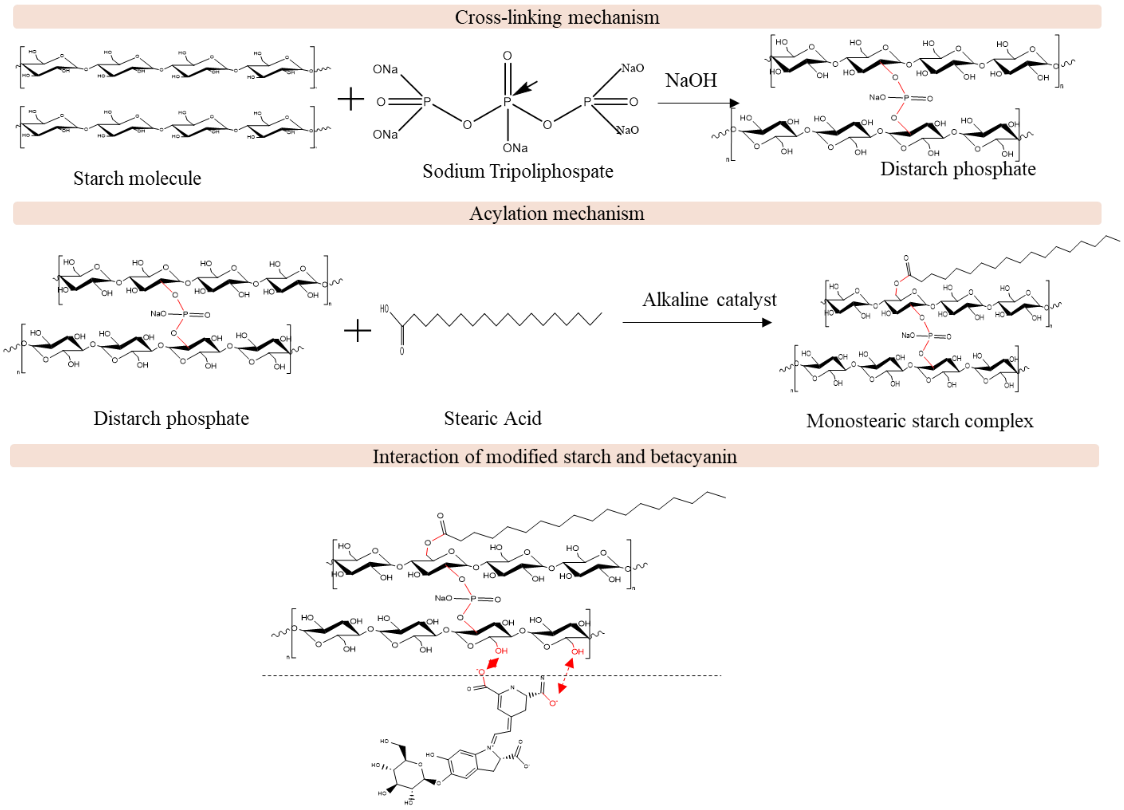
(a) **(b)**

Figure 5. (a) FTIR spectra of modified and unmodified starch-based edible film with varying beetroot extract amounts; and **(b)** FTIR spectra of modified starch-based edible film with varying beetroot extract amounts and pH levels.

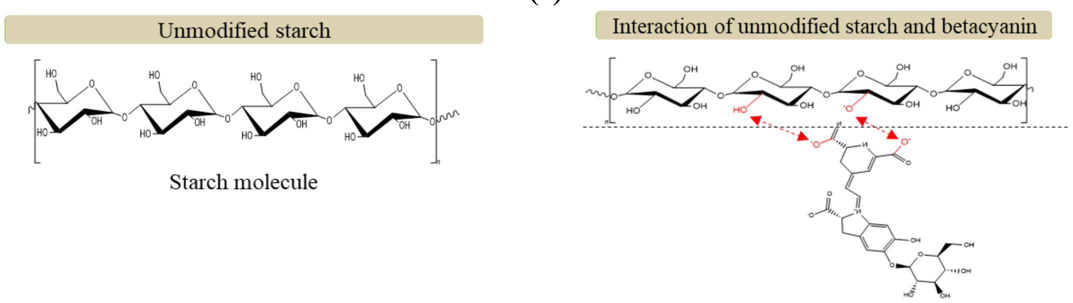
reflects hydrogen bonding that enhances mechanical strength and water resistance. Carbonyl peaks, associated with lipids, proteins, or plasticisers, reveal cross-linking or esterification processes that improve thermal stability and reduce water vapour permeability. Similarly, amide bands in proteins signify interactions with plasticisers or additives, influencing elasticity and tensile properties (Rahmawati *et al*., 2024). In FTIR spectra, the O–H stretching and C–H stretching bands observed in the film spectrum indicated the interaction between the film molecules and anthocyanins, primarily through the formation of hydrogen bonds. These functional groups indicated betacyanin groups (betalain) in edible films (Kumar *et al*., 2017). The increase in the intensity of these bands, particularly the shifted O–H band, suggested that the film bound more strongly with anthocyanin. When the film containing anthocyanin was exposed to pH changes, these hydrogen bonds allowed the film to undergo significant colour changes, caused by the structural transformation of anthocyanin, such as the shift from the flavylium form (red) to another form (blue or colourless) at higher pH levels. This change can be used to detect food spoilage or changes in its freshness (Ke *et al*., 2024).

Proposed mechanism

By observing these FTIR spectra (Figures 5a and 5b), we can also propose edible film production mechanisms. The first process involved the production of a modified starch-based edible film, the mechanism of which is shown in Figure 6a. Starch modification was conducted using the cross-linking and acylation methods. Sodium tripolyphosphate (STPP), as a phosphorylation cross-linking agent, reacted with the starch polymer matrix under alkaline conditions. The pH was adjusted by adding NaOH and Na_2CO_3. The reaction, consequently, formed products (distarch monophosphate) that were anionic due to the phosphate group (Lim and Seib, 1993; Sechi and Marques, 2017). Starch modified by cross-linking with STPP was re-modified by adding fatty acids such as stearic acid (Bajner, 2005). This modification formed complex helix compounds between the amylose and lipids of the modified starch products (Oktaviana and Saepudin, 2021). The cross-linking process made starch molecular more hydrophobic due to linkage between hydroxyl groups on the starch and STPP molecules to form ester bonds. The formation of ester bonds with starch molecules improved mechanical properties, making the film denser and stronger (Mehboob *et al*., 2020). The modified starch was used to produce an edible film. The beetroot extract was fortified into the film solution to enhance antioxidant properties. In this case, a repulsive force existed between the hydroxyl groups of starch and betacyanin. The second production was an unmodified starch-based edible film, with the mechanism shown in Figure 6b. In the present work, native cassava starch was used as the basic material for edible film production. Antioxidant properties were obtained by adding beetroot extract to the film solution.



(a)



(b)

Figure 6. Mechanism of **(a)** modified starch-based-edible film production and **(b)** unmodified starch-based-edible film production.

Conclusion

Edible films made from cassava starch with cross-linking acylation modification showed better properties than edible films made using native cassava starch. Micrograph determination showed that modified starch easily was mixed in the film composite. Also, the mechanical properties of modified starch films were significantly higher and better, yet met the JIS standards for edible films. Beetroot extract fortification into edible films improved their mechanical properties and antioxidative activity. Nevertheless, the betalain contents in beetroot extract were sensitive to temperature and acidity. However, this characteristic can be used to detect the freshness of food as they emit acidic substances that indicate that the food is going to spoil. As the present work did not include the application of edible films as smart materials, further research is recommended to explore this area.

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References

Amila, A. and Sembiring, E. 2021. The effect of beetroot biscuits (*Beta vulgaris*) on the hemoglobin level of patients with pulmonary tuberculosis. Indonesian Journal of Public Health 16(1): 43-55.

Bajner, R. E. 2005. Stearic acid addition to maize starch and its influence on pasting viscosity behaviour. South Africa: University of Pretoria, PhD thesis.

Bergo, P., Sobral, P. J. A. and Prison, J. M. 2010. Effect of glycerol on physical properties of cassava starch films. Journal of Food Processing and Preservation 34: 401-410.

Borjan, D., Šeregelj, V., Andrejč, D. C., Pezo, L., Šaponjac, V. T., Knez, Ž., … and Marevci, M. K. 2022. Green techniques for preparation of red beetroot extracts with enhanced biological potential. Antioxidants 11(5): 805.

Cai, Y., Sun, M. and Corke, H. 2001. Identification and distribution of simple and acylated betacyanins in the Amaranthaceae. Journal of Agricultural and Food Chemistry 49(4): 1971-1978.

Chaari, M., Elhadef, K., Akermi, S., Ben Akacha, B., Fourati, M., Chakchouk Mtibaa, A., … and Smaoui, S. 2022. Novel active food packaging films based on gelatin-sodium alginate containing beetroot peel extract. Antioxidants 11(11): 2095.

Chatpapamon, C., Wandee, Y., Uttapap, D., Puttanlek, C. and Rungsardthong, V. 2019. Pasting properties of cassava starch modified by heat-moisture treatment under acidic and alkaline pH environments. Carbohydrate Polymers 215: 338-347.

Chen, H., Wu, C., Feng, X., He, M., Zhu, X., Li, Y. and Teng, F. 2022. Effects of two fatty acids on soy protein isolate/sodium alginate edible films: Structures and properties. LWT - Food Science and Technology 159: 113221.

Colivet, J. and Carvalho, R. A. 2016. Hydrophilicity and physicochemical properties of chemically modified cassava starch films. Industrial Crops and Products 95: 599-607.

Colivet, J., Garcia, V. A. D. S., Lourenço, R. V., Yoshida, C. M. P., Oliveira, A. L., Vanin, F. M. and Carvalho, R. A. 2022. Characterization of films produced with cross-linked cassava starch and emulsions of watermelon seed oils. Foods 11(23): 3803.

Dai, L., Zhang, J. and Cheng, F. 2019. Effects of starches from different botanical sources and modification methods on physicochemical properties of starch-based edible films. International Journal of Biological Macromolecules 132: 897-905.

Ebrahimi, S. E., Koocheki, A., Milani, E. and Mohebbi, M. 2016. Interactions between *Lepidium perfoliatum* seed gum - grass pea (*Lathyrus sativus*) protein isolate in composite biodegradable film. Food Hydrocolloids 54: 302-314.

Elbandy, M. A. and Abdelfadeil, M. G. 2008. Stability of betalain pigments from a red beetroot (*Beta vulgaris*). Egyptian Journal of Food Science 36: 49-60.

Esfahani, A., Mohammadi Nafchi, A., Baghaei, H. and Nouri, L. 2022. Fabrication and characterization of a smart film based on cassava starch and pomegranate peel powder for monitoring lamb meat freshness. Food Science and Nutrition 10(10): 3293-3301.

Ghanbar Soleiman Abadi, F., Bazargani-Gilani, B., Emamifar, A. and Nourian, A. 2024. Beet root peel extract as a natural cost-effective pH indicator and food preservative in edible film: Shelf life improvement of cold-stored trout fillet. Food Science and Nutrition 12(12): 10561-10575.

Gutiérrez, T. J., Tapia, M. S., Pérez, E. and Famá, L. 2015. Structural and mechanical properties of edible films made from native and modified cush-cush yam and cassava starch. Food Hydrocolloids 45: 211-217.

Iahnke, A. O. S., Costa, T. M. H., Rios, A. O. and Flôres, S. H. 2016. Antioxidant films based on gelatin capsules and minimally processed beet root (*Beta vulgaris* L. var. Conditiva) residues. Journal of Applied Polymer Science 133(10): 43094.

Karabagias, V. K., Karabagias, I. K. and Gatzias, I. 2018. The impact of different heating temperatures on physicochemical, color attributes, and antioxidant activity parameters of Greek honeys. Journal of Food Process Engineering 41(3): 1-9.

Kavalcová, P., Bystrická, J., Tomáš, J., Karovičová, J., Kovarovič, J. and Lenková, M. 2015. The content of total polyphenols and antioxidant activity in red beetroot. Potravinarstvo Slovak Journal of Food Sciences 9: 77-83.

Ke, F., Liu, D., Qin, J. and Yang, M. 2024. Functional pH-sensitive film containing purple sweet potato anthocyanins for pork freshness monitoring and cherry preservation. Foods 13(5): 736.

Kong, I., Degraeve, P. and Pui, L. P. 2022. Polysaccharide-based edible films incorporated with essential oil nanoemulsions: Physico-chemical, mechanical properties and its application in food preservation - A review. Foods 11(4): 555.

Kramer, M. E. 2009. Structure and function of starch-based edible films and coatings. In Huber, K. C. and Embuscado, M. E. (eds). Edible films and coatings for food applications, p. 113-134. United States: Springer.

Kumar, S. N. A., Ritesh, S. K., Sharmila, G. and Muthukumaran, C. 2017. Extraction optimization and characterization of water soluble red purple pigment from floral bracts of *Bougainvillea glabra*. Arabian Journal of Chemistry 10: S2145-S2150.

Li, M., Wang, J., Wang, F., Wu, M., Wang, R., Strappe, P., … and Zhou, Z. 2022. Insights into the multi-scale structure of wheat starch following acylation: Physicochemical properties and digestion characteristics. Food Hydrocolloids 124: 107347.

Lim, S. and Seib, P. A. 1993. Preparation and pasting properties of wheat and corn starch phosphates. Cereal Chemistry 70: 137-139.

Lin, H.-Y. and Chou, C.-C. 2004. Antioxidative activities of water-soluble disaccharide chitosan derivatives. Food Research International 37(9): 883-889.

Liu, H., Ramsden, L. and Corke, H. 1999. Physical properties of cross-linked and acetylated normal and waxy rice starch. Starch 51(7): 249-252.

Manchali, S., Murthy, K. N. C., Nagaraju, S. and Neelwarne, B. 2012. Stability of betalain pigments of red beet BT - red beet biotechnology: Food and pharmaceutical applications. In Neelwarne, B. (ed). Red Beet Biotechnology - Food and Pharmaceutical Applications, p. 55-74. United States: Springer.

Mehboob, S., Ali, T. M., Sheikh, M. and Hasnain, A. 2020. Effects of cross linking and/or acetylation on sorghum starch and film characteristics. International Journal of Biological Macromolecules 155: 786-794.

Mostafavi, F. S. and Zaeim, D. 2020. Agar-based edible films for food packaging applications - A review. International Journal of Biological Macromolecules 159: 1165-1176.

Naghdi, S., Rezaei, M. and Abdollahi, M. 2021. A starch-based pH-sensing and ammonia detector film containing betacyanin of paperflower for application in intelligent packaging of fish. International Journal of Biological Macromolecules 191: 161-170.

Oktaviana, D. and Saepudin, E. 2021. The effect of cross-linking and fatty acid addition on the functional properties and digestibility of tapioca starch. Journal of Physics - Conference Series 1788(1): 12005.

Otálora González, C. M., Schelegueda, L. I., Pizones Ruiz-Henestrosa, V. M., Campos, C. A., Basanta, M. F. and Gerschenson, L. N. 2022. Cassava starch films with anthocyanins and betalains from agroindustrial by-products: Their use for intelligent label development. Foods 11(21): 3361.

Polturak, G. and Aharoni, A. 2019. Advances and future directions in betalain metabolic engineering. New Phytologist 224(4): 1472-1478.

Rahmawati, S., Yassaroh, Y., Theodora, M., Tahril, T., Afadil, A., Santoso, T., … and Nurmayanti, Y. 2024. Antioxidant edible films derived from Belitung taro tubers (*Xanthosoma sagittifolium*) incorporated with moringa leaf extract (*Moringa oleifera*). Preventive Nutrition and Food Science 29(2): 210-219.

Rajha, H. N., Jaoude, N. A., Louka, N., Maroun, R. G. and Vorobiev, E. 2014. Industrial byproducts valorization through energy saving processes. Alkaline extraction of polyphenols from vine shoots. In International Conference on Renewable Energies for Developing Countries 2014, p. 89-94. Beirut, Lebanon.

Roy, S. and Rhim, J.-W. 2021. Anthocyanin food colorant and its application in pH-responsive color change indicator films. Critical Reviews in Food Science and Nutrition 61(14): 2297-2325.

Sawicki, T., Bączek, N. and Wiczkowski, W. 2016. Betalain profile, content and antioxidant capacity of red beetroot dependent on the genotype and root part. Journal of Functional Foods 27: 249-261.

Sechi, N. S. M. and Marques, P. T. 2017. Preparation and physicochemical, structural and morphological characterization of phosphorylated starch. Materials Research 20: 174-180.

Sondari, D., Aspiyanto, Amanda, A. S., Triwulandari, E., Ghozali, M., Septiyanti, M. and Iltizam, I. 2018. Characterization edible coating made from native and modification cassava starch. AIP Conference Proceedings 2049: 030013.

Tongdeesoontorn, W., Mauer, L. J., Wongruong, S., Sriburi, P., Reungsang, A. and Rachtanapun, P. 2021. Antioxidant films from cassava starch/gelatin biocomposite fortified with quercetin and TBHQ and their applications in food models. Polymers 13(7): 1117.

Tshamisane, M. and Adeyemi, J. O. 2025. Materials advances bio-based edible films using encapsulated beetroot powders for smart packaging. Materials Advances 6: 1051-1066.

Ulyarti, U., Nazarudin, N., Surhaini, Lisani, Ramadon, R. and Lumbanraja, P. 2020. Cassava starch edible film with addition of gelatin or modified cassava starch. IOP Conference Series - Earth and Environmental Science 515: 012030.

Vargas, O. L. T., Loaiza, Y. V. G. and González, M. L. 2021. Effect of incorporating extracts from natural pigments in alginate/starch films. Journal of Materials Research and Technology 13: 2239-2250.

Viana, R. M., Sá, N. M. S. M., Barros, M. O., Borges, M. F. and Azeredo, H. M. C. 2018. Nanofibrillated bacterial cellulose and pectin edible films added with fruit purees. Carbohydrate Polymers 196: 27-32.

Yan, H. and Zhengbiao, G. U. 2010. Morphology of modified starches prepared by different methods. Food Research International 43(3): 767-772.

Yulistiani, F., Khairunisa, N. and Fitiana, R. 2020. The effect of glycerol concentration and breadfruit flour mass on edible film characteristics. Journal of Physics - Conference Series 1450: 12001.

Zhelyazkov, S., Zsivanovits, G., Stamenova, E. and Marudova, M. 2022. Physical and barrier properties of clove essential oil loaded potato starch edible films. Biointerface Research in Applied Chemistry 12(4): 4603-4612.