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ORIGINAL RESEARCH PAPER

EFFECTS OF GELATIN CONCENTRATION AND CO-PLASTICIZER TYPE ON PHYSICAL, MECHANICAL AND MICROSTRUCTURAL PROPERTIES OF CORN STARCH-GELATIN COMPOSITE EDIBLE FILMS

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Abstract

In the current study, corn starch-gelatin composite edible films varying in gelatin content (0, 5, 20%) were prepared by using glycerol, and citrate esters; tributyl citrate (TBC) or triisodecyl citrate (TIDC). Edible films formulated with different gelatin concentration and co-plasticizer type were characterized in terms of physical, mechanical, microstructural properties and FTIR spectra. Gelatin addition and increasing the gelatin concentration in the film formulation provided significant increases in surface hydrophobicity and tensile strength and significant decreases in thickness, and elongation at break values. Although the addition of gelatin significantly decreased the ΔE values, gelatin concentration did not affect the ΔE values of edible films. Co-plasticizer addition to the film formulation significantly increased the WCA, and elongation at break values, whereas it significantly decreased the thickness, ΔE and tensile strength values. Co-plasticizer type did not significantly affect the thickness, ΔE, WCA, and tensile strength of edible films. Gelatin addition to the film formulation increased the peak intensity at 1640 cm^{-1}, 1550 cm^{-1} and 1240 cm^{-1} bands and decreased the peak intensity at around 1000 cm^{-1} band. According to the SEM results, addition of gelatin and/or co-plasticizer provided more compact and denser microstructure to the edible films. Overall, gelatin and co-plasticizer addition led to produce thinner edible films with more hydrophobic surface, more transparent appearance, and more ordered microstructure.

Keywords: citrate ester, composite edible film, co-plasticizer, corn starch, film properties, gelatin

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Introduction

Edible films and coatings are defined as environmentally friendly, cost-effective, innovative, and sustainable packaging alternatives for the food industry applications (Hellebois *et al.*, 2020). These biodegradable materials protect foods from physical, chemical, and biological spoilage, increasing their quality, shelf life and safety (Gemeda *et al.*, 2019). The main biopolymers used in edible films are polysaccharides, proteins, and lipids (Chen *et al.*, 2021). The mostly used polysaccharides are starches. Since an edible film made of a single biopolymer (*e.g.*, starch or gelatin) cannot meet all the requirements expected from a packaging material, in recent years, extensive research have been focused on multicomponent edible films. Combination of two or more film forming materials provides better physical, mechanical and barrier properties than one-component film matrices (Galus *et al.*, 2020). Corn starch is one of the promising components of edible film formulations due to its favorable properties, such as biodegradability, renewability, non-toxicity and affordability (Calderón-Castro *et al.*, 2018; Mohamed *et al.*, 2020). Moreover, edible films made of proteins also have desirable properties such as good barrier properties and strong structure and they are also elastic (Janjarasskul and Krochta, 2010). Gelatin is one of the proteins from animal source containing both hydrophilic (*e.g.*: serine, threonine, asparagine, glutamine, aspartic acid, glutamic acid) and hydrophobic aminoacids (*e.g.*: leucine, valine, phenylalanine, isoleucine and methionine (Pulla-Huillca *et al.*, 2021) and has film forming ability related to its unique sequence of amino acids (glycine, proline and hydroxyproline) and characteristics (Hanani *et al.*, 2012). Furthermore, processability of biopolymers alone has some difficulties, plasticizers (hydrophilic or hydrophobic) are used alone or together with co-plasticizers (hydrophilic or hydrophobic) to minimize drawbacks during and after processing. Plasticizers improve polymer chain flexibility and handling of films, maintain integrity, and avoid cracks and pores in the polymeric matrix (Garcia *et al.*, 2000; Suderman *et al.*, 2018). Also, the use of the main plasticizer with co-plasticizers can improve the film properties (Ahmad *et al.*, 2012; Shirai *et al.*, 2013). The plasticization efficiency depends on the similarity of the chemical structures and the compatibility between the plasticizer and the polymer (Shirai *et al.*, 2013). Glycerol (as main plasticizer) and citrate esters (as co-plasticizer) have been used together in biodegradable film formulations to improve the film characteristics (Shirai *et al.*, 2013). Glycerol is a hydrophilic, nontoxic, and biodegradable plasticizer commonly used in edible film formulations which generally provides improvements in film processibility and characteristics. Citrate esters are derived from citric acid, and they are hydrophobic, nontoxic, and biodegradable (Shi *et al.*, 2012). Shirai *et al.* (2013) used glycerol (hydrophilic) and citrate esters (hydrophobic) together in film formulation to plasticize starch (hydrophilic) and poly(lactic acid) (PLA) (hydrophobic), respectively. The results indicated that citrate esters plasticized PLA and made it possible to obtain homogeneous pellets to produce films by blow extrusion (Shirai *et al.*, 2013).

In literature, starch and gelatin obtained from various sources have been combined with different plasticizers in edible film formulations to improve the compatibility (Zhang *et al.*, 2013). Since corn starch is hydrophilic and gelatin has both hydrophilic and hydrophobic functional groups (Nurul and Sarbon, 2015), glycerol and citrate esters (TBC and TIDC) were chosen to be used together in the current study to improve some of the edible film characteristics. The objectives of this study were to develop edible and biodegradable films consisting of corn starch, gelatin, glycerol and citrate esters (co-plasticizers) and to investigate the effects of gelatin concentration and co-plasticizer type on physical, mechanical and microstructural properties of corn starch-gelatin composite edible films. As a result of a comprehensive review of the scientific literature, we found no published data on this content.

Materials and methods

Materials

The corn starch (CAS: 9005-25-8, Sigma Code: S4126), gelatin from bovine skin (CAS: 9000-70-8, Sigma Code: G9391) and glycerol (CAS: 56-81-5, Sigma Code: G6279) were purchased from Sigma Aldrich. The co-plasticizers (TBC and TIDC) were kindly obtained from Pulcra Chemical Company R&D Center (Kocaeli, Turkey).

Edible film production

In edible film formulations, corn starch and gelatin were used as biopolymers, glycerol was used as main plasticizer and tributyl citrate (TBC) and triizodecyl citrate (TIDC) were used separately as co-plasticizers. The ratios of ingredients used in the edible film formulations, given in Table 1 were decided according to the preliminary experiments. Prior to melt processing, corn starch, gelatin, glycerol and co-plasticizers (TBC or TIDC) were mixed at predefined amounts and stored at room temperature for 24 h.

Table 1. Edible film formulations.

Film Samples	Corn starch (%)	Gelatin (%)	Glycerol (%)	TBC (%)	TIDC (%)
0% G, Co-pl free	70	0	30	-	-
5% G, Co-pl free	65	5	30	-	-
20% G, Co-pl free	50	20	30	-	-
0% G, TBC	70	0	27	3	-
5% G, TBC	65	5	27	3	-
20% G, TBC	50	20	27	3	-
0% G, TIDC	70	0	27	-	3
5% G, TIDC	65	5	27	-	3
20% G, TIDC	50	20	27	-	3

0% G, Co-pl free: gelatin free and co-plasticizer free; 5% G, Co-pl free: 5% gelatin added and co-plasticizer free; 20% G, Co-pl free: 20% gelatin added and co-plasticizer free; 0% G, TBC: gelatin free and TBC added; 5% G, TBC: 5% gelatin and TBC added; 20% G, TBC: 20% gelatin and TBC added; 0% G, TIDC: gelatin free and TIDC added; 5% G, TIDC: 5% gelatin and TIDC added; 20% G, TIDC: 20% gelatin and TIDC added

Extrusion process

The premixed edible film ingredients (20g) were fed into a laboratory scale twin-screw micro compounder (MC 15 HT, Xplore Instruments, The Netherlands), and extruded at 130°C for 5 minutes at 100 rpm screw speed, to prepare compounds by a one-step melt-compounding process. Then the compounds were pressed for 5 minutes by using hot press (Yücel Makine, Turkey) operating at 130 °C and under 15 bar pressure to form films.

Physical properties of obtained films

Film thickness

The thickness of films was measured by Peacock Model H comparator (Japan). Five random measurements were made on each film at different sections. Mean value for each film was reported in millimeters (mm).

Film color

The color of films was measured by a chromameter (Konica Minolta CR-400, Japan). Color values were determined according to CIE L*, a*, b* scale. The L*, a*, b* values of films were measured after they were placed on a white plate having L_0*, a_0*, b_0* values of 93.4, 2.26 and -2.4, respectively. Average of five random measurements at different sections of each film were taken to calculate the total color change (ΔE) values according to the equation given below.

$$\Delta E=\sqrt{(L^{*}-L_0^{*})^2+(a^{*}-a_0^{*})^2+(b^{*}-b_0^{*})^2} \qquad (1)$$

Water contact angle (WCA)

WCA values were measured by using an Attension Theta Lite (USA) model contact angle instrument. Deionized water was used as a probe liquid in order to determine the hydrophilicity of the edible films. Average of five measurements from each film were recorded.

Mechanical properties of obtained films

The mechanical properties (tensile strength and elongation at break) of the films were determined using an Instron Universal Testing (Model 3345, USA) machine following the procedure described in ASTM D 882-18 (ASTM, 2018). The crosshead speed of 10 mm / min was used. The films were cut into strips with the width and the length of 2 cm and 8 cm, respectively. At least five samples from each film were tested and the values were averaged.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR spectrums of films were collected by using a spectrophotometer (Perkin Elmer Spectrum 100, USA) in attenuated total reflectance (ATR) mode, operating at 4000-600 cm^{-1} IR-region.

Microstructural properties

The surfaces of mechanically tested film samples were examined using a scanning electron microscope (SEM) (JSM-7500F, Japan) device after gold coating by Edwards Sputter Coater S150B.

Statistical Analysis

The analysis of variance (ANOVA) and Tukey' s tests (MINITAB, version 16) were performed to determine the significant differences in edible film properties ($p<0.05$).

Results and discussion

Film thickness and color

Thickness of edible films can influence several physical properties of the material, such as water vapor permeability, mechanical properties, color, and opacity (Gontard *et al.*, 1992).

The thickness and color values of composite edible films are given in Table 2 and Table 3. The thickness of edible films ranged from 0.14 mm to 0.65 mm. Film thickness is highly associated with the alignment, sorting and compacting of molecules and intensively affected by the film formulation (Hoque *et al.*, 2011; Wang *et al.*, 2017a).

Table 2. Effect of gelatin concentration and co-plasticizer type on thickness (mm) of composite edible films.

	Co-pl free	**TBC**	**TIDC**	**Overall gelatin concentration effect**
0% G	0.65 ± 0.02^{Aa}	0.30 ± 0.00^{Ab}	0.29 ± 0.00^{Ab}	0.41 ± 0.06^{A}
5% G	0.50 ± 0.00^{Ba}	0.25 ± 0.01^{Ab}	0.25 ± 0.01^{Ab}	0.33 ± 0.04^{B}
20% G	0.41 ± 0.01^{Ca}	0.15 ± 0.02^{Bb}	0.14 ± 0.01^{Bb}	0.23 ± 0.05^{C}
Overall plasticizer type effect	0.52 ± 0.04^{a}	0.23 ± 0.02^{b}	0.22 ± 0.02^{b}	

$^{A, B, C}$ Mean values with different letters in the same column differ significantly from each other ($p\leq0.05$)
$^{a, b, c\ d}$ Mean values with different letters in the same row differ significantly from each other ($p\leq0.05$)
All values were given as mean ± standard error

As gelatin concentration in the edible film formulation increased thickness of the edible films decreased. Similar results were found by Sun *et al.* (2013), reporting that the addition of peanut protein isolate to pea starch films decreased the thickness of films. That result was related to the less sticker structure of protein films than starch films, which resulted in thinner films (Sun *et al.*, 2013). Furthermore, gelatin addition to the film formulation, especially at 20% gelatin concentration, improved the spreadability of composite edible films under hot press, which resulted in thinner films (Figure 1 and Table 2). Although co-plasticizer addition significantly decreased the thickness values, co-plasticizer type did not significantly change the thickness of edible films. The significant decrease in the thickness of gelatin and/or co-plasticizer containing edible films in the present study may be due to the interaction between gelatin and starch biopolymers (Wang *et al.*, 2017a).

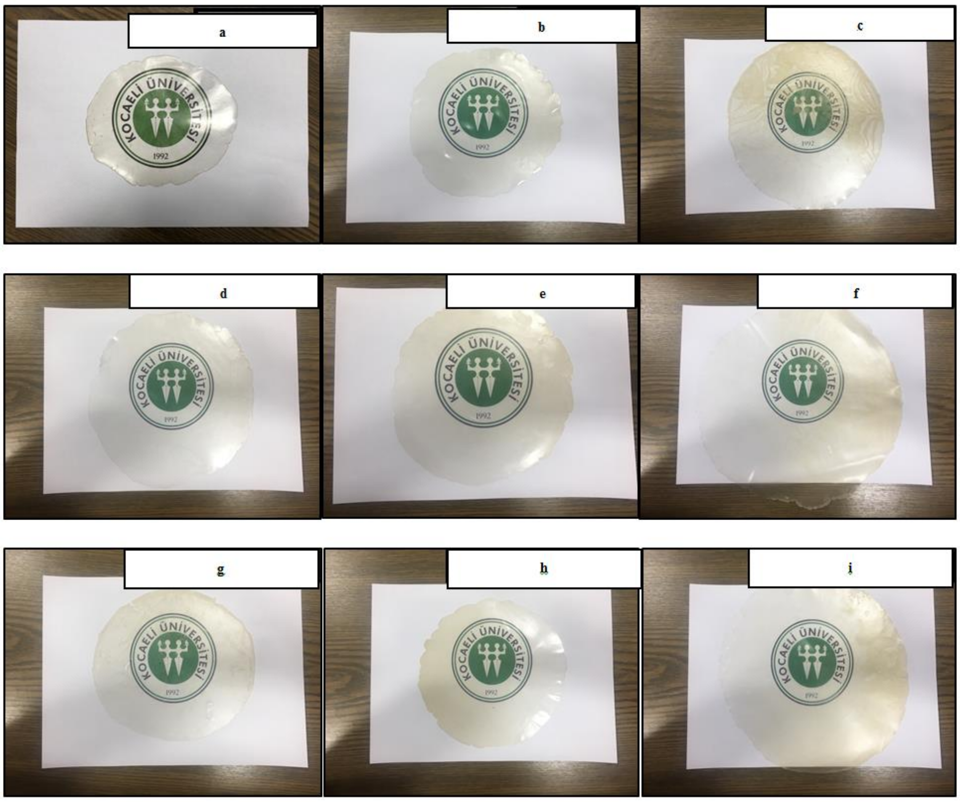

Figure 1. Photos of composite edible films. a) 0% G, Co-pl free b) 5% G, Co-pl free c) 20% G, Co-pl free d) 0% G, TBC e) 5% G, TBC f) 20% G, TBC g) 0% G, TIDC h) 5% G, TIDC i) 20% G, TIDC.

When the color values of composite edible films were compared, it was observed that gelatin and/or co-plasticizer addition to the formulation decreased the ΔE values (Table 3). The color of films depends on type, nature and concentration of biopolymers in the film matrix (Ahmad *et al.*, 2015). Lower ΔE values, which mean color parameters closer to the white standard plate in this study, are considered a desirable property for edible films in the literature (Li *et al.*, 2022). Gelatin and/or co-plasticizer addition provided desirable color for the edible films. On the other hand, co-plasticizer type or gelatin concentration did not significantly affect the ΔE values of composite edible films (Table 3).

Table 3. Effect of gelatin concentration and co-plasticizer type on ΔE of composite edible films.

	Co-pl. free	**TBC**	**TIDC**	**Overall gelatin concentration effect**
0% G	16.39±0.32^{Aa}	16.85±0.32^{Aa}	16.33±0.51^{Aa}	16.52±0.21^{A}
5% G	16.27±0.19^{Aa}	12.65±0.33^{Bb}	12.34±0.09^{Bb}	13.75±0.64^{B}
20% G	15.45±0.23^{Aa}	11.26±0.02^{Cb}	11.72±0.20^{Bb}	12.81±0.67^{B}
Overall plasticizer type effect	16.03±0.19^{a}	13.59±0.85^{b}	13.47±0.74^{b}	

$^{A, B, C}$ Mean values with different letters in the same column differ significantly from each other ($p \leq 0.05$)
$^{a, b, c\ d}$ Mean values with different letters in the same row differ significantly from each other ($p \leq 0.05$)
All values were given as mean ± standard error

Water contact angle (WCA)

WCA measurements help to obtain information about hydrophilicity and hydrophobicity of the film surfaces. If the WCA of a surface is lower than 90°, the surface is regarded as hydrophilic (Crick and Parkin, 2010). The WCA measurement results demonstrated that all composite edible films were hydrophilic, but addition of gelatin to the film formulation increased the surface hydrophobicity of the composite edible films (Table 4).

Table 4. Effect of gelatin concentration and co-plasticizer type on WCA (°) of composite edible films.

	Co-pl. free	**TBC**	**TIDC**	**Overall gelatin concentration effect**
0% G	56.0±0.7^{Bb}	64.3±1.9^{Ca}	63.3±2.2^{Bab}	61.2±1.6^{C}
5% G	59.0±1.1^{Bb}	74.0±2.1^{Ba}	70.9±1.6^{Ba}	68.0±2.4^{B}
20% G	69.0±1.0^{Ab}	87.9±1.1^{Aa}	89.4±1.8^{Aa}	82.1±3.3^{A}
Overall plasticizer type effect	61.3±2.0^{b}	75.4±3.5^{a}	74.5±4.0^{a}	

$^{A, B, C}$ Mean values with different letters in the same column differ significantly from each other ($p \leq 0.05$)
$^{a, b, c\ d}$ Mean values with different letters in the same row differ significantly from each other ($p \leq 0.05$)
All values were given as mean ± standard error

Moreover, as gelatin concentration in the film formulation increased WCA of the films increased, resulting in more hydrophobic surfaces, being in line with the earlier reported results (Zhang *et al.*, 2013; Shi *et al.*, 2018). That result was related with the crosslinking of hydrophilic groups in gelatin with hydroxyl groups of corn starch which decreased surface hydrophilicity (Shi *et al.*, 2018). The WCA of edible films formulated with co-plasticizers were found to be higher than that of co-plasticizer-free ones, which can be related to the hydrophobic character of the co-plasticizers. Furthermore, hydrophobic amino acids of gelatin can interact with hydrophobic co-plasticizers and realign towards the air-side interface, contributing to the formation of a more hydrophobic surface (Pulla-Huillca *et al.*, 2021). On the other hand, co-plasticizer type did not significantly affect the WCA values of the composite edible films (Table 4).

Mechanical properties

Gelatin addition to the edible film formulation increased the tensile strength values of the films significantly. Increasing the gelatin concentration from 5% to 20% provided a significant increase in tensile strength values (Table 5). That can be related to the formation of intermolecular hydrogen bonds between hydroxyl groups of starch and amine groups of gelatin resulting in higher tensile strength values (Tongdeesoontorn *et al.*, 2012). On the other hand, as expected, co-plasticizer addition decreased the tensile strength of composite edible films significantly (Table 5), which can be related to the interaction of co-plasticizers with polymer chains. That interaction decreased the intermolecular interactions of polymer chains by increasing the free volume of polymers or by interacting directly with functional groups in their backbone (Donhowe and Fennema, 1993; McHugh and Krochta, 1994; Irissin-Mangata *et al.*, 2001; Silva *et al.*, 2009; Sanyang *et al.*, 2015; Arham *et al.*, 2016). On the other hand, co-plasticizer type did not significantly affect the tensile strength values of edible films (Table 5).

Table 5. Effect of gelatin concentration and co-plasticizer type on tensile strength (MPa) of composite edible films.

	Co-pl. free	**TBC**	**TIDC**	**Overall gelatin concentration effect**
0% G	1.81±0.10^{Ba}	0.44±0.00^{Cb}	0.40±0.02^{Bb}	0.88±0.23^{C}
5% G	2.42±0.09^{Aa}	0.88±0.03^{Bb}	0.97±0.03^{Ab}	1.42±0.25^{B}
20% G	2.58±0.01^{Aa}	1.57±0.07^{Ab}	1.03±0.04^{Ac}	1.73±0.23^{A}
Overall plasticizer type effect	2.27±0.12^{a}	0.96±0.17^{b}	0.80±0.10^{b}	

$^{A, B, C}$ Mean values with different letters in the same column differ significantly from each other ($p \leq 0.05$)
$^{a, b, c\ d}$ Mean values with different letters in the same row differ significantly from each other ($p \leq 0.05$)
All values were given as mean ± standard error

Mechanical test results demonstrated that the tensile strength and elongation at break values of composite films were inversely related (Tables 5 and 6). Gelatin addition significantly decreased the elongation at break values of the films and as gelatin concentration increased elongation at break values of the films decreased (Table 6). The reduction in elongation at break values can be related to the decrease in mobility of polymer matrix with the addition of gelatin to the film formulation (Tongdeesoontorn *et al.*, 2012). The elongation at break values of composite edible films containing co-plasticizer were found to be higher than that of co-plasticizer-free ones (Table 6). Interaction of co-plasticizers with gelatin chain decreased the intermolecular interactions of polymer chains by increasing the free volume of polymers or by interacting directly with functional groups in their backbone

(Donhowe and Fennema, 1993; McHugh and Krochta, 1994; Irissin-Mangata *et al.*, 2001; Silva *et al.*, 2009; Sanyang *et al.*, 2015; Arham *et al.*, 2016).

Table 6. Effect of gelatin concentration and co-plasticizer type on elongation at break (%) values of composite edible films.

	Co-pl. free	**TBC**	**TIDC**	**Overall gelatin concentration effect**
0% G	62.49±3.52^{Ab}	95.33±4.18^{Aa}	98.00±1.15^{Aa}	85.27±5.93^{A}
5% G	52.62±6.57^{Ab}	86.33±0.33^{Aa}	57.67±2.03^{Bb}	65.54±5.61^{B}
20% G	23.50±0.06^{Bc}	38.21±0.41^{Ba}	30.67±1.33^{Cb}	30.79±2.16^{C}
Overall plasticizer type effect	46.20±6.24^{c}	73.29±8.95^{a}	62.11±9.81^{b}	

$^{A, B, C}$ Mean values with different letters in the same column differ significantly from each other ($p \leq 0.05$)
$^{a, b, c\ d}$ Mean values with different letters in the same row differ significantly from each other ($p \leq 0.05$)
All values were given as mean ± standard error

Elongation at break values of TBC added composite edible films, except gelatin-free ones, were found to be higher than that of films formulated with TIDC (Table 6). According to those results, it can be concluded that TBC is more compatible than TIDC for gelatin containing edible films, especially for the ones containing gelatin at low concentration (5%).

FTIR analysis

The changes in FTIR spectrum of composite matrices as band shifting and/or broadening reflects a distinct interaction between the chains of two polymers as hydrogen bonding or dipolar interaction, reflecting the compatibility between the two polymers (Tomasik *et al.*, 1995; Ahmad *et al.*, 2012).

Figures 2a, 2b and 2c show the FTIR spectra of the composite edible films. It is known from literature that bands observed at 1750-1735 cm^{-1} is related to ester carbonyl groups, 1640 cm^{-1} is related to C=O (peptide bond) stretching, 1550 cm^{-1} is related to C-N-H stretching, 1240 cm^{-1} is related to C-N and N-H groups and 3600-3000 cm^{-1} is related to free and bound O-H and N-H groups (Muyonga *et. al.*, 2004; Soares *et. al.*, 2005; Wang *et. al.*, 2017b). Furthermore, it has been stated in literature that the band changed in the amide I region (about 1650 cm^{-1}), corresponds to the formation of covalent bonds between carbonyl group and amino group, resulting in the generation of typical Maillard reaction products (*i.e.*, a Schiff base imine group and enaminol group) (Han *et al.*, 2017).

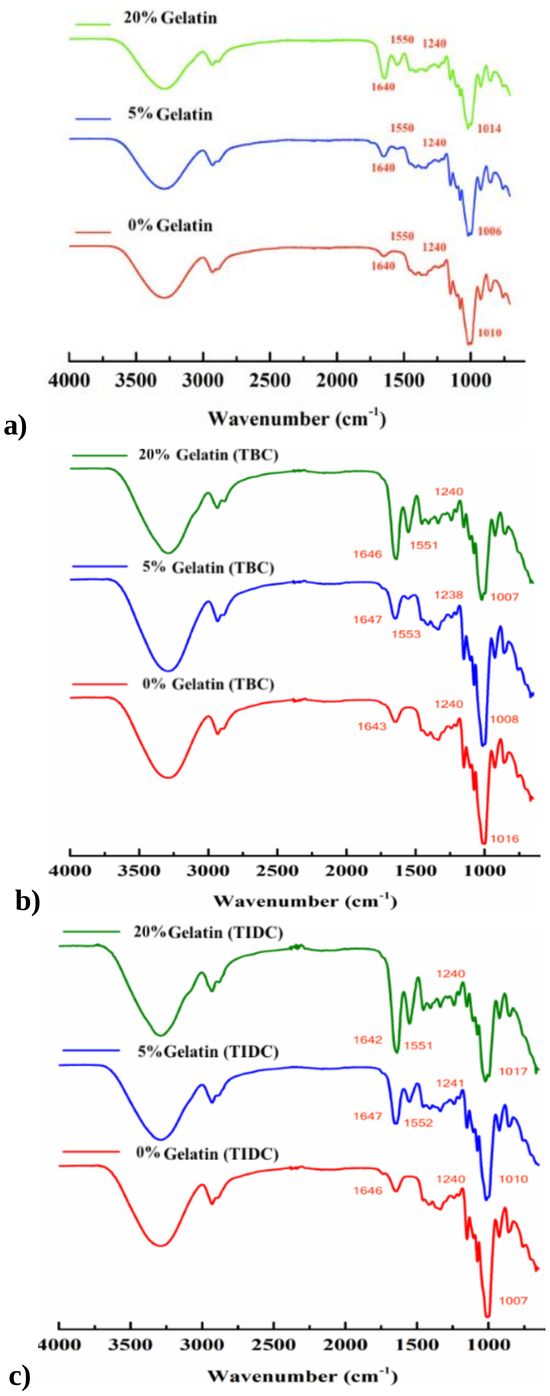

Figure 2. FTIR spectrums of composite edible films. (a) Co-pl. free (b) TBC added (c) TIDC added.

Peaks observed in this study at 3290-3295 cm^{-1} band range corresponded to the stretching vibration of the O-H groups and the intermolecular hydrogen bonding of gelatin. 1750-1735 cm^{-1} band were attributed to the esters (co-plasticizers, TBC and TIDC), peaks observed at 1640 cm$^{−1}$, 1550 cm$^{−1}$ and 1240 cm$^{−1}$ were related to gelatin and starch-gelatin interaction and peaks observed at 1000 cm$^{−1}$ (known as saccharide band) were attributed to the hydrogen bonds between starch chains. As gelatin concentration increased peak intensity at 1640 cm$^{−1}$, 1550 cm$^{−1}$ and 1240 cm$^{−1}$ bands increased and peak intensity at around 1000 cm$^{−1}$ band decreased (Figures 2a, 2b and 2c). That can be associated with the decrease in starch concentration and increase in gelatin concentration in the edible film formulation. The increased intensity at 1640 cm^{-1} bands and the decreased intensity at around 1000 cm$^{−1}$ band through gelatin addition could be related to the Maillard reaction between corn starch and gelatin (Han *et al.*, 2017). Furthermore, the FTIR results are in perfect correspondence with the findings of the previous studies (Wang *et al.*, 2017a; Channa *et al.*, 2022).

Microstructural properties

SEM images of tensile fracture surfaces of edible films are presented in Figures 3.

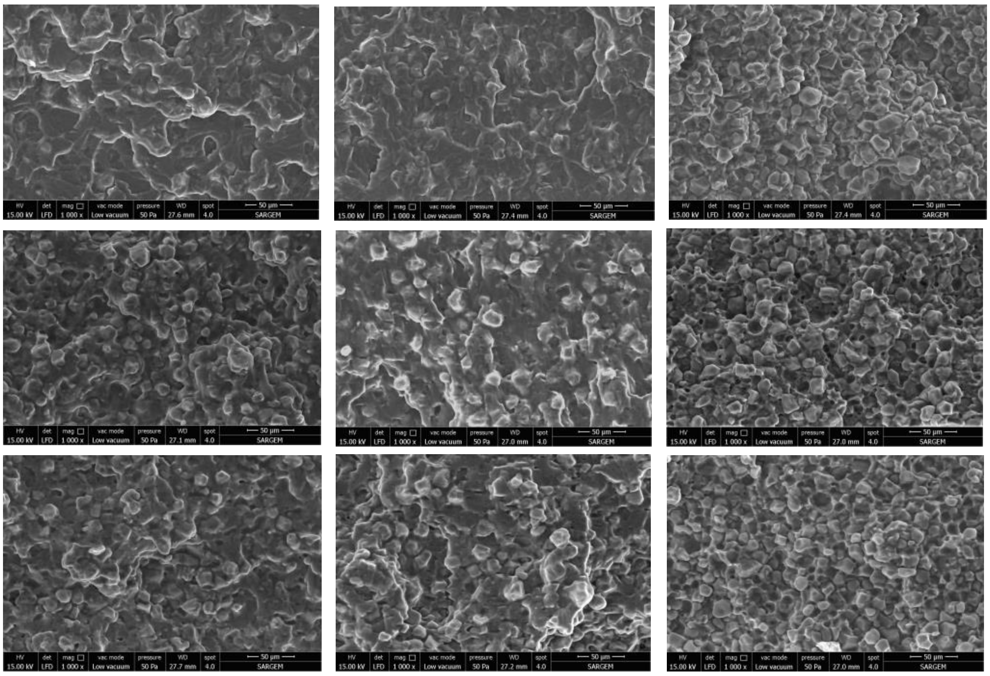

Figure 3. SEM photos of composite edible films; a) 0% G, Co-pl. free b) 5% G, Co-pl. free c) 20% G, Co-pl. free d) 0% G, TBC e) 5% G, TBC f) 20% G, TBC g) 0% G, TIDC h) 5% G, TIDC i) 20% G, TIDC.

Co-plasticizer and gelatin-free (0%G, Co-pl. free) films had more continuous microstructure, indicating the good interaction of corn starch with the main plasticizer, glycerol (Figure 3a). That continuity was lower for co-plasticizer added edible films, because of the hydrophobic nature of co-plasticizers (Figure 3d and 3g). On the other hand, gelatin and/or co-plasticizer addition or increasing the gelatin concentration in the film formulation provided more compact and denser microstructure (3b-3i). The compact and dense structure observed with the addition of gelatin to cassava starch films was previously noted by Tongdeesoontorn *et.al.* (2012). It can be hypothesized that gelatin interacted with corn starch and acted as a filler in the film matrix, providing more ordered structure. The observed microstructures also supported the improvements in tensile strength provided by the addition of gelatin to the corn starch films (Table 5).

Conclusions

Corn starch-gelatin-glycerol-citrate ester composite edible films were successfully produced by a micro compounder and hot press. The addition of gelatin and co-plasticizers to the film formulation improved the tensile strength and elasticity of edible films, respectively. Moreover, gelatin and co-plasticizer added edible films were found to have higher WCA values, providing more hydrophobic surface to the starch-based edible films. FTIR spectra and morphology of the films confirmed the intermolecular interactions among corn starch, gelatin and the co-plasticizers. Data related to physical, mechanical and microstructural properties of films demonstrated that corn starch-gelatin-glycerol-citrate ester composite edible films had potential to be used as edible packaging materials.

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