

Article

Characterization of Caseinate–Carboxymethyl Chitosan-Based Edible Films Formulated with and without Transglutaminase Enzyme

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Abstract: Edible composite packaging has the advantage of complementary functional properties over its individual bio-components. However, film composites made from caseinate (CA) and carboxymethyl chitosan (CMCH) have not yet been well explored. In this study, four types of CA-CMCH composite films were prepared and evaluated with and without transglutaminase (TGase) supplement. Aqueous CA (8%, *w/v*) and CMCH solutions (2%, *w/v*) were mixed in different volume ratios of CA: CMCH as 100:0, 75:25, 50:50, 75:25, and 25:75. Those to be supplemented with TGase were incorporated at 10 U/g of caseinate protein level. Results revealed that CMCH incorporation to CA facilitated a smooth and uniform surface microstructure on films and markedly improved the transparency, water barrier properties, mechanical properties, and solubility of the composite film. Furthermore, addition of TGase resulted in an improvement in the water vapor permeability. TGase successfully enforced the formation of CA-CMCH composites with some enhanced functional properties. The resulting composite film offers potential for applications as an alternative edible film or in the preparation of edible packaging films.



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Keywords: edible films; composite films; chitosan; caseinate; transglutaminase

1. Introduction

Currently there is a growing interest in the formulation of edible films from natural polymers such as proteins, carbohydrates, lipids, and/or appropriate combinations of these components because of the demand for renewable and biodegradable packaging and coating materials for containing and improving the quality/shelf-life of foods. In general, proteins and carbohydrates are widely used to make edible films and packaging materials, which improve food quality and safety by providing selective barrier properties to oxygen, moisture and aromas, and to reduce lipid oxidation by facilitating incorporation of antioxidant and antimicrobial agents [1].

Caseinates form films from aqueous caseinate solutions due to their random coil nature and ability to form extensive intermolecular hydrogen, electrostatic, and hydrophobic bonds, resulting in an increase in the inter-chain cohesion [1]. Caseinates are manufactured by precipitating the casein from milk by lowering the pH to 4.6, either by the addition of dilute mineral acid or by adding a microbial culture. The casein curd is then washed with water, dissolved in an appropriate alkali to increase the pH to about 7.0, and the dispersion is then spray-dried. When NaOH is used to adjust the pH, the product formed will be sodium caseinate which, when dispersed in water, dissociates into casein molecules and some small casein aggregates [2]. When Ca(OH)₂ is used to adjust the pH, calcium caseinate is obtained. Sodium caseinate (NaCAs) and calcium caseinate (CaCAs) are the most common caseinates available commercially. Because of the high affinity of casein to calcium ions, calcium caseinate generally contains casein aggregates of varying sizes [1].

Chitosan (CH) is the second most abundant biopolymer in nature, and been found to possess strong film-forming capabilities coupled with other beneficial properties such as biodegradability, biocompatibility, low oxygen permeability, and desirable mechanical properties [3]. However, its poor water-solubility largely restricts its application. Carboxymethyl chitosan (CMCH) is a water-soluble derivative of chitosan. It is an excellent biomaterial because it is biodegradable, has low toxicity, good biocompatibility, biological activity, and stability in food. The strength of protein networks can be increased by an actively improved cross-linking of proteins. The presence of reactive functional groups in the amino acid side chain of proteins allows cross-linking via chemical reactions with substances such as acids, alkalis or formaldehyde, glutaraldehyde or glyoxal [4]. Recently, chitosan has attracted considerable attraction as an important component of edible films [5].

In addition, some enzymes such as transglutaminase (TGase) can crosslink protein by catalyzing acyl-transfer reactions between “-carboxamide” groups of glutamine residues (acyl donor) and “-amino” groups of lysine residues (acyl acceptor), resulting in the formation of intra- and inter-molecular bonds [6]. Moreover, the reactive lysine in the protein may be substituted by several compounds containing primary amino groups, and TGase can also incorporate small primary amines into protein substrates, giving rise to a variety of protein-(glutamyl) derivatives [7]. TGase has demonstrated potential as a catalyzing agent in the preparation of edible composite films. Di Pierro et al. [8]. employed TGase to prepare chitosan–whey protein edible films with markedly improved mechanical resistance and barrier efficiency toward oxygen and carbon dioxide. Diaz-Montes and Castro-Munoz [9]. and Shahidi and Hossain [10]. provide excellent reviews and current status of edible films and coatings for food preservation purposes.

Chitosan and sodium caseinate composite films have been evaluated in one of the earlier studies [11] using glycerol as plasticizer. Glycerol as plasticizer was shown to increase the elongation by 60%. They found the interaction between sodium caseinate and chitosan to significantly improve the film strength. Volpe et al. [12] evaluated the influence of different concentrations of sodium alginate and chitosan affect the structure and physical properties of the blended films and reported that the blended films demonstrated a marked influence in mechanical properties of the films.

Based on the catalytic characteristics of TGase and the advantage of CMCH with available amino groups as well as its film forming ability, in this presented work, it is proposed to prepare composite films constituted by CA and CMCH with and without TGase treatment. The composite films were made using CA film as a control, and CMCH and TGase as additional inclusion variables to evaluate their influence on the functional properties of resulting composite films, with the objective of finding out the more suitable composite films for packaging/coating applications. No such work has been reported in the literature.

2. Materials and Methods

2.1. Materials

Carboxymethyl chitosan (CMCH) (DAC degree $\leq 85\%$) was obtained from Nutrakey Industries, Inc. (Qingdao, China), glycerol, anhydrous calcium chloride, acetic acid, and sodium chloride were obtained from Sigma-Aldrich (Canada). Sodium caseinate (NaCa) ($\sim 92.0\%$ protein) and calcium caseinate (CaCa) ($\sim 92.6\%$ protein) were provided by CALDIC Canada Inc., Mississauga, ON, Canada).

2.2. Edible Film Formulations

2.2.1. Preparation of Films

Carboxymethyl chitosan (CMCH) edible film was prepared at room temperature by dispersing $2\% w/w$ CMCH in water. The dispersion was stirred at 300 rpm for 4 h to dissolve the CMCH. After dissolution, the CMCH solution was kept in a refrigerator ($4\text{ }^{\circ}\text{C}$) overnight. Caseinate edible films were prepared by dispersing in distilled water $8\% w/w$ of sodium caseinate or calcium caseinate. Caseinate dispersions were heated at $80 \pm 2\text{ }^{\circ}\text{C}$ for

30 min in order to solubilize them, and then the solutions were cooled to room temperature. Carboxymethyl chitosan and caseinates solutions were filtered through a mesh 600 μm . Caseinates solutions (NaCA or CaCA) and carboxymethyl chitosan (CMCH) were mixed in different proportions: 100:0, 75:25, 50:50, and 25:75 w/w CA:CMCH. Glycerol at 5% level was added to all mixtures and mixed for 15 min, and then the enzyme transglutaminase (10 U g^{-1} of caseinates protein) was added to all prepared solutions (marked as TGase sample) by stirring at room temperature for 4 h. Control film solutions were prepared likewise but in the absence of TGase. For setting the film, 14 mL prepared solutions were poured into polystyrene Petri dishes and air dried at $45 \text{ }^\circ\text{C}$ for 8 h. All films were stored for 48 h at $25 \text{ }^\circ\text{C}$ and 50% RH for equilibration before analysis. Test samples were designated as follows: Caseinate films in general (NaCA or CaCA): combined with chitosan: CMCH:CaCA for carboxymethyl chitosan-calcium caseinate and CMCH:NaCA carboxymethyl chitosan-sodium caseinate solutions, respectively.

2.2.2. Thickness Measurement

The thickness of the film sample was measured using a digital micrometer (Mitutoyo Manufacturing, Tokyo, Japan) with a sensitivity of 0.0001 mm. Thickness measurements were made at least at ten random positions on each film. The averages were used for mechanical properties and water vapor permeability.

2.2.3. Film Solubility in Water

Film solubility (FS) was measured as described by Saberi et al. with some modification [13]. The film strips ($1.5 \text{ cm} \times 4 \text{ cm}$), were dried at $105 \text{ }^\circ\text{C}$ for 24 h weighed (w_i), and then immersed in 50 mL distilled water in a beaker. The beakers were covered to prevent the evaporation of water, and stirred at room temperature for 24 h. The residual film strips were dried at $105 \text{ }^\circ\text{C}$ for 24 h to a constant weight to determine the final dry matter (w_f). The test were done in triplicate. Film solubility (FS) was calculated according to the following equation:

$$\% \text{ FS} = \frac{w_i - w_f}{w_i} \times 100 \quad (1)$$

where: w_i was the initial weight and w_f was the weight of the undissolved film residue.

2.2.4. Moisture Content

Moisture content (MC) of the film samples were determined by weighing the initial sample (W_1) and then drying it at $105 \text{ }^\circ\text{C}$ for 24 h, and weighing again (W_2). MC of the samples were calculated by using the following equation:

$$\% \text{ MC} = \frac{W_1 - W_2}{W_1} \times 100 \quad (2)$$

Measurements of moisture content were made with three specimens of each film, and the average was taken as the moisture content [14].

2.2.5. Color

The color characteristics of the film surfaces were assessed with a colorimeter (Minolta Crop., Ramsey, NJ, USA) using the CIELAB color parameter L^* , a^* , and b^* . The total color difference (ΔE) and white index (WI) were calculated according to the following Equations (3) and (4), respectively:

$$\Delta E = [(L - L_1)^2 + (a - a_1)^2 + (b - b_1)^2]^{1/2} \quad (3)$$

$$\text{WI} = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2} \quad (4)$$

where L_1 , a_1 , and b_1 are the color parameters obtained from the white plate and L , a , and b are the color parameters measured from the film samples [15,16]. The film samples were placed on a white standard plate ($L_1 = 96.58$, $a_1 = -0.21$ and $b_1 = -0.28$).

Four measurements were taken on each film, one at the center and three around the perimeter, and the values were averaged.

2.2.6. Opacity Index

The opacity index of the films was calculated by dividing the value of absorbance at 600 nm by film thickness as described by Wang et al. [17]:

$$OP = \frac{Abs_{-600}}{d} \quad (5)$$

where OP is the opacity, Abs_{-600} the value of absorbance at 600 nm, and d is the film thickness.

2.2.7. Water Vapor Permeability (WVP)

A gravimetric method was used to measure the WVP of the film samples as detailed in [18]. Glass cups were $\frac{1}{4}$ filled with anhydrous calcium chloride to generate relative humidity of ~0% RH. The cups were covered with circular film samples, and stored in desiccator filled with saturated sodium chloride solution (75% RH). Covered cups were periodically weighted (0, 1, 2, 4, 6, 9, and 12 h) at ambient temperature. Linear regression analysis was used to calculate the slope of weight loss versus time. The WVP of film samples was measured from the weight gain of the cups using the following equation:

$$WVP \left(g \ m^{-1} \ s^{-1} \ Pa^{-1} \right) = WVTR \ (L/\Delta P) \quad (6)$$

where WVTR is the water vapor transmission rate ($gm^{-2} h^{-1}$) which is calculated from the slope of the regression curve divided by the area (m^2), L is the film thickness (m), and ΔP is the water vapor pressure differential between the two sides of the film (Pa).

2.2.8. Mechanical Properties (TS, EAB)

The mechanical properties of the film samples such as tensile strength (TS) and elongation at break (EAB) were tested according to the ASTM standard method 638–10 with some modification using an Instron Universal Testing Machine (Model 4500, Instron Corporation, Canton, MA, USA).

The films were cut into pieces (30×10 mm) and the initial distance between grips was adjusted to 50 mm and the crosshead speed for stretching was set at 30 mm/s. Tests were performed at room temperature and a relative humidity of $50 \pm 5\%$ RH. The maximum load and the final extension at break were used for calculation of tensile strength TS (MPa) and elongation at break EAB (%). Five replicates were used for each films:

$$TS = \frac{F}{L \cdot X} \quad (7)$$

2.2.9. Scanning Electron Microscopy (SEM)

The morphology surfaces and cross sections of the films were examined using a scanning electron microscope (TM3000, Hitachi Tabletop Microscope). The film samples ($2 \text{ mm} \times 2 \text{ mm}$) were mounted on aluminum stubs using double sided carbon adhesive tapes and photographed at $600\times$ magnifications for the surface and $1200\times$ magnifications for cross-section at room temperature. The films were observed at an accelerating voltage of 5 kV, with the electron beam directed to the surface at 90° angle and a secondary electron imaging detector.

2.2.10. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectrum of test film samples was obtained using a FTIR spectrometer (Agilent 5500a; Mississauga, ON, USA) over a wavelength range of 400–4000 cm^{-1} equipped with an OMNIC operating system software (Version 7.3, Thermo Electron Corporation, Waltham, MA, USA) and using a resolution of 4 cm^{-1} and 32 scans.

2.2.11. Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range tests ($p < 0.05$) using SPSS (version 23.0) software, (Chicago, IL, USA). The reported results were the average values of three samples ($n = 3$).

3. Results and Discussion

3.1. Film Thickness

Carboxymethyl chitosan-caseinate films were prepared at different combination ratios at natural pH, and their physicochemical properties evaluated are summarized in Table 1. Glycerol was used as a plasticizer at 5% level in order to reduce polymer chain interactions and provide the film more flexibility. Only homogenous films without phase separation and without insoluble particles, and those easy to remove from casting materials were used in the analysis. Films not presenting these characteristics were discarded. Film formation showed good appearance, homogeneity, and easy to remove and handle capability at room temperature and RH about 55–60% at different proportions 100:0, 75:25, 50:50, and 25:75 CA:CMCH (both with Na and Ca) are shown in Table 1. The thickness of individual films made from CMCH-100, NaCA-100, and CaCA-100 were 0.123, 0.167, and 0.162 mm, respectively, thus the sodium and calcium alginate films showed higher thickness than CMCH-100 (0.123 mm). Combining alginates with chitosan resulted in progressive lowering of the film thickness. For example, with NaCA, it decreased from 0.167 mm to 0.163, 0.155, and 0.130 mm at 25, 50, and 75% substitutions, respectively, reaching the 0.130 mm value with 100% CHCH. Similar behavior was also observed with CaCA. Thus, chitosan had a significant lowering effect on thicknesses of films made from sodium and calcium caseinates.

Table 1. Nomenclature and physicochemical properties of edible films.

Sample	Thickness (mm)	Water Solubility (%)	Moisture Content (%)
CMCH 100	0.123 ± 0.09	55.93 ± 0.33	22.58 ± 0.19
NaCA 100	0.167 ± 0.07	39.04 ± 0.40	38.50 ± 0.40
NaCA 75	0.163 ± 0.08	33.84 ± 0.51	24.19 ± 0.26
NaCA 75-TGase	0.151 ± 0.01	40.17 ± 0.57	66.48 ± 0.40
NaCA 50	0.155 ± 0.21	37.60 ± 2.00	21.32 ± 0.27
NaCA 50-TGase	0.165 ± 0.11	29.98 ± 0.06	58.88 ± 0.67
NaCA 25	0.130 ± 0.07	31.88 ± 1.44	17.69 ± 0.16
NaCA 25-TGase	0.148 ± 0.34	30.00 ± 1.79	48.25 ± 0.68
CaCA 100	0.162 ± 0.01	36.48 ± 0.35	33.37 ± 0.23
CaCA 75	0.168 ± 0.05	33.39 ± 0.31	22.98 ± 0.25
CaCA 75-TGase	0.159 ± 0.02	44.48 ± 0.37	71.06 ± 0.71
CaCA 50	0.150 ± 0.01	30.34 ± 0.14	17.07 ± 0.80
CaCA 50-TGase	0.143 ± 0.08	30.79 ± 0.68	64.92 ± 0.86
CaCA 25	0.138 ± 0.01	41.89 ± 1.32	18.12 ± 0.20
CaCA 25-TGase	0.139 ± 0.62	31.82 ± 1.51	50.24 ± 0.17

CHCH = carboxymethyl chitosan; NaCA = sodium caseinate, CaCA = calcium caseinate, 100, 75, 50, and 25 represent the proportion of the individual component, the balance out of 100 being CMCH.

Comparing the cases of test samples with and without TGase, the addition of TGase in edible films resulted in a significant overall difference ($p < 0.05$) in film thickness as compared to films without TGase for CaCA films while they showed mixed results with NaCA. The overall range of thickness of the films with and without TGase failed to show

a real difference varying between 0.138 and 0.168 mm while those with TGase varied between 0.138 and 0.165 (Table 1). When each pair of data with and without TGase for different ratios of composites was analyzed, some differences become more apparent. For example, with NaCA, the addition of TGase appeared to generally increase the film thickness except with NaCA 75, while for CaCA, the TGase addition resulted in a decrease in the resulting thickness.

Film thickness is an important parameter from both quality, performance, and cost point of view. Together with adhesive properties, it represents a critical parameter determining the permeability characteristics and therefore the influence on shelf-life. It also represents a measure of easiness of coating application. If the thickness is too low, the coating may fail to deliver the intended results. The mechanical properties of the film are also dependent on the film thickness. Generally, a limit of 0.3 mm is considered for edible films and coatings [19]. All films in the present study had thicknesses within this maximum limit.

3.2. Water Solubility

Polysaccharides and proteins are hydrophilic, making them sensitive to moisture loss or moisture absorption [20]. Low water solubility property is important in enhancing the overall integrity of the film in different relative humidity environments [21]. However, certain applications require high solubility of the film in water such as dissolvable pouches for a pre-weighed single size serving of instant powders of coffee, tea, soups etc., [22].

CMCH-100 possessed the highest solubility of ~60% which reduced to between 30 and 42% for other samples. Relative to CMCH-100, NaCA 100 and CaCA 100 had lower solubility of 37–39%. Addition of NaCA and CaCA to CMCH at different levels further reduced the solubility to between 31 and 33%. TGase added to NaCA and CaCA resulted in the lowest solubility around 30–31%. So, the solubility depended on the proportion of different components in the formulation.

3.3. Moisture Content

The moisture content (MC) of edible films is an important parameter to characterize, since it works as a plasticizer in a polymeric matrix [23]. The moisture content of edible film samples in presence and absence of TGase are also shown in Table 1. Films with TGase had a higher moisture content. Calcium caseinate at 75% and 50% showed higher moisture content of 71 and 65% compared with sodium caseinate at the same ratio of 67 and 59%, respectively. It is important to note the protein–carbohydrate interaction in the film which can lead to lower availability of hydroxyl and amino groups that can interact with water. As shown in Table 1, a high content of CMCH as control with 56% water solubility, would result in high hydrophilicity which makes the films easier to dissolve in water. In comparison, the caseinates caused a decrease in the water solubility of the composite film, especially with CMCH incorporation of about 50% (*w/w*). Furthermore, the solubility of CA-CMCH films (75:25, and 50:50) was further decreased significantly due to TGase treatment. This was consistent with the results of Di Pierro et al. [7], who also found the chitosan–whey protein films prepared in the presence of TGase achieved low solubility levels across a wide range of pH. At 50:50 ratio with and without TGase treatment showed lower solubility based on the dry base of the film samples. TGase induces covalent cross linking creating new larger molecule polymers, leading to impaired solubility [21].

3.4. Color

Film color is an important factor in the products' acceptability by consumers. Table 2 shows the values of color coordinates, lightness (L^*), a^* , b^* , total difference (ΔE), and whiteness index (WI) of different films. In CMCH composite films, the whiteness index significantly increased with TGase treatment. However, no significant ($p < 0.05$) difference in values for L were observed in films treated with TGase. Overall, color of films treated with TGase was lighter. This is correlated with visual results.

Table 2. L-a-b color parameters, total color difference, and whiteness index of edible films.

Sample	L*	a*	b*	ΔE	WI	Opacity
CMCH 100	90.3 ± 0.67	−0.72 ± 0.01	6.59 ± 0.10	9.35 ± 0.48	88.2 ± 0.62	0.58 ± 0.84
NaCA 100	96.0 ± 0.62	−0.45 ± 0.16	2.18 ± 0.21	2.60 ± 0.17	95.4 ± 0.47	0.22 ± 0.17
NaCA 75	90.4 ± 0.48	−0.94 ± 0.04	5.49 ± 0.66	8.49 ± 0.08	88.9 ± 0.10	0.82 ± 0.21
NaCA75-Gase	90.6 ± 0.06	−1.01 ± 0.04	4.56 ± 0.02	7.68 ± 0.05	89.6 ± 0.05	0.48 ± 0.02
NaCA 50	91.2 ± 0.31	−1.14 ± 0.41	4.75 ± 0.58	7.45 ± 0.72	89.9 ± 0.63	0.62 ± 0.12
NaCA50-Gase	91.0 ± 0.88	−0.77 ± 0.13	2.92 ± 0.25	6.43 ± 0.83	90.5 ± 0.88	0.39 ± 0.32
NaCA 25	91.1 ± 0.76	−1.09 ± 0.12	4.94 ± 0.86	7.69 ± 0.39	89.7 ± 0.43	0.54 ± 0.11
NaCA25-Gase	92.0 ± 0.35	−0.65 ± 0.02	2.21 ± 0.30	5.27 ± 0.16	91.6 ± 0.25	0.84 ± 0.26
CaCA 100	96.2 ± 0.70	−0.81 ± 0.4	2.79 ± 0.25	3.11 ± 0.18	95.6 ± 0.65	0.83 ± 0.04
CaCA 75	90.7 ± 0.31	−1.45 ± 0.13	4.93 ± 0.53	8.24 ± 0.48	89.1 ± 0.43	0.23 ± 0.05
CaCA75-Gase	89.9 ± 0.24	−1.30 ± 0.02	4.76 ± 0.32	8.44 ± 0.16	88.8 ± 0.16	0.54 ± 0.02
CaCA 50	91.8 ± 0.20	−0.95 ± 0.05	3.44 ± 0.09	6.09 ± 0.10	91.1 ± 0.15	0.37 ± 0.03
CaCA50-Gase	90.2 ± 0.62	−0.99 ± 0.06	3.85 ± 0.25	7.58 ± 0.31	89.5 ± 0.36	0.58 ± 0.03
CaCA 25	87.8 ± 0.14	−1.40 ± 0.04	7.63 ± 0.07	11.9 ± 0.82	85.5 ± 0.66	0.45 ± 0.07
CaCA25-Gase	90.4 ± 0.79	−0.75 ± 0.09	2.41 ± 0.25	6.74 ± 0.63	90.1 ± 0.71	0.68 ± 0.04

CHCH = carboxymethyl chitosan; NaCA = sodium caseinate, CaCA = calcium caseinate, 100, 75, 50, and 25 represent the proportion of the individual component, the balance out of 100 being CMCH.

Overall range of L value was very narrow, 88–96. NaCA 100 and CaCA 100 had the highest L value of ~96. Adding these to CMCH slightly reduced their L values. The changes in “a value” were very minor between −0.6 and −1.5 and similarly the “b values” varied between 2.2 and 7.6. Combining these as a total color difference magnified the differences a bit more ranging from 2.6 to 11.9, but there was no specific trend. The whiteness index varied from 85 to 96 somewhat similar to what was observed with L value. ΔEL , the color difference, indicates the degree of total color deviation from a standard color plate, and WI, the whiteness index, indicates degree of whiteness. Films treated with TGase revealed ΔEL values that were significantly ($p < 0.05$) higher than that in control caseinates.

The main variations were observed with opacity which varied between 0.22 and 0.84. The least opaque samples were NaCA 100 (0.22), while CaCA 100 showed the highest opacity (0.83), and CMCH sample was half way in between at 0.58. Mixing these components and adding TGase showed mixed results.

3.5. Mechanical Properties

The mechanical properties of materials are largely associated with the distribution and density of intermolecular and intramolecular interactions in the network that produces the protein film [24]. When most inter protein interactions are of low energy, the films are easily stretched. The elongation properties (tensile strength) of the prepared films are compared in Figure 1 with and without the addition of TGase. The elongation of CA-CMCH films decreased by 9.8% while it increased by 17.6% for TGase-treated composite film at a ratio of 75:25 for sodium caseinate (NaCA) and calcium caseinate (CaCA). Highest elongation was observed with CaCA 75. As the volume ratio of CMCH increased from 25% to 75%, the tensile strength steadily decreased with NaCA, and addition of TGase decreased it further. With CaCA however, there was an increase in tensile strength at CaCA 50, and TGase in this case steadily decreased the tensile strength when CMCH concentration increased. These results indicate that CMC incorporation can be beneficial for the stretching ability of the composite films, especially with CaCA. Daniels. [25]. pointed out that cross-linking can increase film elongation because the structure of the overall material goes from that of individual chains associated with many weak van der Waals forces to chains bound by a few, but strong, covalent bonding sites. On the other hand, each cross-linkage site can act as a rigid node and can tend to restrict the motion of the chain segments adjacent to it. Tensile strength (TS) is a very useful parameter for describing the mechanical properties of films. The tensile strength properties of CA, CA-CMCH films with or without TGase

treatment when compared indicated several useful trends especially with CaCA but also showed that with NaCA a reverse trend could exist.

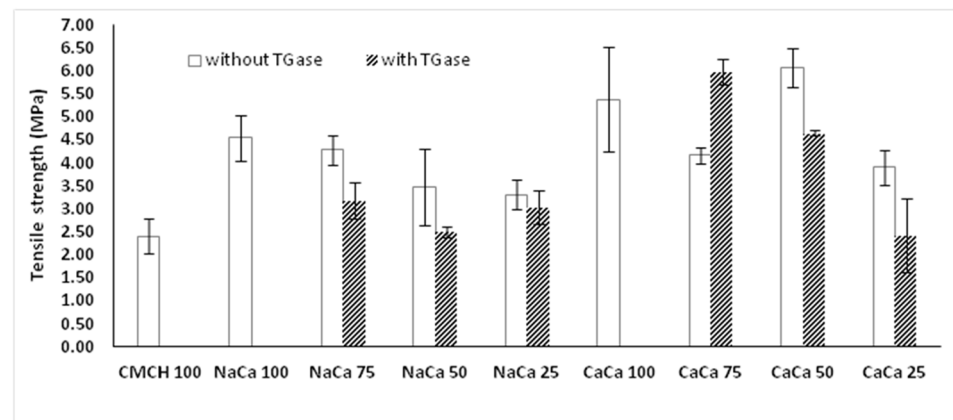


Figure 1. Tensile strength of CA-CMCH composite films [CHCH = carboxymethyl chitosan; NaCA = sodium caseinate, CaCA = calcium caseinate, 100, 75, 50, and 25 represent the proportion of the individual component, the balance out of 100 being CMCH].

Figure 2 compares the elongation at break (EAB) for the different films obtained from CA-CMCH formulations. NaCA 100 and CaCA 100 had EAB values higher than for CMCH 100. While addition of CMCH to NaCA had the best influence at NaCA 50, with CaCA, the best results were obtained with CaCA 75. TGase slightly enhanced the EAB with NaCA 25, NaCA 75, and CaCA 75, but in other situations, it had a negative influence. Higher elongation properties are desirable since the film can be stretched to a higher level before breakage can occur. In general, the CMCH addition helped to extend the elongation properties of both NaCA and CaCA films. The morphology of films determines the corresponding physicochemical properties to some extent. This will be described in the next section.

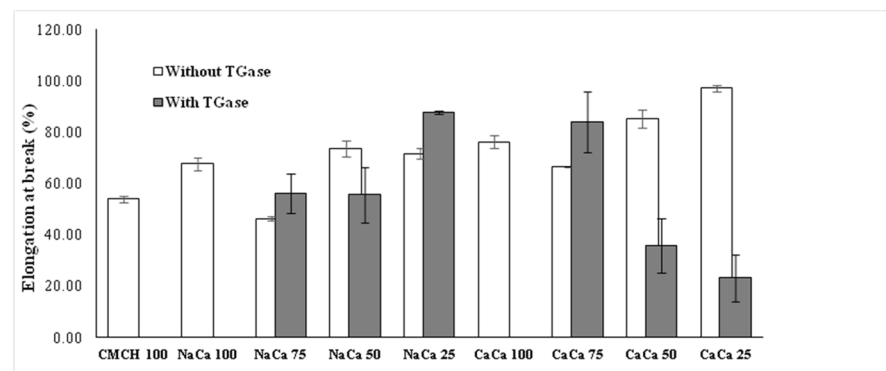


Figure 2. Elongation at break of CA-CMCH composite films [CHCH = carboxymethyl chitosan; NaCA = sodium caseinate, CaCA = calcium caseinate, 100, 75, 50, and 25 represent the proportion of the individual component, the balance out of 100 being CMCH].

3.6. SEM

SEM photographs of the surfaces and cross section of caseinates carboxymethyl chitosan composites films are shown in Figure 3a,b. All film surfaces were observed to have a very smooth and homogenous structure containing no crack or holes, which indicates that the mixtures of caseinates, carboxymethyl chitosan, and glycerol can produce homogenous surfaces with no phase separations. SEM micrographs also show that films incorporated with TGase were flatter and had smoother appearances and good compact structures. Jiang et al. [21] also showed that whey protein-carboxymethylated chitosan composites film had a smooth surface which confirmed the high miscibility between the film component.

It was observed that CA films had a rougher surface with protein granules, whereas the CMCH film was observed to be very smooth and even. CA-CMCH composite films gradually took on an increasing smooth surface with CMCH incorporation. The composite films prepared at the volume ratio of CA to CMCH (75:25) had a comparable surface to the CMC film, which suggested high miscibility between WPC and CMC, and there was little phase separation in the two composites in terms of film-forming solution. These results can also be verified by the visual images of the different films as shown in Figure 4.

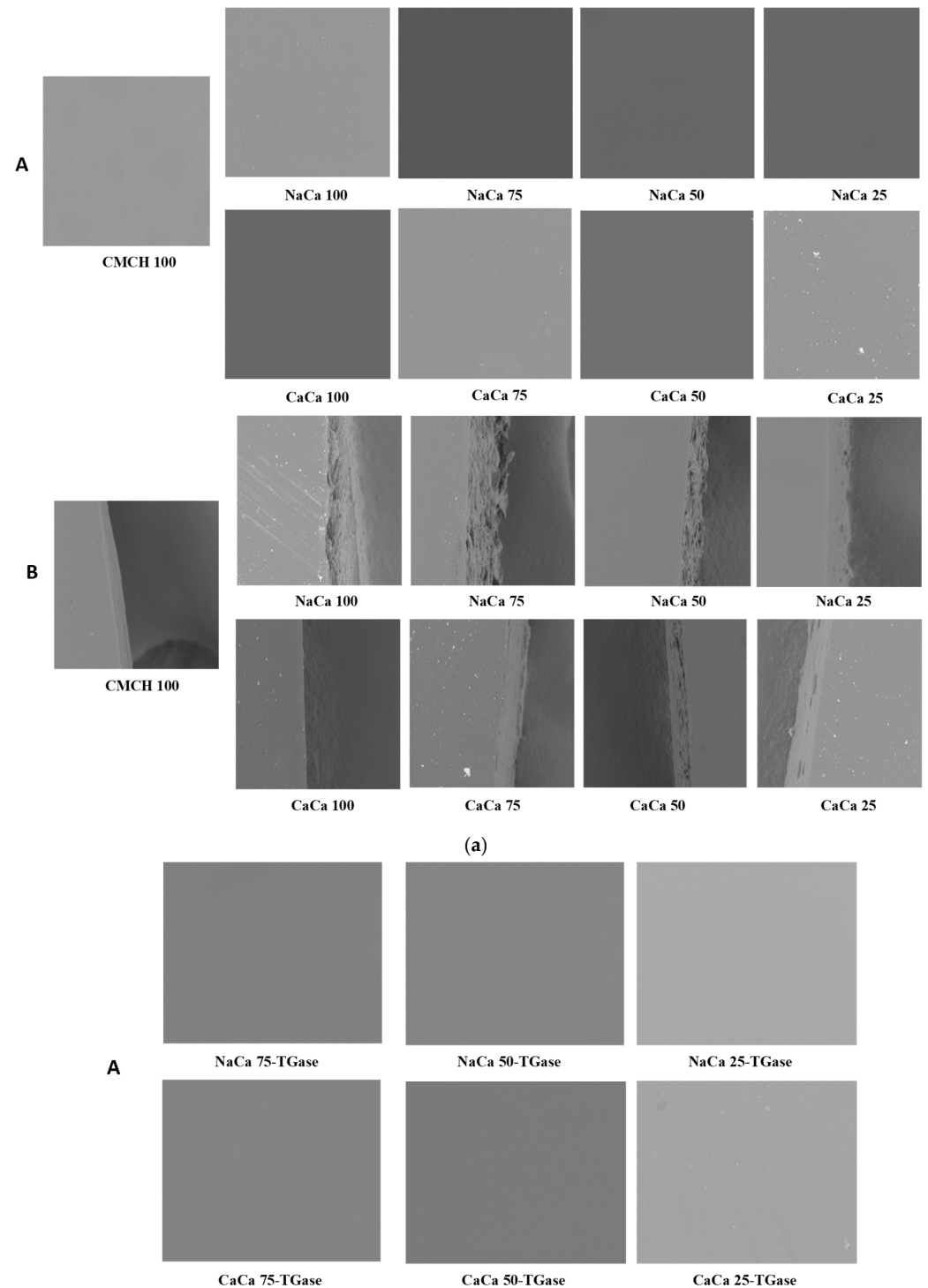


Figure 3. Cont.

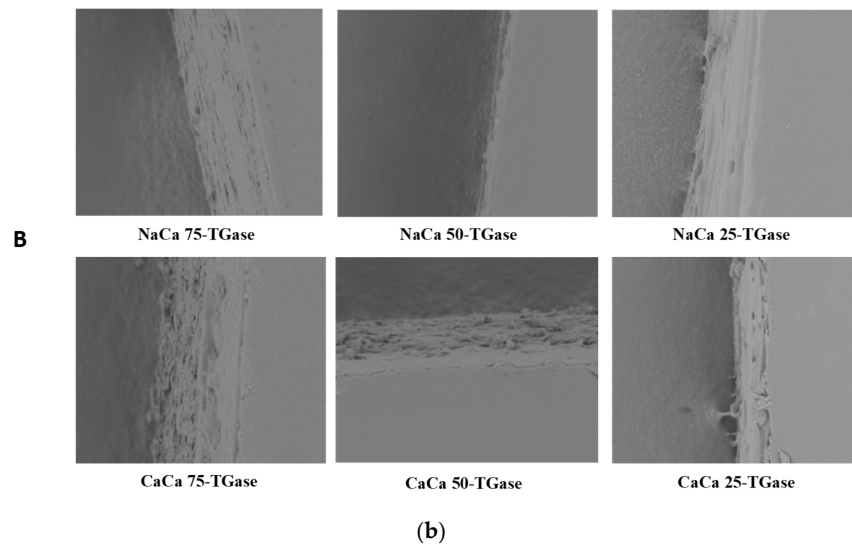


Figure 3. (a) Scanning electron micrographs of (A) surfaces (magnification $\times 600$) and (B) cross-sections (magnification $\times 1200$) of caseinates-carboxymethyl chitosan without transglutaminase [CHCH = carboxymethyl chitosan; NaCA = sodium caseinate, CaCA = calcium caseinate, 100, 75, 50, and 25 represent the proportion of the individual component, the balance out of 100 being CMCH]. (b) Scanning electron micrographs of (A) surfaces (magnification $\times 600$) and (B) cross-sections (magnification $\times 1200$) of caseinates-carboxymethyl chitosan with transglutaminase [CHCH = carboxymethyl chitosan; NaCA = sodium caseinate, CaCA = calcium caseinate, 100, 75, 50, and 25 represent the proportion of the individual component, the balance out of 100 being CMCH].

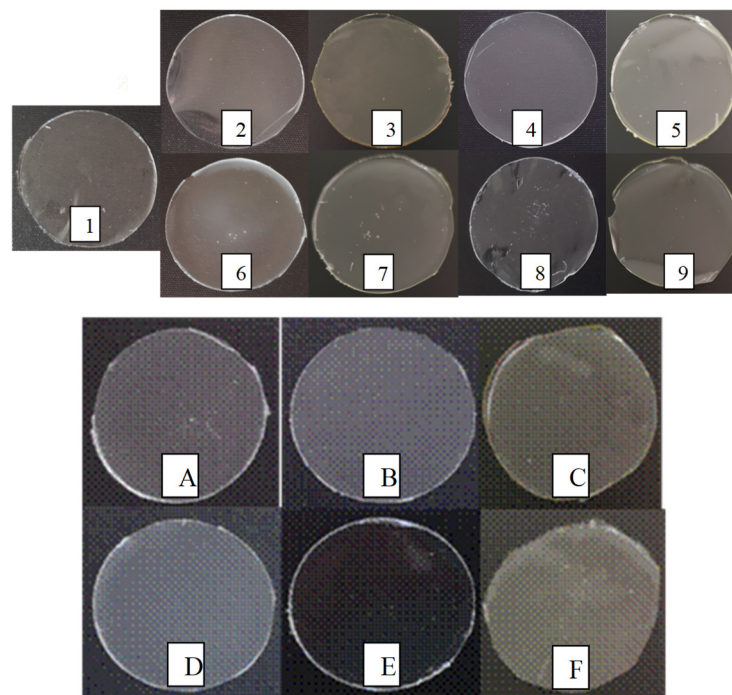


Figure 4. Visual appearance of edible film samples with and without transglutaminase: (1) CMCH 100 (left); (2) NaCA 100, (3) NaCA 75, (4) NaCA 50, (5) NaCA 25, (6); CaCA 100, (7) CaCA 75, (8) CaCA 50, (9) CaCA 25, (A) NaCA 75-TGase, (B) NaCA 50-TGase, (C) NaCA 25-TGase, (D) CaCA 75-TGase, (E) CaCA 50-TGase, (F) CaCA 25-TGase [CHCH = carboxymethyl chitosan; NaCA = sodium caseinate, CaCA = calcium caseinate, 100, 75, 50, and 25 represent the proportion of the individual component, the balance out of 100 being CMCH].

3.7. Water Vapor Permeability

Water vapor permeability (WVP) is one of the most important properties of edible film in food coating and packaging applications. Lower the water permeability of the film, the better ability of edible film to control moisture loss of the packaged food [21]. The water vapor permeability of different films are compared in Figure 5. In general, it can be observed that although by incorporating CMCH 100, the water vapor permeability of both NaCA and CaCA films were generally decreased, however, the WVP values of the composite films remained higher than the CMCH 100 film. With the addition of TGase to the formulation combinations of CMCH 100 and NaCA and CaCA, a large reduction in WVP values was observed, especially with NaCA-based composites. The maximum reduction due to TGase was with NaCA 75 and NaCA 50 which resulted in over 50% reduction in WVP values. Reduction was also evident with CaCA combinations, but the degree of lowering was much smaller (<15%).

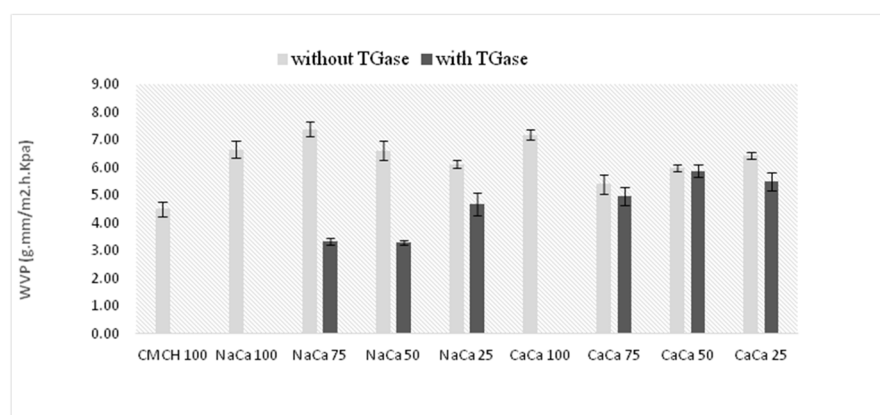


Figure 5. Water vapor permeability of CA-CMCH composite films [CHCH = carboxymethyl chitosan; NaCA = sodium caseinate, CaCA = calcium caseinate, 100, 75, 50, and 25 represent the proportion of the individual component, the balance out of 100 being CMCH].

The WVP lowering could be attributed to the decrease in mobility of the chains in the film because TGase induced the crosslinking or carboxymethyl chitosan. The range of WVP observed was 3.3 to 4.7 $\text{g mm m}^{-2} \text{h}^{-1} \text{kPa}^{-1}$. With NaCA combination while with CaCA, the values were between 4.9 and 5.9 $\text{g mm m}^{-2} \text{h}^{-1} \text{kPa}^{-1}$. Composite films with NaCA with TGase clearly demonstrated advantage of lower WVP.

3.8. FTIR

The interaction between functional groups in caseinates carboxymethyl chitosan composite films with and without TGase was evaluated through their FTIR spectra. As shown in Figure 6a,b, amide I, amide II, and amide III are the main absorption bands in the FTIR region of the spectrum to study the conformation in proteins structural changes, especially when hydrogen bonds are formed between water molecules and/or organic compounds and proteins by NH and OH groups as donors and C = O, C-O, and C-N groups as acceptors leading to the band shifts or changing in the intensity of those chemical groups. In Figure 6a, TGase spectra showed the main bands at 3276.9, 2925.5, 1638.4, 1516.4, and 1237.5 cm^{-1} , corresponding to amides A (NH-stretching), amide B (asymmetric stretching vibration of =C-H and $-\text{NH}^3+$), amide I (C = O stretching), amide II (C-N stretching and N-H bending), and amide III (C-N stretching). FTIR spectra corresponding to the caseinate powder, TGase powder and films with glycerol are shown in Figure 6a,b. Due to the caseinates random coil nature and their ability to form extensive intermolecular hydrogen bonds, their blending with glycerol resulted in a general increase in the macromolecules cohesion [26]. Thus, the broad absorption band observed in the 3600–3000 cm^{-1} range can be attributed to hydrogen bonds formed between SC and glycerol hydroxyl groups as well as to the presence of unbonded N-H groups [11,27,28]. As expected, the intensity of films

with TGase increased since the number of available hydroxyl groups was proportionally higher. Similar comments could be applied to the asymmetric and symmetric stretching vibrations of C–H bonds at 2925 cm^{-1} .

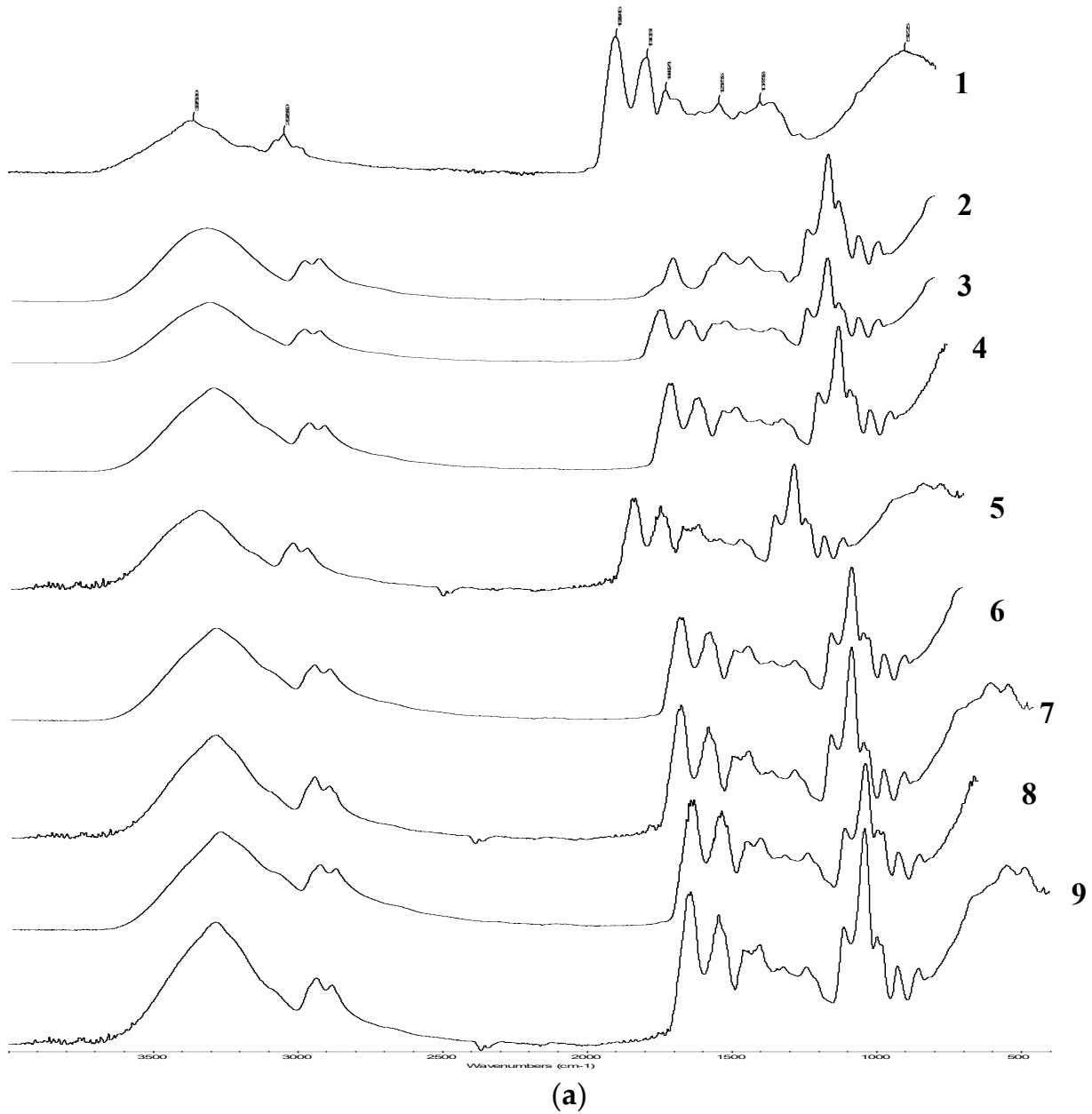


Figure 6. Cont.

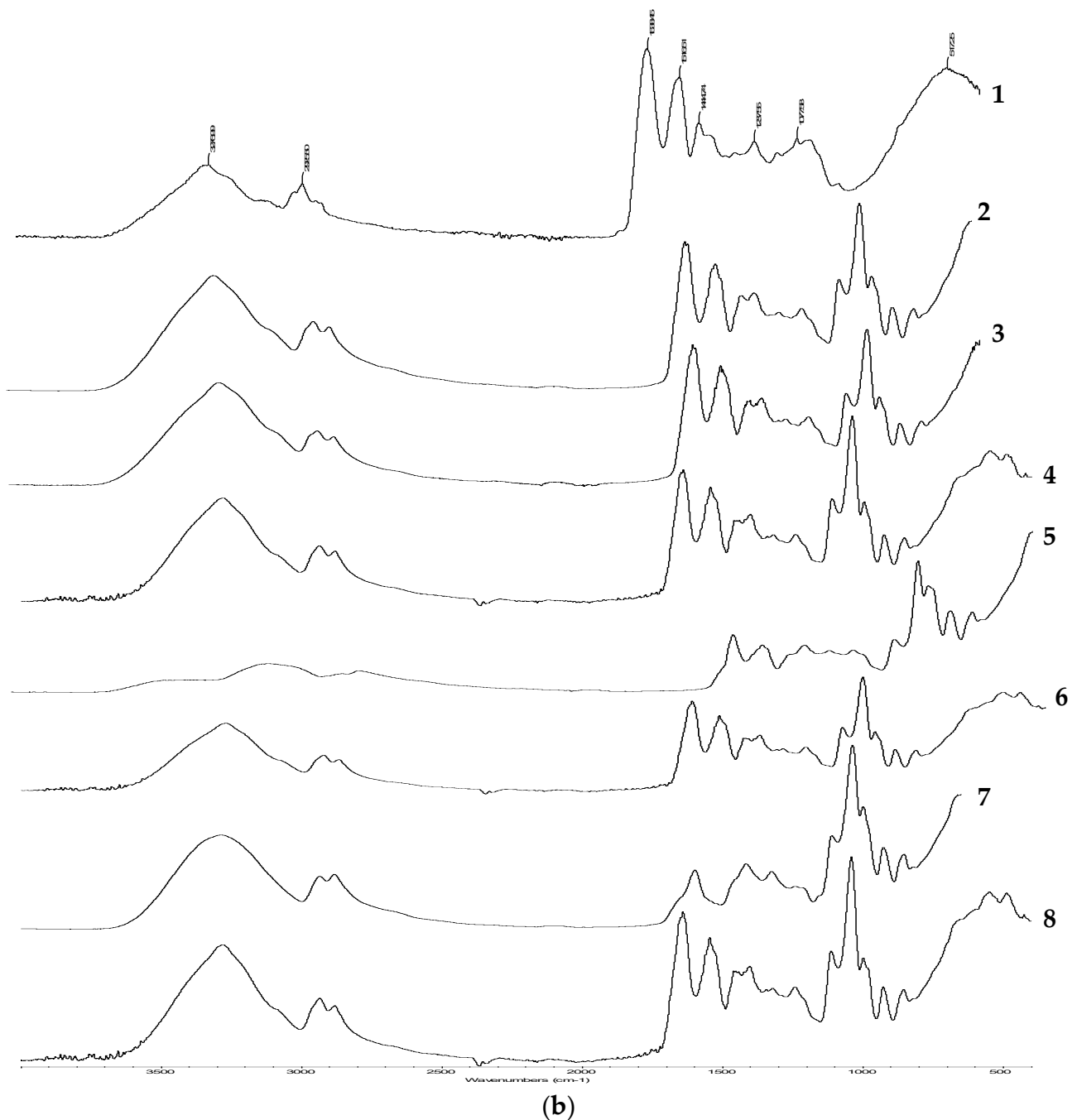


Figure 6. (a) FTIR spectra of edible film samples with and without transglutaminase (1) TGase powder, (2) CMCH 100, (3) NaCA 100, (4) NaCA 75, (5) NaCA 75-TGase, (6) NaCA 50, (7) NaCA 50-TGase, (8), NaCA 25, and (9) NaCA 25-TGase [CHCH = carboxymethyl chitosan; NaCA = sodium caseinate, CaCA = calcium caseinate, 100, 75, 50, and 25 represent the proportion of the individual component, the balance out of 100 being CMCH]. (b) FTIR spectra of edible film samples with and without transglutaminase (1) TGase powder, (2) CaCA 100, (3) CaCA 75, (4) CaCA 75-TGase, (5) CaCA 50, (6) CaCA 50-TGase, (7), CaCA 25, and (8) CaCA 25-TGase [CHCH = carboxymethyl chitosan; NaCA = sodium caseinate, CaCA = calcium caseinate, 100, 75, 50, and 25 represent the proportion of the individual component, the balance out of 100 being CMCH].

4. Conclusions

In this study, several functional properties of composite films formulated with sodium and calcium caseinates (CA) combined with carboxymethyl chitosan (CMCH) some with

and others without TGase treatment. CMCH incorporation markedly influenced the transparency, the water barrier properties, mechanical properties, and solubility of composite film. Furthermore, TGase resulted in an improvement in the water vapor barrier properties and the mechanical properties of selected CA-CMCH composite films (75:25, and 50:50). It can therefore be concluded that CA-CMCH composite films can be seen as alternatives to other films from proteins and carbohydrates.

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