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Edible Films of Whey and Cassava Starch: Physical, Thermal, and Microstructural Characterization

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Received: 8 October 2020; Accepted: 28 October 2020; Published: 2 November 2020

Abstract: The present work aimed to obtain and characterize edible films produced with liquid whey and cassava starch. The films were produced with different proportions of whey (63.75–67.50%) and cassava starch (7.50–11.25%) and characterized in relation to physical, thermal, and microstructural properties. The films showed reduced solubility with increasing concentrations of cassava starch, and those with the highest proportions of whey were more stable to thermal decomposition. The increase in concentration of cassava starch altered the microstructure of the films, making them more irregular and with an accumulation of matter. The production of biodegradable polymer blend films is an important step in the development of films for use in packaging, with the formulation of 67.50/7.50% whey/cassava starch being the best film for continued future work.

Keywords: coproduct; thermal degradation; reuse

1. Introduction

Packaging is an essential tool to ensure containment and preservation of food. Traditionally, these packages are based on polymers derived from oil, such as polypropylene, polyethylene, and polystyrene. Different types of plastics are utilized due to their low cost and easy manufacture. However, serious environmental problems are caused due to the nonbiodegradability of these materials, thus increasing the interest of researchers in biodegradable packaging production using natural polymers extracted from renewable sources for application in food packaging [1–4].

Traditionally, biodegradable films are produced from organic macromolecules, such as polysaccharides, proteins, or lipids. Films formulated from polysaccharides have good barrier properties to O_2 and CO_2; however, their water barrier and mechanical properties are weak. Films formulated from proteins also have low water-related properties but good mechanical properties. Due to their hydrophobic nature, lipids have good moisture barrier properties. However, because they are not biopolymers, they are not capable of being used individually for film production and are instead used as additives in the formulation of composite films. In this sense, a mixture of macromolecules for

the production of biodegradable films seems to be a strategy to improve the properties of these films for application in food [4,5].

Among biopolymers, whey protein and starch are promising biomaterials for the development of biodegradable and edible food packaging. Whey is an important coproduct of the cheese agroindustry and is used to produce whey protein concentrate (WPC), which has 25–80% protein content [6]. The main proteins found in whey are α-lactalbumin and β-lactoglobulin, representing about 50 and 20% of total proteins, respectively. Films and coatings developed with these proteins have excellent gas barrier properties [7,8].

Starch, a polysaccharide naturally present in several plants, has been widely explored for the production of films and coatings for food due to its properties such as transparency, high availability, and low cost [9]. Among starches from different vegetable sources, cassava starch is a good raw material for the production of edible and biodegradable films [10]. However, the high hydrophilicity of hydroxyl groups in the starch matrix results in weak moisture barrier properties [9]. Starch modifications can be made or other biopolymers can be added to improve the water barrier properties [11,12].

A promising alternative strategy is the combination of polysaccharides and proteins to improve the properties of the developed films. The interactions between these two components create a continuous network [13]. Combining hydrocolloid components in composite coatings can mask or reduce the limitations of each macromolecule (polysaccharide, protein, or lipid) [14]. Several researchers have focused on the development of biodegradable and/or edible composite films due to the benefits of combining compounds to improve their properties [13,15,16]. The present study aimed to evaluate the effect of a mixture of cassava starch and whey protein on the physical, thermal, and microstructural properties of edible films.

2. Materials and Methods

2.1. Film Production

The cassava starch was solubilized in liquid whey and homogenized until complete solubilization. Liquid whey contained 0.19 g of lipids, 1.01 g of proteins, 4.32 g of lactose, and 0.65 g of casein, totaling 6.17 g of dry extract per 100 g of fresh matter. Afterward, the plasticizer (glycerol) and acetic acid were added in the proportions described in Table 1. The concentrations of cassava starch and liquid whey (Table 1) were defined according to previous tests so that homogeneous, flexible and without fragility and/or bubbles films were formed. For this, the proportions of whey: cassava starch ranged from 79.4: 30.6 (F1), 77.9: 21.1 (F2), 76.5: 23.5 (F3), and 75:25 (F4).

Table 1. Film formulations with liquid whey, cassava starch, and glycerol plasticizer.

Ingredients (%)	Formulations			
	F1	F2	F3	F4
Liquid whey	67.50	66.25	65.00	63.75
Cassava starch	7.50	8.75	10.00	11.25
Glycerol	5.00	5.00	5.00	5.00
Acetic acid	20.00	20.00	20.00	20.00

The filmogenic solutions were heated at 90 ± 3 °C for 30 min in a water bath for protein denaturation. The solution was homogenized with a mixer (Walita, São Paulo, Brazil) every five minutes until reaching temperature of 85 °C for gelatinization of the cassava starch. Then, 40 mL of film-forming solutions were spread on each Petri dish (90 mm in diameter). The films were dried in a forced-air circulation oven (Solab SL-102, Piracicaba, São Paulo, Brazil) at 42 °C for 60 h. After drying, the films were stored in a desiccator at 25 °C and 51% relative humidity (controlled by the addition of saturated magnesium nitrate solution) until evaluation [1].

2.2. Film Characterization

The film thickness was determined with a portable difetgital micrometer (Mitutoyo Co., Kawasaki-Shi, Japan) to the nearest 0.001 mm. Measurements were carried out at a minimum of seven points of each film.

Water solubility was measured as described by Kavoosi et al. [17] with modifications described by Filho et al. [1]. The initial dry weight of 2 cm^2 square film samples was determined by weighing them before and after drying at 105 ± 1 °C for 24 h. The samples were then immersed in 50 mL distilled water for 24 h at 23 ± 2 °C and dried at 100 ± 5 °C for 24 h, and the final dry weight was measured. The water-soluble content was then calculated as the percentage weight that remained after water immersion. The water solubility of the film was calculated using Equation (1).

$$\text{Water solubility } (\%) = \frac{initial\ weight - final\ weight}{initial\ weight} * 100 \quad (1)$$

The color of the films was determined using a Color Quest II spectrophotometer (Hunter lab, Reston, VA, USA) with the CIELab system as described by Filho et al. [18]. The hue angle (h°), chroma (C*), and total color difference (ΔE*) were calculated according to Equations (2)–(4), respectively.

$$h^0 = tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$C^* = \left((a^*)^2 + (b^*)^2\right)^{1/2} \quad (3)$$

$$\Delta E^* = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (4)$$

The thermal degradation profiles of the films were determined in a TGA Shimadzu, (model DTG 60/60H, Japan) with a heating rate of 10 °C min^{-1} between 25 and 600 °C. The nitrogen rate was 100 mL min^{-1}. The weight loss (%) and the derivative (%/°C) were determined as functions of temperature.

The microstructure of the films was evaluated using an electronic scanning electron microscope (acceleration voltage 2.5 kV, JSM 6610, JEOL, São Paulo, Brazil) equipped with EDS (NSS Spectral Imaging, Thermo Scientific, Waltham, MA, USA) with increments of 1000×.

2.3. Statistical Analysis

Results were calculated as mean ± standard deviation. The experiment was replicated three times with analyses conducted in triplicate. Data were analyzed by one-way analysis of variance (ANOVA), whereas Tukey's test ($p < 0.05$) was used for testing differences between the means using Assistat software version 7.7 (Professor Francisco de A. S. e Silva, Federal University of Campina Grande, Brazil).

3. Results and Discussion

3.1. Physical and Optical Properties

Table 2 presents results of the water solubility of the whey and cassava starch films. The formulations with the highest percentage of whey (F1: 66.7% and F2: 66.25%) showed the highest values of solubility in water ($p > 0.05$). Whey proteins are hydrophilic polymers with hydroxyl groups, so water can easily solubilize the film [19]. On the other hand, replacing whey with cassava starch reduced the water solubility of the films. Azevedo et al. [16] also reported that higher concentrations of whey protein in starch-based films obtained by extrusion result in films that are more hydrophilic but with weaker water-related properties. Similar values of solubility have been described for films of *Salvia hispanica* mucilage and whey protein concentrate (48.30–63.96%) [20].

Table 2. Solubility, thickness, chroma (C), hue (h°), and total color variation (ΔE) of films of whey and cassava starch.

Parameters	Formulations			
	F1	F2	F3	F4
Solubility (%)	53.63 ± 4.64^{ab}	57.06 ± 6.04^{a}	42.95 ± 9.84^{c}	46.43 ± 6.92^{bc}
Thickness (mm)	0.70 ± 0.10^{a}	0.79 ± 0.22^{a}	0.71 ± 0.07^{a}	0.68 ± 0.11^{a}
h°	1.07 ± 0.48^{a}	1.12 ± 0.43^{a}	0.40 ± 0.66^{a}	0.89 ± 0.53^{a}
Chroma	1.86 ± 0.69^{a}	1.98 ± 0.62^{a}	0.98 ± 0.34^{b}	1.16 ± 0.36^{b}
ΔE*	58.38 ± 3.27^{b}	62.32 ± 2.35^{a}	56.20 ± 1.77^{c}	59.02 ± 1.32^{b}

^{a,b,c} Different letters on the line differ from each other according to the Tukey's test ($p < 0.05$). The percentage of whey/cassava starch in the formulations were as follows: F1 = 67.50/7.50%, F2 = 66.25/8.75%, F3 = 65.00/10.00%, and F4 = 63.75/11.25%. All films contained 5% glycerol and 20% acetic acid.

In studies of barrier properties, thickness control becomes a critical variable because the barrier properties of films can be negatively affected by increasing thickness [21]. In the present work, the thickness of the films showed no significant difference and varied from 0.68 to 0.79 mm. Similar behavior has been described for films of corn starch and whey protein obtained using extrusion [16].

The hue angle values define red at 0°, yellow at 90°, green at 180°, and blue at 270°. In the present work, the hue values decreased significantly with the increase in concentration of cassava starch in the films, resulting in films with red coloring. In addition, the C* values decreased with the concentration of cassava starch in the films, indicating that saturation of the red color of the films decreased. F2 and F3 films showed significantly different values for the total color difference (ΔE*) of the films. Similar behavior has been reported in whey protein films and Lotus starch [15].

3.2. Thermal Analysis

The thermogravimetric analysis (TGA) curves and their first derivatives (DTG) are presented in Figure 1 and were determined to assess the thermal stability of the films (Table 3). The weight loss occurred in three main stages. The first stage, from 25 to 200 °C, is attributed to the evaporation of water and molecules with molecular weight. The mixture of polymers did not influence this stage. The second stage, around 200–350 °C, can be attributed to the thermal decomposition of the components present in the films. Protein breakdown starts at around 225 °C [22,23], while the decomposition of starch occurs at 230–326 °C [24,25]. In the third stage, above 350 °C, degradation of carbonaceous residues formed during the second stage occurs, with complete oxidation of these materials [16,23].

Table 3. Temperature and weight loss related to stages of TG/DTG curves of whey and cassava starch films.

Films	First Stage		Second Stage		Third Stage	
	Temperature (°C)	Weight Loss (%)	Temperature (°C)	Weight Loss (%)	Temperature (°C)	Weight Loss (%)
F1	32	99.5 − 90.2 = 9.3	201	74.5 − 54.5 = 20	267	55 − 37 = 18
F2	31	99 − 90 = 9	193	79.2 − 58 = 21.2	264	56 − 32 = 24
F3	31	99 − 89 = 9	183	80 − 69 = 11	263	57.2 − 31 = 26.2
F4	30.5	99.3 − 91.2 = 8.1	160	85.4 − 70.5 = 14.9	260	62 − 32 = 30

The percentage of whey/cassava starch in the formulations were as follows: F1 = 67.50/7.50%, F2 = 66.25/8.75%, F3 = 65.00/10.00%, and F4 = 63.75/11.25%. All films contained 5% glycerol and 20% acetic acid.

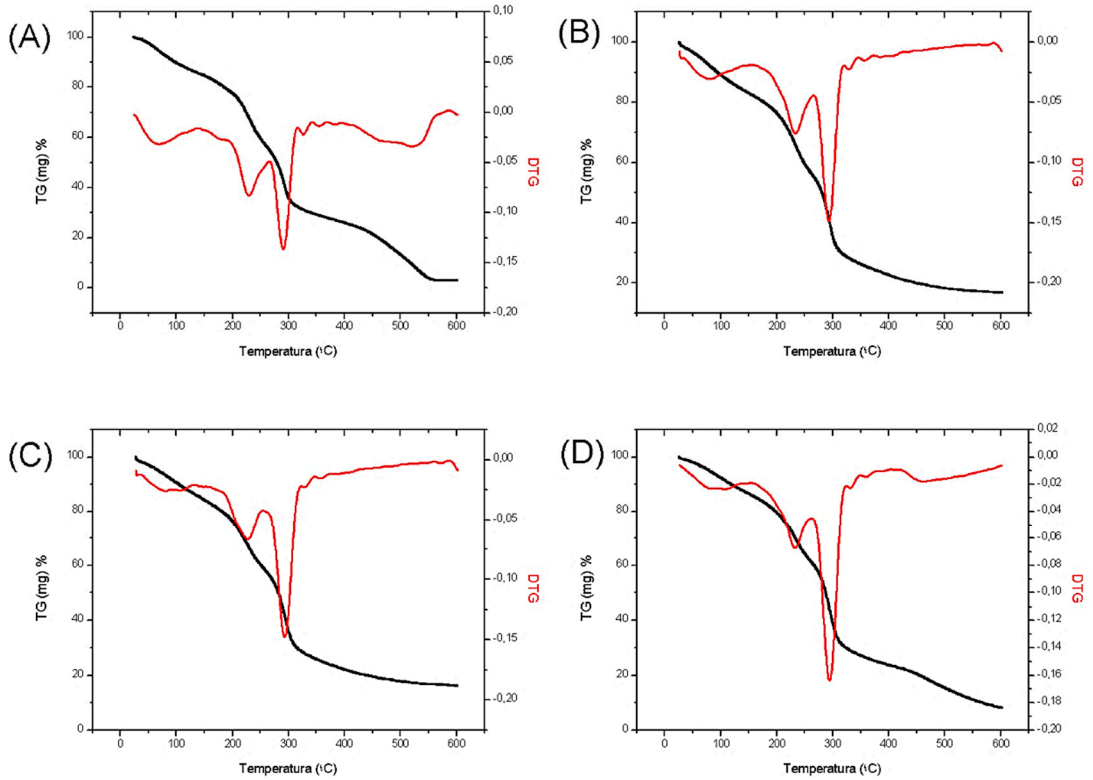

Figure 1. Thermogravimetric analysis (TGA) (black) and derivative thermogravimetry (DTG) (red) curves of films of whey and cassava starch: (**A**) = F1, (**B**) = F2, (**C**) = F3, and (**D**) = F4. F1 = 67.50/7.50 (whey/cassava starch), F2 = 66.25/8.75 (whey/cassava starch) F3 = 65.00/10.00 (whey/cassava starch), and F4 = 63.75/11.25 (whey/cassava starch). All films contained 5% glycerol and 20% of acetic acid.

The increased concentration of cassava starch in the films appeared to reduce the temperature range for the second peak of DTG (Table 2). The peaks with temperature of degradation of 294.9–310.8 °C and 463.8–543.21 °C are attributed to starch and whey degradation, respectively [16]. Both peaks (starch and whey) seemed to have occurred in this work. When cassava starch was replaced by whey, the initial degradation temperature of the films decreased (Table 3), indicating a reduction in the thermal stability of the film. Thus, films with a higher concentration of whey showed higher thermal stability. Similar behavior has been described for whey protein and corn starch films, where it was demonstrated that the thermal stability of the films was directly related to the concentration of whey protein [16].

3.3. Scanning Electron Microscopy (SEM)

Figure 2 shows the SEM micrographs of whey and cassava starch films. F1 presented a smooth surface without surface cracks and with the presence of small clusters. With the increase in cassava starch concentration, the microstructure of the films became more irregular with greater presence of material accumulation (Figure 2B–D). This behavior can be attributed to the interactions between starch and whey. Whey proteins can adhere to the surfaces of starch granules, resulting in a clumping effect [26]. Another hypothesis is that the denaturation of proteins during heating resulted in their aggregation and formation of agglomerates in the films [27]. Similar results have been reported by Huntrakul et al. [28] in cassava starch films with isolated pea protein. The presence of discontinuities and agglomerates in the film matrix can affect its properties [29], as seen in the properties evaluated in this work (Tables 2 and 3).

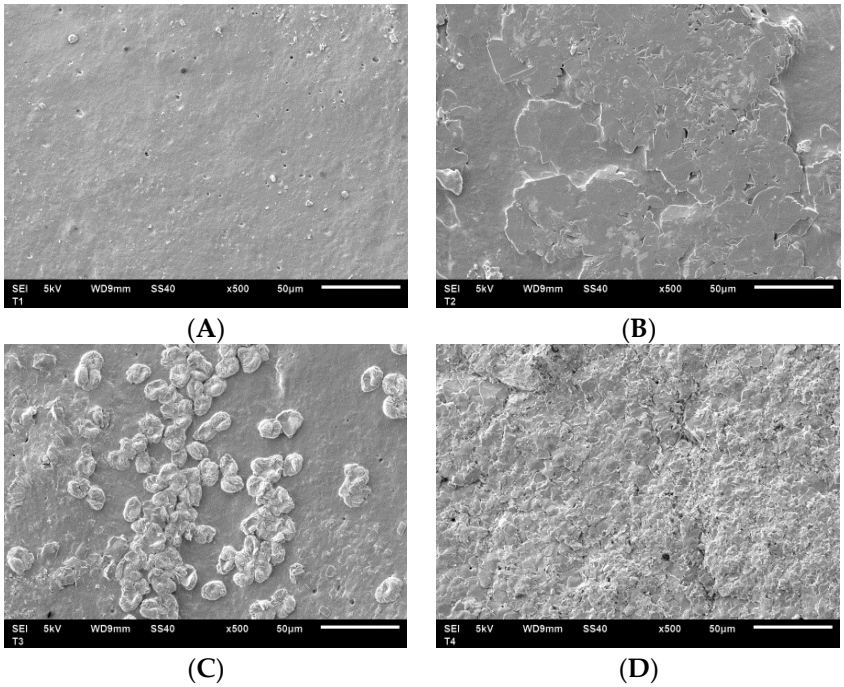

Figure 2. SEM micrographs (500× magnification) of whey and cassava starch films. (**A)** F1, (**B)** F2, (**C**) F3; and (**D**) F4. The percentage of whey/cassava starch in the formulations were as follows: F1 = 67.50/7.50%, F2 = 66.25/8.75%, F3 = 65.00/10.00%, and F4 = 63.75/11.25%.

4. Conclusions

The films studied in this work showed reduced solubility with increasing cassava starch concentration. The films with the highest proportion of whey were more stable to thermal decomposition. The increase in concentration of cassava starch altered the microstructure of the films, making them more irregular and with an accumulation of matter. The production of biodegradable polymer blend films is an important step for the development of films for use in packaging, and F1 with 67.50/7.50% whey/cassava starch is the best film for continued future work. Future studies should be carried out to evaluate permeability to water vapor and gases as well as the mechanical properties and antioxidant and antibacterial activities of the films.

Author Contributions: Y.A.A.M., S.V.F., N.M.S., M.F.B.S., and P.V.T.L. performed the experimental part of the work and contributed to the tabulation, discussion of the results obtained, and revision of the final content to be submitted. J.G.O.F., K.M.L., E.S.N., G.R.P., M.B.E., and M.A.P.d.S carried out the planning and orientation of the experimental part and contributed to the critical review of the final work. All authors have read and agreed to the published version of the manuscript.

Funding: To the Goiano Federal Institute of Education, Science and Technology, Campus Rio Verde, for the financial contribution granted through the Notice nº 21/2015—Proposal for Cooperative Projects of Applied Research Proposals, Foundation of Support to Research of the State of Goiás (Fapeg), Coordination for the Improvement of Higher Education Personnel (CAPES), National Council for Scientific and Technological Development (CNPq) and Financier of Studies and Projects for supporting research.

Conflicts of Interest: The authors declare no conflict of interest.

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