



The effect of fatty acids on the physicochemical properties of edible films composed of gelatin and gluten proteins



Farayde Matta Fakhouri^{a, b, *}, Silvia Maria Martelli^a, Thiago Caon^c, José Ignacio Velasco^d, Rodolfo Cardoso Buontempo^e, Ana Paula Bilck^f, Lúcia Helena Innocentini Mei^b

^a Faculty of Engineering, Federal University of Grande Dourados, Dourados, Brazil

^b Department of Chemical Engineering, Campinas State University, Campinas, Brazil

^c Department of Pharmaceutical Sciences, Federal University of Santa Catarina, Florianópolis, Brazil

^d Centre Català del Plàstic, Universitat Politècnica de Catalunya, Spain

^e Faculty of Technology of São Paulo State, Mogi Mirim, Brazil

^f Londrina State University, Londrina, Brazil

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ABSTRACT

In this study, edible films were prepared with different wheat gluten (GLU) and gelatin (GEL) ratios by the solvent casting technique using glycerol (GLY) or sorbitol (SOR) as plasticizers. Fatty acids (caproic, caprylic, capric, lauric, myristic or palmitic) were also added to a previously selected GLU/GEL/sorbitol film, and the effect of GLU:GEL proportion and type of fatty acid on the film properties were tested. Films plasticized with GLY presented a more significant reduction in the elongation at break (EB). In contrast, films plasticized with SOR did not show a significant difference in the EB. The film's water vapor permeability (WVP) and acid solubility increased with increasing proportions of GEL while the water solubility was decreased. On the other hand, the elongation at break of the films decreased with increasing GEL content, which may be associated with its more rigid structure. The addition of fatty acids resulted in lower WVP and the plasticizing effect was dependent on the degree of interaction with the proteins of the film (identified by thermal analysis). The GLU:GEL proportion and the type of fatty acid affect the film properties (mechanical, solubility, opacity, water vapor barrier), allowing the development of new materials with different and useful functional properties according to the desired application.

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1. Introduction

Edible packaging has been recognized as a potential alternative to conventional packaging since it reduces waste and may improve product stability, quality and safety (Janjarasskul & Krochta, 2010). They provide a semi-permeable barrier against oxygen, carbon dioxide and moisture, reducing respiration, water loss and oxidation rates (Lee, Park, Lee, & Choi, 2003). These last generations of edible films are not expected to replace petrochemical polymer films, but they can help in limiting moisture, aroma, and lipid migrations within composite foods or between food and headspace of conventional packaging (Benbettaieb, Gay, Karbowiak, & Debeaufort, 2016; Vieira, Silva, Santos, & Beppu, 2011). Edible coatings may be

prepared from proteins (eg. gluten and gelatin), polysaccharides, lipids and combination of edible constituents (Fakhoury et al., 2012; Han, Hwang, Min, & Krochta, 2008; Martelli et al., 2017; Moore, Martelli, Gandolfo, Sobral, & Borges Laurindo, 2006).

Gluten proteins are plant proteins and consists of monomeric gliadins and polymeric glutenins (Shewry, Halford, Belton, & Tatham, 2002). They are divided into two groups based on their extractability in aqueous alcohols: the gliadins (extractable) and glutenins (no extractable) (Shewry & Tatham, 1997). The cohesiveness and elasticity of gluten provide integrity and facilitate film formation. Wheat gluten-based films exhibit significant gas-barrier properties and selectivity at high relative humidity (RH) (Angellier-Coussy, Torres-Giner, Morel, Gontard, & Gastaldi, 2008). The mechanical properties of gluten-based films are strongly affected by pH and gluten concentration while water vapor permeability may be correlated with pH and ethanol level (Gontard, Duchez, Cuq, & Guilbert, 1994). Although the effects of plasticizers and hydrophobic constituents on properties of these

* Corresponding author. Department of Chemical Engineering, Campinas State University, Av. Albert Einstein, 500, CEP 13083-852, Campinas, SP, Brazil.

E-mail address: farayde@gmail.com (F.M. Fakhouri).

films have not tested, the addition of these constituents is suggested to optimize the mechanical and moisture barrier properties. Fakhouri, Tanada-Palmu, and Grosso (2004), for example, studied composite films of wheat gluten and cellulose acetate phthalate and concluded that the composite films present more significant barrier properties to water than single films produced from the same materials. Curiously, in another study, it was showed that the addition of thyme oil as the active agent in gluten films by thermoplastic processing provide both antioxidant and antimicrobial properties. Although this constituent also leads to heterogeneous films with hydrophobic discontinuities that reduce the mechanical performance in terms of strength and modulus, at the same time, it enhances the flexibility of the film (Ansorena, Zubeldía, & Marcovich, 2016).

Gelatin is a product of collagen degradation, which involves the breakdown of the triple-helix structure into random coils (Kozlov & Burdygina, 1983) and its film-forming ability is well-established (Yakimets et al., 2007). Gelatin, which is able to form clear and strong films, is often used for microencapsulation and capsule coatings in both food and pharmaceutical industry (Lens et al., 2003). Bertan, Tanada-Palmu, Siani, and Grosso (2005) have studied the effect of fatty acids and 'Brazilian elemi' on composite films based on gelatin. Films containing fatty acids presented higher water vapor barrier properties than gelatin/triacetin films. The mechanical resistance was decreased by the addition of lipids while the opacity and soluble matter increased. Composite edible protein films combined with lipids can present more significant barrier properties than those films prepared only with proteins (Galus & Kadzińska, 2015, 2016; Otoni, Avena-Bustillos, Olsen, Bilbao-Sáinz, & McHugh, 2016).

Taking into account the advantages of the above materials, this study focused on the development of edible gluten-gelatin films containing plasticizers and fatty acids to improve the mechanical and water barrier properties. The fatty acids selected were caproic, caprylic, capric, lauric, myristic and palmitic in order to consider the hydrophobicity effect on film properties. Thermal and water barrier properties, solubility and opacity of the films were considered.

2. Materials and methods

2.1. Materials

Gelatin type A, wheat gluten, glycerol and sorbitol were obtained from Leiner Davis Gelatin, Rhodia, Synth and Getec, respectively (Brazilian suppliers). Caproic, caprylic, capric, lauric, myristic and palmitic fatty acids were obtained from Sigma-Aldrich (Brazil).

2.2. Film preparation and composition

Films were obtained by the casting solvent technique proposed by Gontard, Guilbert, and Cuq (1992) with minor modifications (citric acid was used to adjust the pH). Prior to the preparation of the protein blend that would form the films, gluten and gelatin solutions were prepared separately. After this step of solubilization of the protein and inclusion of the plasticizer, the solutions were mixed in the desired proportions so that the final formulations of each film may be obtained. GLY or SOR were used as plasticizer because they are edible, inexpensive and compatible with the film macromolecules (Vanin, Sobral, Menegalli, Carvalho, & Habitante, 2005).

The gluten solution contained 318.8 mL of distilled water, 431.2 mL of ethanol, 37.5 g or 75 g of gluten (5 or 10% solution of GLU, respectively → 5 or 10GLU10GEL) and 0.2 g plasticizer/g

gluten. The pH was adjusted to 3.0 by the addition of citric acid, and the mixture was heated at 70 °C under magnetic stirring. Subsequently, the gluten solution was centrifuged at 3500 rpm for 25 min. The liquid phase was separated from the solid (viscous) phase and stored for later use.

The gelatin solution was obtained in two steps. In the first step, 10 g of gelatin type A was hydrated in 100 mL of water. Afterward, this solution was heated at 70 °C for 10 min, and the plasticizer (sorbitol or glycerol) was included in a proportion of 0.05 g plasticizer/g gelatin.

To obtain the filmogenic solution, gluten and gelatin solutions were mixed on a heating plate at 60 °C under magnetic stirring at volume ratios of 1:1, 1:4 and 4:1 (gluten solution/gelatin solution). Films were obtained by pouring 20 mL of filmogenic solution into Petri dishes (11.8 cm diameter) and drying at 25 °C for 24 h. After drying, films were stored at 25 °C and 55± 2% relative humidity (RH) for at least 48 h prior to analysis. Films containing different plasticizer are summarized in Table 1. The composition of films containing fatty acids is 10GLU10GEL/SOR (1:1) with different FA amounts (5, 15, 25 and 50 g/100 g polymer).

Fatty acids were added to the solution under magnetic stirring (30 min, 200 rpm) at 60 °C. The mixture showed to be stable and no apparent phase separation in the films was observed. No exudation of fatty acids from the films was also observed during the drying process, probably due to an interaction of these constituents with the protein matrix, which involved hydrogen bonds and physical entanglement interactions.

2.3. Physicochemical characterization

2.3.1. Visual aspect and thickness

A visual trial was performed to select only homogeneous and flexible films (presenting a homogeneous color and no insoluble gelatin particles). Sample thickness was measured by using a micrometer (Model MDC-25 M, Mitutoyo, MFG, Japan). A minimum of 10 points was measured for each sample. After that, films were kept at 25 °C and 50% RH for 48 h before analysis.

2.3.2. Water and acid solubility

The water solubility assays were carried out according to (Gontard et al., 1994). The solubility was expressed as the percentage of dry matter of the film solubilized after a 24 h immersion in water. The solubility in acidic solution was determined in a chloride acid solution (1 mol/L) for 24 h. The measurements were performed in triplicate.

2.3.3. Film opacity

Film opacity was determined by using a HunterLab colorimeter (Colorquest II, Fairfax, USA). The calibration of the instrument was performed with black and white backgrounds. Opacity was determined according to the equation below (HUNTERLAB, 1997):

$$Op = \left(\frac{Op_N}{Op_B} \right) 100 \quad (1)$$

where: Op = film opacity (%), Op_N = film opacity on a black background, Op_B = film opacity on a white background.

2.3.4. Water vapor permeability (WVP)

The water vapor permeability (WVP) of films was measured according to a modified ASTM E96 standard method. Films were placed in aluminum cells containing calcium chloride (relative humidity close to 0.01% at 25 °C), and sealed with paraffin. Permeation cells were placed in desiccators maintained at 25 °C and 75% RH. The amount of water vapor migrating through the film

Table 1

Films prepared with gluten, gelatin and plasticizers. Three GLU/GEL proportions were used (4:1, 1:1 and 1:4) for each formulation.

Sample Code	GLU (g/100 g of solution)	GEL (g/100 g of solution)
5 GLU10GEL/GLY	5	10
10 GLU10GEL/GLY	10	10
5GLU10GEL/SOR	5	10
10GLU10GEL/SOR	10	10

GEL = gelatin; GLU = gluten, GLY = glycerol, SOR = sorbitol.

The amounts of plasticizers in all formulations were 0.2 g/g gluten and 0.05 g/g gelatin.

was determined indirectly by the gain in mass of the calcium chloride.

2.3.5. Mechanical properties

The mechanical properties were evaluated in a TA-XT2 Texture Analyzer (SMS, Surrey, UK), which operated according to the ASTM method D882-83. The initial grip separation and the crosshead speed were set at 50 mm and 1 mm/s, respectively. At least ten samples presenting an area of 100 × 25 mm were selected for the measurements.

2.3.6. Thermal properties

The thermal properties of pure gluten films and films containing fatty acids (5 g/100 g of macromolecules) were studied by differential scanning calorimetry (DSC) and thermogravimetry (TGA). DSC measurements were performed in a calorimeter from TA Instruments (USA) (model TA 2010). Approximately 8–10 mg of samples were sealed in aluminum hermetic pans and heated from 30 to 140 °C at a rate of 10 K/min in a nitrogen atmosphere (gas flow rate was set at 50 mL/min). TGA assays were performed in the thermogravimetric analyzer (SDT 2960, TA Instruments, EUA). Samples (15–20 mg) were scanned at 10 K/min (25–600 °C) under nitrogen atmosphere at 100 mL/min flow rate. The material used as reference was the aluminum oxide (Al₂O₃), which does not show weight loss in the temperature range considered.

2.4. Statistical analysis

Analysis of variance (ANOVA) and Tukey's multiple comparison tests ($p < 0.05$) were performed with Statistica[®] 5.5 (Stasoft, USA).

3. Results

3.1. Gluten-gelatin films plasticized with glycerol or sorbitol

Water vapor permeability (WVP) data as well as solubility in acid and water of the edible films containing gelatin (GEL), gluten (GLU), and glycerol (GLY) or sorbitol (SOR) as plasticizer are presented in Table 2. WVP values of films increased with increasing GEL concentration, except for the film 10GLU10GEL/GLY. Overall, lower WVP values were observed when sorbitol was used as the plasticizer. The GLU films containing 10 g/100 g of GLY or SOR showed lower WVP values in a 1:1 proportion, which it was still much lower than those found by Tanada-Palmu and Grosso (2002). Lower WVP values were also obtained when blends of wheat gluten and cellulose acetate in a 1:1 proportion were tested (Fakhouri et al., 2004). The WVP values of 5GLU10GEL/SOR films in the proportion 1:1 (1.96 kg/mskPa) were higher and lower than those found for edible films based on wheat gluten and bees wax, which were 0.0048 (Gontard et al., 1994) and 5.80 kg/mskPa (Tanada-Palmu, 2003), respectively. Specific combinations of constituents in the films result in different intra and intermolecular interactions,

which would explain these differences.

Similar to WVP values, the acid solubility also increased with GEL content for both plasticizers studied, which was not found for water solubility. GEL-GLU films plasticized with GLY from another study presented improved water and acid solubility when the amount of gelatin was increased (Tanada-Palmu & Grosso, 2002). For systems plasticized with SOR, the same formulations typically had higher water and acid solubility. An increase in GLU concentration from 5 to 10 g/100 g improved the water solubility of samples for both plasticizers in three GLU/GEL proportions. Solubility in water is an important property of edible films. Potential applications may require water insolubility to enhance product integrity and water resistance. On the other hand, high solubility in water and acid medium is required during the consumption to ensure a rapid solubilization of films and maintenance of nutritional properties of the product (Pérez-Gago, Nadaud, & Krochta, 1999).

In general, thickness values of the protein films increased with the concentration of their constituents (protein or plasticizer). While thickness and tensile strength were improved by increasing concentrations of GEL, a reduction of the elongation at break (EB) was observed (Table 3). Similar results were reported in the literature for wheat gluten films plasticized with GLY (Tanada-Palmu & Grosso, 2002). Films plasticized with GLY presented a more significant reduction in the elongation at break when the GLU/GEL proportion was changed from 4:1 to 1:4. In contrast, films plasticized with SOR did not show a significant difference in the EB. 5GLU10GEL/GLY films showed higher tensile strength (TS) values than 10GLU10GEL/GLY films. Samples plasticized with SOR and containing 5 and 10 g/100 g of GLU showed significant differences in the TS only at medium proportions (1:1). The TS clearly increased with the concentration of GEL, regardless of the concentration of GEL and GLU used.

The opacity of GLU/GEL films plasticized with GLY increased from 5 to 20% when the GLU concentration increased from 5 to 10 g/100 g (4:1 proportion) (Fig. 1). When SOR was considered, the opacity increased approximately 333% in this proportion. Only the formulations containing the maximum concentration of GLU presented a significant reduction in the opacity when GEL concentration was increased. For the 10GLU10GEL/GLY and 10GLU10GEL/SOR samples, the opacity decreased by approximately 60.5 and 66.7%, respectively.

3.2. Gluten-gelatin films containing fatty acids

The 10GLU10GEL/SOR sample (1:1 GLU:GEL proportion) was used to evaluate the effect of the addition fatty acids on the properties of the films. This film was selected to incorporate the fatty acids considering their improved water vapor barrier properties and mechanical properties. Although no gas permeability experiment has been considered in this study, the greater thickness of these films containing higher GLU ratio and SOR as the plasticizer (10GLU10GEL/SOR) could reduce the gas access to film, avoiding degradative reactions such as oxidation.

The GLU/GEL films containing fatty acid were more homogeneous and easy to handle. For all cases, the film thickness increased with an increase in fatty acid amounts (Table 4). The addition of palmitic acid increased the film thickness in approximately 179% for increasing concentrations of this constituent while films containing myristic, lauric and caprylic acid had an increase of 104, 137 and 34%, respectively. The largest thickness variation of films was found after addition of capric acid (from 0.036 mm to 0.146 mm). Conversely, films containing caproic acid showed the lowest thickness variation (from 0.029 to 0.056 mm for films composed of 5 and 50% fatty acid).

Table 2

Water vapor permeability, solubility values in water and acid of GLU/GEL films plasticized with GLY or SOR.

Sample	Proportion	WVP (gmm/m ² dkPa)	Water solubility (g/100 g)	Acid solubility (g/100 g)
5GLU10GEL/GLY	4:1	2.96 ± 0.50 ^{de}	32.23 ± 1.32 ^{efg}	27.41 ± 1.76 ^e
	1:1	3.37 ± 0.68 ^{cd}	31.65 ± 1.97 ^{fg}	40.37 ± 0.52 ^{bcd}
	1:4	5.91 ± 0.21 ^b	25.82 ± 0.04 ^h	47.49 ± 2.95 ^{ab}
10GLU10GEL/GLY	4:1	7.67 ± 0.07 ^a	39.99 ± 1.42 ^{bc}	35.52 ± 2.87 ^{de}
	1:1	3.05 ± 0.39 ^{de}	37.57 ± 1.11 ^{cde}	37.64 ± 3.76 ^{cd}
	1:4	4.01 ± 0.29 ^{cd}	30.83 ± 1.20 ^g	49.89 ± 0.56 ^a
5GLU10GEL/SOR	4:1	1.43 ± 0.13 ^f	44.28 ± 2.92 ^b	41.31 ± 2.79 ^{abcd}
	1:1	1.69 ± 0.20 ^f	38.86 ± 1.68 ^{bcd}	43.14 ± 0.67 ^{abc}
	1:4	4.52 ± 0.23 ^c	31.59 ± 0.67 ^g	49.15 ± 1.91 ^a
10GLU10GEL/SOR	4:1	2.32 ± 0.23 ^{ef}	51.41 ± 2.83 ^a	38.49 ± 2.21 ^{cd}
	1:1	1.50 ± 0.08 ^f	40.69 ± 0.60 ^{bc}	37.24 ± 0.99 ^{cd}
	1:4	3.66 ± 0.07 ^{cd}	34.95 ± 1.23 ^{def}	47.76 ± 0.55 ^{ab}

GEL = gelatin; GLU = gluten, GLY = glycerol, SOR = sorbitol.

Data were presented as mean ± standard deviation.

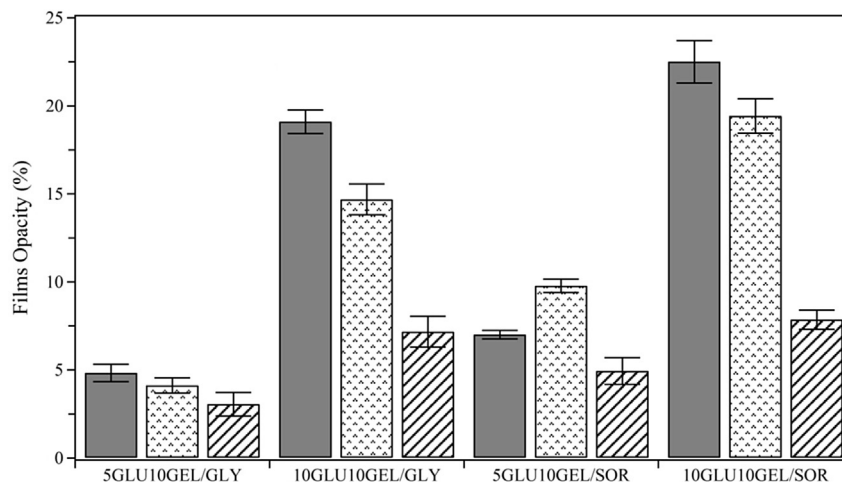
a-h Mean values in the same column with different letters are significantly different (Tukey, $p \leq 0.05$).**Table 3**

Thickness and mechanical properties of GLU/GEL films plasticized with GLY or SOR.

Sample	Proportion GLU/GEL	Thickness (mm)	Tensile Strength (MPa)	Elongation to Break (%)
5GLU10GEL/GLY	4:1	0.049	25.77 ± 1.41 ^g	31.19 ± 5.58 ^b
	1:1	0.063	87.03 ± 5.10 ^d	4.90 ± 0.47 ^c
	1:4	0.073	106.84 ± 5.27 ^{ab}	5.39 ± 0.36 ^c
10GLU10GEL/GLY	4:1	0.093	2.45 ± 0.27 ^h	96.71 ± 4.59 ^a
	1:1	0.065	40.68 ± 2.36 ^f	29.01 ± 5.79 ^b
	1:4	0.077	102.23 ± 4.43 ^{bc}	5.88 ± 0.73 ^c
5GLU10GEL/SOR	4:1	0.052	24.68 ± 1.66 ^g	6.09 ± 0.56 ^c
	1:1	0.055	96.54 ± 6.86 ^c	5.68 ± 0.56 ^c
	1:4	0.075	109.74 ± 1.99 ^{ab}	5.27 ± 0.77 ^c
10GLU10GEL/SOR	4:1	0.081	24.41 ± 0.86 ^g	4.81 ± 0.99 ^c
	1:1	0.082	75.59 ± 2.69 ^e	6.00 ± 0.44 ^c
	1:4	0.084	112.23 ± 3.97 ^a	5.89 ± 0.65 ^c

GEL = gelatin; GLU = gluten, GLY = glycerol, SOR = sorbitol.

Data were presented as mean ± standard deviation.

a-h Mean values in the same column with different letters are significantly different (Tukey, $p \leq 0.05$).**Fig. 1.** Opacity of GLU/GEL films plasticized with GLY and SOR. The GLU/GEL proportion is ■ 4:1/▨ 1:1/▩ 1:4.

Overall, WVP is lower in the presence of fatty acids. WVP values ranging from 0.93 to 3.37 (Table 5) or 2.96 to 7.67 (Table 2) gmm/m²dkPa were found for films prepared with and without fatty acids, respectively. The WVP pattern was more concentration-dependent than the fatty acid type. WVP was maintained constant for concentrations of capric acid of up to 15% while other fatty acids

preserved low and constant WVP values up to 25% (Table 5). The WVP of these fatty acid-based films may be compared those obtained for hydroxypropyl methylcellulose and methylcellulose films (Kamper & Fennema, 1984), which values are 1.92 and 1.48 gmm/m²dkPa, respectively. These WVP values also are lower than those of cellophane films (7.27 gmm/m²dkPa) (McHugh, Huxsoll, &

Table 4
Thickness (mm) of GLU/GEL-fatty acid films.

Fatty acid	Number of carbons in the chain	Fatty acids amount (g/100 g of GLU/GEL macromolecule)			
		5	15	25	50
Palmitic	16	0.039	0.069	0.086	0.109
Myristic acid	14	0.049	0.068	0.089	0.100
Lauric	12	0.054	0.059	0.119	0.128
Capric	10	0.036	0.071	0.085	0.146
Caprylic	8	0.055	0.056	0.058	0.074
Caproic	6	0.029	0.045	0.051	0.056

Table 5
WVP (gmm/m²dkPa) of GLU/GEL-fatty acid films plasticized with sorbitol*.

Fatty acid	Fatty acid amount (g/100 g polymer)			
	5	15	25	50
Palmitic	1.19 ± 0.18 ^b	2.08 ± 0.17 ^{ab}	2.01 ± 0.18 ^{ab}	2.40 ± 0.59 ^a
Myristic	1.08 ± 0.01 ^b	1.74 ± 0.21 ^b	1.31 ± 0.29 ^b	3.37 ± 0.33 ^a
Lauric	1.16 ± 0.46 ^b	1.40 ± 0.36 ^b	1.25 ± 0.12 ^b	1.72 ± 0.29 ^a
Capric	0.93 ± 0.14 ^b	1.42 ± 0.16 ^b	3.24 ± 0.42 ^a	2.76 ± 0.11 ^a
Caprylic	1.07 ± 0.01 ^b	1.23 ± 0.10 ^b	1.55 ± 0.31 ^b	2.61 ± 0.04 ^a
Caproic	1.78 ± 0.01 ^c	2.19 ± 0.32 ^b	2.74 ± 0.36 ^b	3.21 ± 0.08 ^a

Data were presented as mean ± standard deviation.

The WVP of unplasticized GLU/GEL film (without FA) is 1.50 ± 0.08 (gmm/m²dkPa). a-h Mean values in the same column with different letters are significantly different (Tukey, $p \leq 0.05$).

Table 6
Water solubility (g/100 g of GLU/GEL solution) of 10GLU10GEL/SOR-fatty acid films.

Fatty acid	Fatty acid amount (g/100 g)			
	5	15	25	50
Palmitic	29.02 ± 1.48 ^b	28.13 ± 0.55 ^b	30.05 ± 1.74 ^a	34.64 ± 1.34 ^a
Myristic	35.03 ± 2.39 ^a	34.07 ± 0.36 ^a	35.24 ± 2.49 ^a	31.56 ± 1.76 ^a
Lauric	39.99 ± 0.42 ^a	41.90 ± 1.93 ^a	42.57 ± 2.49 ^a	43.08 ± 0.63 ^a
Capric	37.07 ± 0.04 ^a	35.84 ± 0.41 ^a	39.36 ± 2.24 ^a	36.60 ± 0.65 ^a
Caprylic	37.24 ± 0.26 ^c	40.54 ± 1.07 ^b	43.56 ± 1.08 ^a	41.01 ± 0.41 ^{ab}
Caproic	38.68 ± 0.87 ^a	39.86 ± 2.12 ^a	35.52 ± 0.57 ^a	39.91 ± 1.21 ^a

Data were presented as mean ± standard deviation.

a-h Mean values in the same column with different letters are significantly different (Tukey, $p \leq 0.05$).

Krochta, 1996).

The water solubility of samples containing palmitic and caprylic fatty acids (Table 6) showed a concentration-dependent effect, which increased from 29.0 to 34.6% and from 37.2 to 41.0%, respectively. The addition of other fatty acids, regardless of concentration, did not cause any change in this parameter ($p > 0.05$). The opacity of films was also associated with the fatty acid concentration (Fig. 2). Lower opacity values were found for films prepared with caproic acid (ranged from 5 to 31%). Higher variation in this parameter was obtained for caprylic acid.

The DSC and TGA traces of GLU/GEL films are shown in Fig. 3. Films containing lauric and palmitic acids presented a transition between 100 and 250 °C in TGA analysis, which could be a pre-thermal degradation of biocompound. The increased hydrophobicity of these two fatty acids could lead to less interaction with the protein matrix, providing a thermal transition of gelatin and gluten faster than other acids.

Biofilms containing myristic and capric acid into GLU/GEL matrix are apparently more thermally stable, presenting a single stage of thermal degradation. The degradation step for all films was completed in the region close to 500 °C, presenting 20% ash content as a solid waste (80% is converted to volatile gases). These two films

seem to have a more significant structural cohesion; where fatty acids, gelatin and wheat gluten would interact more strongly.

The DSC trace of GLU/GEL film showed a first endothermic transition between 85 and 100 °C, which may be associated with the sol-gel transition of the gelatin (Sobral, Carvalho, Moraes, Bittante, & Monterrey-Quintero, 2011). In the region close to 60 °C, an unclear transition is observed, which may be related to an increased mobility of the chains or modification in the tertiary structure of the protein. Another explanation for this early phase transition may be the gelatinization process of gluten, which occurs close to 65 °C (Eliansson & Hegg, 1980).

All biofilms containing fatty acids showed a significant reduction in the intensity of their transitions or broader transition temperature range, suggesting a reduction of crystallinity or phase transition phenomena. Moreover, a transition temperature displacement was observed in some situations, which may be associated with a plasticizing effect of fatty acids. The less intense transition is observed in the biofilm with myristic acid, which started in 90 °C and ended around 115 °C. The transition of the biofilm with palmitic acid was earlier and more intense (it started in the range between 70 and 85 °C). Transitions of biofilms containing caproic, caprylic and capric occurred in the same region and with the same intensity in the range of 80–95 °C. The biofilm with lauric acid showed a very subtle and late transition in the region between 110 and 120 °C.

3.3. Discussion

The WVP of the films increased with the reduction of GLU concentration, and even lower values for this parameter were obtained when the SOR was used as the plasticizer. The GEL is more hydrophilic than GLU, which explains higher WVP in cases where a higher amount of GEL was considered. Regarding the plasticizer type, GLY is more hygroscopic and has low molar weight (MW) (Sothornvit & Krochta, 2001), which may explain their higher WVP values obtained. Thus, the low MW of GLY and its greater amount of related water increase its effectiveness as the plasticizer. Given that SOR presents more –OH hydrophilic groups in its structure (GLY-3 vs. SOR-6), more molecular interaction with the GEL/GLU matrix is expected (H-bonds). Based on this fact, fewer hydroxyl groups from the plasticizer would be available to absorb water, explaining the lower values of WVP when the SOR was considered (Sanyang, Sapuan, Jawaid, Ishak, & Sahari, 2016). These results are consistent with literature data (Thomazine, Carvalho, & Sobral, 2005).

In film solubility analysis, it is known that the dry matter solubilized in water is likely composed of plasticizer together with a small amount of protein. Film insolubility is associated with a high interaction density and, more specifically, the presence of intermolecular covalent bonds or “physical knots” (i.e., chain entanglements). Moreover, the presence of non-covalent bonds is also extremely important and plasticizers will reduce these interactions (Cuq, Gontard, Cuq, & Guilbert, 1997). In addition to intra- and intermolecular forces among the film proteins or among proteins and plasticizer, the solubility of proteins also depends on their isoelectric point (pI) and ionic strength of the medium. The interaction between the charged molecules and the molecules of the polar solvent increases with the increasing chain charge. The maximum protein solubility is obtained at pH away from its pI (Wittaya, 2012). At pH values away from the pI, protein denatures, unfolds, and solubilizes. Although GEL is soluble in water, high concentrations of this protein increase the ionic strength of the medium, reducing the chances to solubilize the GLU. Wheat gluten dissolves in aqueous solutions of high or low pH at low ionic strength (Krull & Inglett, 1971). In the acid medium, both GLU and GEL are more positively charged (pI 7.5 and 9, respectively), which

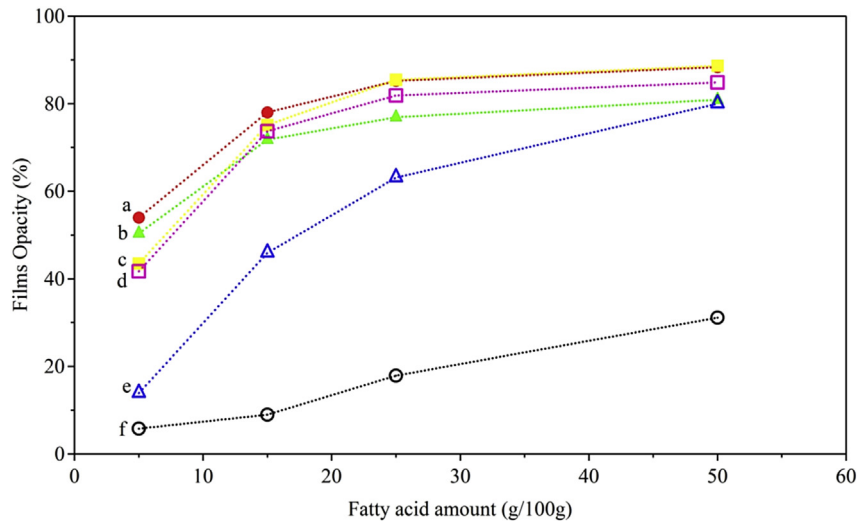
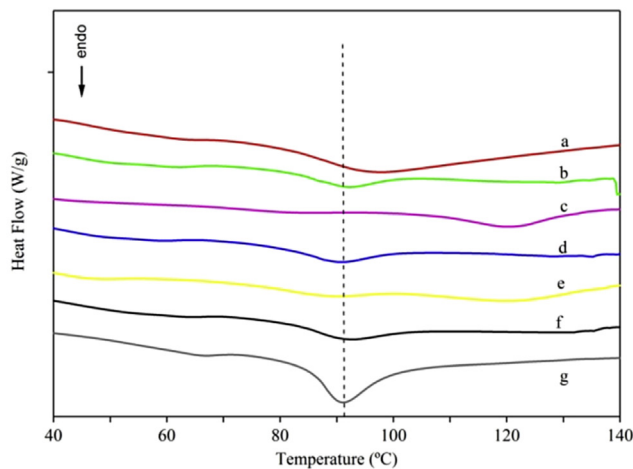
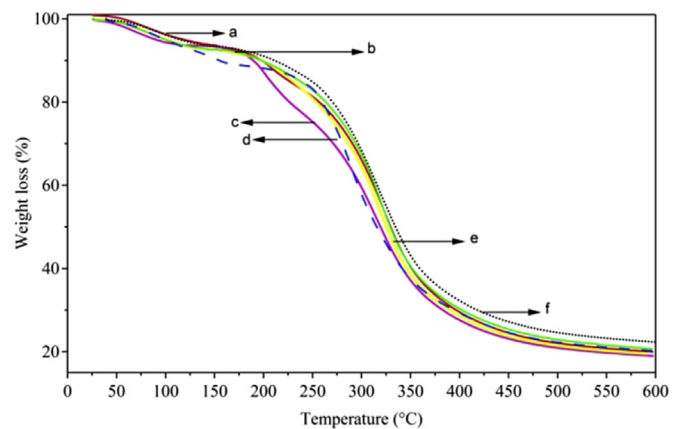


Fig. 2. Opacity of GLU/GEL films containing fatty acids; a) palmitic; b) capric; c) myristic; d) lauric; e) caprylic and f) caproic acids.



(a)



(b)

Fig. 3. DSC (a) and TGA (b) analyses of GLU/GEL films containing 5 g/100 g of fatty acids. For TGA: a) palmitic; b) capric; c) lauric; d) caprylic; e) myristic and f) caproic acids and g) GLU/GEL.

increase the repulsive forces between these two proteins and also reduce coagulation phenomena. As consequence, less protein-protein chemical interaction occurs and the solubility is increased.

The effect of adding fatty acids to the film formulation also was considered. Although the type of fatty acid did not have a significant effect on the WVP, which is in agreement with the literature (Zahedi, Ghanbarzadeh, & Sedaghat, 2010), this parameter was dependent on the concentration of fatty acids (except for caproic acid). At high concentrations of fatty acids, a disruption of the polymer matrix is caused by the hydrophobic molecules, increasing WVP values (these compounds would present a lower compatibility with the matrix). At low amount of fatty acids, the matrix disruption does not occur and the hydrophobic effect of these compounds is more significant. A heterogeneous distribution of lipids and low stability of the formed emulsion were observed for films composed of high FA concentration. Furthermore, other studies have shown that fatty acids have a poor detachment from the Petri plates, causing disruption of the constituents that form the

film, resulting in increased WVP values. The WVP of the films containing fatty acids also depends on the degree of saturation, polarity, chain length and degree of branching thereof. Longer lipid molecules, non-polar, saturated and linear tend to produce more cohesive films, rigid and less permeable compared to polar lipid molecules, presenting short and unsaturated chain as well as higher degree of branching (Baldwin, Nisperos, Hagenmaier, & Baker, 1997; Kamper & Fennema, 1984; Kester & Fennema, 1989).

The intended use of edible coatings or films requires a clear understanding of mechanical properties such as tensile strength (TS) and elongation at break (EB), which are strictly associated with the film composition (Bourbon et al., 2011). The chemical interactions among bioactive compounds and polymeric matrix also play an important role on the mechanical properties of films (Sivaroban, Hettiarachchy, & Johnson, 2008). Increasing the amount of GEL in the mixture, an increase in the TS and reduction in the EB may be observed (Table 3), most likely due to the intrinsic properties of both proteins used in the blend. Glutenins are

important for the elasticity of dough and thus they can influence on the strength of the film. HMM subunits of glutenins have also been associated with the formation of 'elastic backbone' in gluten, which are stabilized by disulfide bonds. The central part of these subunits presents glutamine-rich repetitive sequences, which would originate extensive arrays of interchain hydrogen bonds, contributing to the elastic properties via a 'loop and train' mechanism (Shewry et al., 2002). In contrast, the gelatin provides more rigid structures, improving TS and reducing EB at higher concentrations.

Although films were prepared considering lower amounts of plasticizer than protein, the presence of plasticizer seems to affect EB. Overall, SOR-plasticized films presented lower EB values than those GLY-based films (except for films prepared with higher GEL proportions), as already reported by Al-Hassan and Norziah (2012). The plasticizers cause the weakening of intermolecular forces among the chains of macromolecules, increasing the free volume and causing a reduction of mechanical resistant (Sobral, Menegalli, Hubinger, & Roques, 2001). Given that the SOR exhibits more chemical interaction with the GLU-GEL matrix than GLY via hydrogen bonds, as already mentioned in the discussion of the WVP analyzes, this fact would contribute to explain lower EB provided by this plasticizer. Moreover, GLY has lower molecular size, which facilitates its penetration into protein chains, weakening the GLU-GEL interaction.

The increase in the concentration of fatty acids increased the opacity of films, which can be associated with the low water content. It is well known that the water and crystalline content affect this parameter (Matsuguma et al., 2009). The addition of fatty acids reduces the water absorption into the films due to hydrophobic character of these molecules, changing the molecular organization of GLU and GEL and, therefore, an increase in optical properties is observed.

DSC traces of biofilms indicate that fatty acids had a plasticizing effect on GLU/GEL films, interacting with the protein matrix and interfering in the structural organization by decreasing the crystallinity of the material. Films containing fatty acids presented broader, lower-intensity melting peaks, as well as phase transition peak displacement, supporting this hypothesis. A late low-intensity phase transition was observed for lauric acid and myristic acid (lower intensity for this later), suggesting an interaction between fatty acid and protein matrix. Films containing palmitic acid showed a strong phase transition before that of the gluten films, suggesting higher compatibility between this fatty acid and protein matrix or higher plasticizing efficiency. Biofilms composed of caprylic, caproic and capric acid showed similar thermal transitions (intensity and position), which suggest a similar plasticizing effect. The transitions of these last fatty acids were observed in the region close to the original transition of the gluten, suggesting that plasticizers were not very efficient.

4. Conclusions

Edible gluten-gelatin films containing fatty acids and sorbitol or glycerol as plasticizers can be prepared by the solvent casting technique. The gluten: gelatin proportion and the type of fatty acid affect the film properties (mechanical, solubility, opacity, water vapor barrier and thermal properties), allowing the development of new materials with different and useful functional properties according to the desired use.

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