INFLUENCE OF LOCUST BEAN GUM ADDITION ON PROPERTIES OF SOY PROTEIN BASED FILMS

INFLUÊNCIA DA ADIÇÃO DE GOMA DE ALFARROBA NAS PROPRIEDADES DE FILMES À BASE DE PROTEÍNA DE SOJA

Kayque Antonio Santos Medeiros¹, Laís Ravazzi Amado² and Keila de Souza Silva¹*.

1. Department of Technology, UEM – Maringa State University, Angelo Moreira da Fonseca Avenue, 1800, 87506-370 – Umuarama, PR, Brazil.
2. Department of Food Engineering and Technology, São Paulo State University (UNESP), Institute of Biosciences, Humanities and Exact Sciences, Cristovão Colombo Street 2265, 15054-000 - São José do Rio Preto, SP, Brazil.

RESUMO
A proteína de soja é o mais importante representante da proteína de leguminosa e tem apresentado alto potencial para a fabricação de filmes. As misturas entre proteínas e polissacáridos podem melhorar o desempenho, estabilidade, funcionalidade e resistência de filmes de biopolímero em comparação com filmes simples. Este trabalho tem como objetivo desenvolver filmes compostos com isolado protéico de soja (SPI) e concentrado protéico de soja (SPC) e diferentes concentrações de goma de alfarroba (LBG), bem como, avaliar o efeito da adição de polissacarídeo nas propriedades óticas e mecânicas, barreira, cor, microestrutura, solubilidade e umidade dos filmes SPI e SPC. Para tanto, foram elaborados filmes compostos variando a concentração de LBG (0; 0,05; 0,1; 0,2; 0,3; 0,4%) e fixando a concentração de SPI e SPC (5%) em solução. O glicerol foi utilizado em todas as formulações como plastificante. Foram avaliadas a microestrutura, solubilidade, umidade, permeabilidade ao vapor de água, propriedades óticas e mecânicas dos filmes simples e compostos. A solubilidade e barreira do filme à luz foram aumentadas em filmes SPI com adição de LBG, mas não foram alteradas em filmes SPC com adição de goma. Todos os filmes compostos apresentaram valores de resistência à tração mais elevados do que os filmes simples. A leveza dos filmes compostos foi menor do que os filmes simples, porém a barreira de vapor de água foi semelhante.

Palavras-Chave
concentrado de proteína de soja, filmes compostos, interações de biopolímeros.

ABSTRACT
Soy protein is the most important representative of legume protein and has presented high potential to manufacture films. Blends between protein and polysaccharide can to improve the performance, stability, functionality and resistance of biopolymer films in comparison to simple films. This work aims to develop composite films with soy protein isolate (SPI) and soy protein concentrate (SPC) and different locust bean gum (LBG) concentration, as well as, to evaluate the effect of polysaccharide addition on optical and mechanical properties, water vapor barrier, color, microstructure, solubility and moisture of the SPI and SPC films. For this purpose, composite films were elaborated varying LBG concentration (0; 0.05; 0.1; 0.2; 0.3; 0.4%) and fixing SPI and SPC (5%) concentration in
solution. Glycerol was used in all formulations as plasticizer. Microstructure, solubility, moisture, water vapor permeability, optical and mechanical properties of the simple and composite films were evaluated. The solubility and film barrier to the light was increased in SPI films with LBG addition, but was not changed in SPC films with gum addition. All composite films presented higher tensile strength values than simple films. The lightness of composite films was smaller than simple films, however water vapor barrier was similar.

**KEY WORDS**
soy protein concentrate, composite films, biopolymer interactions.

1. INTRODUÇÃO.

The importance of sustainable and green alternatives has been discussed in recent years, and the concern for replacing conventional plastics with ecologically profitable alternatives has been studied by several authors, mainly in the production and application of biodegradable films for use in foods. Interest in biodegradable protein based films has increased with time due to better oxygen barrier and mechanical properties than polysaccharide based films (CUQ, AYMARD, CUQ, & GUILBERT, 1995). Soy protein, the most important representative of legume protein (SONG, TANG, WANG & WANG, 2011), has presented high potential to manufacture films with good mechanical properties and oxygen barrier in comparison to polysaccharides based films, in addition it is a raw material with low cost, high availability and biodegradability (CHO, PARK, BATT & THOMAS, 2007; CIANNNAMEA, STEFANI, & RUSECKAITE, 2014; REDDY & YANG, 2013; GARRIDO, ETXABIDE, GUERRERO & DE LA CABA, 2016). This protein is obtained from defatted dry soybean meal and it can be commercially available as soy flour (SF, 54% protein), soy protein concentrate (SPC, 65-72% protein) and soy protein isolate (SPI, ≥ 90% protein) (Song et al., 2011).

Functional properties of protein based films are determined by its microstructure which strongly depends on the structure and content of protein (CIANNNAMEA ET AL., 2014). The nonprotein fraction of soy protein concentrate can adversely affect the film formation (KUNTE, GENNADIOS, CUPPETT, HANNA & WELLER, 1997) and reduce mechanical properties and gas barrier due to lower formation of disulfide bonds between proteins (GENNADIOS & WELLER, 1991). Despite of this presented disadvantage, SPC is a good option to manufacture films on a large scale because it is cheaper than SPI (CIANNNAMEA ET AL., 2014).
Blends between different biopolymers have been studied by several authors in order to form composite films with higher stability and functionality in relation to simple films (OSÉS ET AL., 2009; SILVA, MAURO, GONÇALVES & ROCHA, 2016; SILVA, FONSECA, AMADO & MAURO, 2018). Composite SPI/guar-gum films, with 5% (w/v) SPI and 0.25% (w/v) guar-gum, for instance, were more resistant and less oxygen permeable than SPI films without gum (SUI, ZHANG, YE, LIU & YU, 2016). Guerrero, Garrido, Leceta, & de la Caba, (2013) observed that 3.3 % (w/w) of gelatin (bovine or fish) addition increased the resistance and barrier to UV light of 5% (w/w) SPI film. However, it is not always the interactions between the different components of the film matrix improve all the properties of films. The composition and the nature of each component are also important to manufacture composite films with higher functionality than simple films. Garrido et al. (2016) observed that the interaction between SPI and agar, proven by conformational changes in the second structure of the protein with polysaccharide addition, resulted in composite films opaquer and with lower tensile strength values than SPI films.

Locust bean gum (LBG) also known as carob gum, is a heterogeneous and non-ionic polysaccharides found in the endosperm of fruit pod of the carob tree and commonly used in the food industry. This gum is a thickening agent able to form viscous solutions at relatively low concentration and to be little affected by pH and thermal treatment (CERQUEIRA ET AL., 2011; DAKIA, BLECKER, ROBERT, WATHELET, & PAQUOT, 2008; GOYCOOLEA, MORRIS & GIDLEY, 1995; SITTIKIJJYOTHIN, SAMPAIO & GONÇALVES, 2005). At ambient temperature, LBG present low solubility and for this reason it is necessary heating to its fully hydration (Pollard et al., 2006).

LBG film is indicated by some authors as alternative to synthetic materials (BOZDEMIR & TUTAS, 2003; CERQUEIRA, SOUZA, TEIXEIRA & VICENTE, 2012). Some researchers reported that the interaction between LBG and other biopolymer is able to form composite films with better properties than simple films. Mostafavi, Kadkhodaee, Emadzadeh & Koocheki (2016) reported improvement of up to 26% in water vapor barrier and of up to 96% in tensile strength of tragacanth films as LBG concentration increased. Kurt, Toker & Tornuk, (2017) observed increasing of 80% tensile strength of LBG/xanthan-gum/40% glycerol films as LBG concentration increased. Silva et al. (2016) suggested that interaction between LBG and whey protein isolate (WPI) could be used to tune properties of WPI edible films, since gum addition improved mechanical properties and oxygen barrier of the films.
The aim of this work was to develop composite films with SPI and SPC and different LBG concentration, as well as, to evaluate the effect of polysaccharide addition on optical and mechanical properties, water vapor barrier, color, microstructure, solubility and moisture of the SPI and SPC films.

2. MATERIALS AND METHODS

2.1. MATERIALS

Soy protein concentrate (SPC) with 83.4% minimum protein content on dry basis, 6.7% moisture, 5% ash and 1.5% fat and soy protein isolate (SPI) with 91% total protein, 6.5% of moisture, 3.5% ash, 0.4% fat, approximately, was kindly supplied by Maxsoy® fibras & ingredientes (Hortolândia, São Paulo, Brasil). Locust bean gum (LBG) (GRINDSTED® LBG 246, São Paulo, Brasil) was kindly supplied by Danisco (Cotia, Brazil). Glycerol was supplied by Sigma, Co (St. Louis MO, USA).

2.2. FILM PREPARATION

Simple films constituted by SPI or SPC and composite films constituted by SPI or SPC + LBG (in different concentration) were developed for the purpose of this study.

Stock solution of 1% (w/w) LBG was prepared with stirring at 25°C/1hour and after heated at 80°C/30min (Silva et al., 2016).

Film solutions were prepared adding 5% SPI or SPC (powder), required amounts of LBG stock solution, 2% glycerol and distilled water. Six different LBG concentrations (0%; 0.05%; 0.1%; 0.2%; 0.3% e 0.4%) were tested, following the same concentrations tested by Silva et al., (2016) and considering that the highest concentration of LBG to form a homogeneous gel was 0.5%. The pH of mixtures was adjusted to 11 with NaOH solution (40% w/w) addition and heated at 65°C/10min in thermostatic bath (Simétrica, SI/6Aneis/18L, Brazil) to complete dissolution of soy protein (Jaramillo, Roberts & Coupland, 2011; Lam, Paulsen & Corredig, 2008). Then, the mixtures were stirred at room temperature for 2 hours and after thermally treated at 75°C/20min in thermostatic bath (Simétrica, SI/6Aneis/18L, Brazil) to denature the protein fraction. Next, solutions were cooled, poured onto tray (casting method) and dried on a convective oven (Marconi, model MA 035) for 16h at 35±2°C. Dried films were equilibrated in desiccator contained magnesium.
nitrate-6-hydrate saturated solution (53% relative humidity) at 25°C for one week before carry out
the analyses.

2.3. FILM THICKNESS

The thickness of the simple and composite films was measured at 10 different points for each
film using a digital micrometer (Mitutoyo, Japan).

2.4. MICROSTRUCTURE

Simple and composites films microstructures were studied by Scanning Electron Microscopy
– SEM (SEI model Quanta 250) using a working distance (WD) between 9.5 mm and an accelerating
voltage of 5 kV. The protein films (without and with LBG addition) were coated with a sputtered
carbon thin film and analyzed at 1000× magnification.

2.5. WATER SOLUBILITY

The simple and composite film solubility in water was determined in triplicate using the
modified method described by Cuq, Gontard, Cuq, & Guilbert (1996). Pieces (2 x 2 cm) of dried
films were weighted (\(W_0\)) and immersed in water at 25°C, with agitation (60 rpm). After 24h of
solubilization, the pieces were taken to water and dried in an oven (Logen, model 1.3) at 105°C until
constant weight (\(W_f\)). The solubility was determined according to Equation (1).

\[
S = \frac{W_0 - W_f}{W_0} \times 100
\]  
\[\text{Equation (1)}\]

2.6. MOISTURE CONTENT

Moisture content was gravimetrically determined in triplicate an oven (Logen, model 1.3) at
105°C until constant weight (AOAC, 1995).

2.7. WATER VAPOR PERMEABILITY MEASUREMENT

Water vapor permeability (WVP) of simple and composite films were performed
gravimetrically in triplicate based on ASTM E96-95 method (ASTM E96-95, 1995). For this purpose,
SPI or SPC films with and without LBG were placed in permeation cell partially filled with calcium
chloride (2% RH) and placed in a desiccator, with a miniature fan inside, containing water (100%
RH), at 25 °C. The permeation cells were weighed every 40 minutes for 7 hours. The WVP, presented as g·(m·s·Pa)⁻¹, was estimated using regression analysis from Equation (2) as described for McHugh, Avena-Bustillos & Krochta (1993).

\[ WVP = \frac{WVPR \cdot x}{A \cdot \Delta P} \]  

(2)

where WVPR is the water vapor permeability rate (g· s⁻¹), \( x \) is the film thickness (m), \( A \) is the permeation area and \( \Delta P \) is the difference of the water vapor partial pressure at 20°C across the two sides of the film.

2.8. MECHANICAL PROPERTIES

Tensile strength (TS) and elongation-at-break (E), were measured using a texture analyzer (TA.XT2, Stable Micro Systems, Surrey, UK) equipped with tensile test attachments, at 25°C with the crosshead speed of 0.8 mm s⁻¹, according to the guidelines of ASTM D882-91 standard method (ASTM D882-91, 1991). Tests were performed replicated twenty times for each type of film.

2.9. OPTICAL PROPERTIES

Color measurement was evaluated in five replicates using a colorimeter (Konica Minolta, model CR-400) previously calibrated on white surface. The response was expressed in the form of the parameters L* (lightness or darkness), a* (redness or greenness) and b* (yellowness or blueness).

The visible light barrier properties of the SPI or SPC (with and without LBG) films were measured in triplicate, using a quartz colorimetric cell and an UV-VIS (GENESYS 10S UV-VIS, Thermo Scientific, EUA), according to Equation (3).

\[ Op = \frac{A_{600}}{X} \]  

(3)

where Op is opacity (mm⁻¹); A600 is absorbance at 600 nm; X is mean thickness of films (mm).

2.10. STATISTICAL ANALYSES
The data expressed in mean ± standard deviation was statistically analyzed by an analysis of variance (ANOVA) and Tukey’s test at a 5% significance level, using Statistic 7.0 software version 7.0 (StatSoft, Inc, Tulsa, USA).

3. RESULTS AND DISCUSSION

3.1 MORPHOLOGICAL PROPERTIES

In order to study the microstructure and surface characteristics of SPI and SPC films with 2% glycerol (w/w) and different LBG concentrations, scanning electron microscopy (SEM) analysis was performed. Both SPI and SPC films (Fig 1a) presented more homogeneous, denser and continuous structure than films with polysaccharide addition. The surface becomes rougher and less homogeneous as LBG concentration increased.

In Figure b2 it is possible to observe accumulation of matter in specific areas of the film surface, which may indicate phase separation between gum and SPI. For SPC films, this separation is more evident in Figure c1. The phase separation can have lead to the appearance of two zones, being one protein-enriched and other gum-enriched (GRINBERG & TOLSTOGUZOV, 1997; SILVA et al., 2016).

Figure 1. Surface structure (x 1000) of the SPI, SPC, SPI-LBG, SPC-LBG films. From left to right (from 1 to 2): SPC (Soy Protein Concentrate) and SPI (Soy Protein Isolate) films. In the first column SPC movies and in the second column SPI movies. In the first line films without adding LBG (a1, a2); 0.05% LBG (b1, b2); 0.1% LBG (c1, c2); 0.2% LBG (d1, d2); 0.3% LBG (e1, e2) and 0.4% LBG (f1, f2).
3.2. SOLUBILITY AND MOISTURE

Moisture and solubility values obtained for soy protein films with and without addition of LBG, are presented in Table 1.

Table 1. Values of solubility and moisture content for SPC (Soy Protein Concentrate) and SPI (Soy Protein Isolate) films, with glycerol, varying LBG (Locust Bean Gum) concentration.

| Treatment | SPC | SPI |
|-----------|-----|-----|
|           | Solubility (%) | Moisture (%) | Solubility (%) | Moisture (%) |
| LBG 0%    | 62.48 ± 2.80 a,A | 36.69 ± 0.20 a,A | 48.40 ± 0.77 a,B | 29.81 ± 0.54 a,B |
| LBG 0.05% | 61.65 ± 0.15 a,A | 36.44 ± 0.43 a,A | 49.93 ± 1.35 ab,B | 33.61 ± 0.48 b,B |
| LBG 0.1%  | 61.54 ± 0.54 a,A | 34.14 ± 0.89 b,B | 49.50 ± 1.67 ab,B | 33.54 ± 0.78 b,B |
| LBG 0.2%  | 61.45 ± 1.20 a,A | 34.78 ± 0.16 ab,A | 50.68 ± 0.43 ab,B | 30.58 ± 0.44 a,B |
| LBG 0.3%  | 60.72 ± 0.78 a,A | 35.61 ± 1.21 ab,A | 49.99 ± 1.28 ab,B | 31.20 ± 0.47 a,B |
| LBG 0.4%  | 60.82 ± 0.26 a,A | 34.11 ± 0.82 a,A | 52.00 ± 0.77 b,B | 28.06 ± 0.66 c,B |

Means with the same capital letter, for the same response variable, in the same line did not differ significantly at p < 0.05 according to the Tukey test. Means with the same lower case letter, in the same column, did not differ significantly at p < 0.05 according to the Tukey test.

SPC film presented moisture and solubility values 23 and 29%, respectively, higher than SPI films. As SPI has higher protein content, more hydrophobic groups are exposed during thermal treatment (NISHINARI, FANG, GUO & PHILLIPS, 2014) and, consequently, smaller solubility and moisture of the films. Moreover, SPC has higher amounts of contaminants (such as lactose) and, probably, for this reason SPC films were wetter and soluble than SPI films. Even though it is more soluble, both SPC and SPI films, with or without LBG addition, did not dissolve completely in water during 24h of immersion under agitation.

It was possible to observe a significant increase in SPI films moisture with 0.05% LBG addition, however none change was observed for SPC films. As LBG concentration increased, SPI and SPC films moisture reduced until to reach values below of the film without gum.

Some researchers reported increasing of moisture content of protein films with polysaccharide addition. Guerrero et al. (2013) and Tonyali, Cikrikci & Oztop (2018) observed significant increase of 13% in moisture content of SPI films with agar addition and 5% in WPI films with gum tragacanth addition. Authors related the observed results to hygroscopic character of gum added. Despite of polysaccharide presents a great affinity by water, its addition in solutions contained protein does not always increase moisture content of composite films. Sui et al., (2016) for instance, observed that films manufactured with the higher SPI concentration presented reduction of moisture content as guar
gum concentration increased. The authors related this result to an increase in film surface hydrophobicity evidenced by increasing contact angle value. Probably, the separation between LBG and SPI or SPC in different zones, observed in SEM analysis (Figure 1), also increased the film surface hydrophobicity, which reduced water interaction in the enriched phase in protein (region more hydrophobic), facilitating water expulsion by evaporation during drying of the films and reducing, in this way, the moisture content of the films.

Despite LBG has hydrophilic nature, its concentration in the polysaccharide enriched phase was not high enough to influence significantly the solubility of SPC films. The same behavior was observed for SPI films with up the 0.3% gum. Nevertheless, with the LBG concentration increase, the polysaccharide-enriched phase seems becomes more influent on solubility of SPI films, since that the 0.4% polysaccharide addition resulted films 7.4% more soluble than ones without gum.

Silva et al. (2016) also evaluated the influence LBG addition on solubility whey protein isolate (WPI) films. Authors manufactured films with 5% WPI + 2% glycerol and different LBG concentrations (0; 0.025; 0.05 and 0.1%). They observed a little but significant reduction of 18% the solubility of the films with gum addition. In our study, none significant change was observed in solubility of SPC and SPI films with 0.05 and 0.1% LBG addition.

3.3. WATER VAPOR PERMEABILITY MEASUREMENT

Water vapor permeabilities (WVP) of SPI and SPC films with or without LBG are shown in Table 2. The order of $10^{-10}$ g·(m·s·Pa)$^{-1}$ was coherent with previous soy protein films studies (GARRIDO ET AL., 2016; SILVA ET AL., 2016; ZHANG, HSIEH & VARDHANABHUTI, 2014). Both SPC and SPI films (with and without gum addition) present WVP values close to ones obtained by Arvanitoyannis, Biliaderis, Ogawa & Kawasaki (1998) for low density polyethylene film ($8.64 \times 10^{-10}$ g·(m·s·Pa)$^{-1}$) and smaller than the ones obtained to whey protein isolate (WPI)+LBG by Silva et al. (2016) ($1.57 - 1.66 \times 10^{-9}$ g·(m·s·Pa)$^{-1}$).

Water vapor permeability of SPC film was statistically similar to SPI film, presented in Table 2. LBG addition did not change significantly the water vapor barrier of both films, even though, it was observed a trend of water vapor permeability of SPC and SPI films increase as LBG concentration increased, 15% and 8% respectively. This trend was more pronounced in SPC films.
Table 2. Water vapor permeabilities (WVP) of SPI and SPC films with or without LBG.

| Treatment | SPC $\text{WVP} \times 10^{-10}$ (g·m⁻¹·s⁻¹·Pa⁻¹) | SPI $\text{WVP} \times 10^{-10}$ (g·m⁻¹·s⁻¹·Pa⁻¹) |
|-----------|---------------------------------------------|---------------------------------------------|
| LBG 0%    | 7.66 ± 0.01 a,A                           | 7.78 ± 0.31 a,A                             |
| LBG 0.05% | 7.87 ± 0.61 a,A                           | 7.93 ± 0.92 a,A                             |
| LBG 0.1%  | 8.25 ± 0.74 a,A                           | 7.91 ± 0.06 a,A                             |
| LBG 0.2%  | 8.31 ± 0.66 a,A                           | 7.58 ± 0.53 a,A                             |
| LBG 0.3%  | 8.87 ± 0.37 a,A                           | 7.76 ± 0.31 a,B                             |
| LBG 0.4%  | 8.99 ± 0.25 a,A                           | 8.42 ± 0.13 a,A                             |

Means with the same capital letter, for the same response variable, in the same line did not differ significantly at p < 0.05 according to the Tukey test. Means with the same lower case letter, in the same column, did not differ significantly at p < 0.05 according to the Tukey test.

The hydrophilic nature of the polysaccharide added to protein film can reduce the protein network density and increases free volume and tortuosity of the structure. This change in network organization can result in reduction of water vapor barrier of films (SILVA et al., 2018). For instance, Sui et al. (2016) observed significant increase of 29% in WVP value of films containing 5g SPI when 0.5g guar gum was added. It was expected that more heterogeneous films and less cohesive (films with LBG addition) also presented higher porosity and, consequently, smaller water vapor barrier. However, the phase separation and increase of heterogeneity of films observed with LBG addition (Figure 1) seems do not influence structural cohesivity of the matrix protein-protein on protein enriched phase, and, therefore, it was not able to increase the free volume to the vapor diffusion in films. Silva et al. (2016) also observed that LBG addition did not also significantly affect WVP of whey protein isolate films. Mostafavi et al. (2016) observed that the increase of 0 to 100% (w/w) LBG concentration increased the compact structure of tragacanth gum-LBG based films, but reduced WVP values them. These results can indicate that the way of LBG interact in different matrix do not significant increase the free volume and the tortuosity of structure of the films. One of the possible explanations for this behavior is related to the mobility of LBG in the matrix and structure of films. Kurt & Kahyaoglu (2014) observed that LBG films presented water vapor barrier 25% greater than guar gum film due to greater molecular mobility of guar gum in structure of film. The reduction on water vapor barrier in SPI/guar-gum films observed by Sui et al. (2016) can be also related to higher mobility of guar gum in comparison with LBG in SPI films. Possibly, for this reason, LBG does not weaken the cohesiveness of the protein matrix and consequently do not increase WVP in protein based films.
3.4. MECHANICAL PROPERTIES

Tensile strength (TS) and elongation-at-break (EB) of SPI and SPC films with or without LBG addition are shown in Table 3.

Table 3. Values of tensile strength (TS), elongation at break (EB) for SPC (Soy Protein Concentrate) and SPI (Soy Protein Isolate) films, with glycerol, varying LBG (Locust Bean Gum) concentration.

| Treatment | SPC TS (MPa) | SPC EB (%) | SPI TS (MPa) | SPI EB (%) |
|-----------|--------------|------------|--------------|------------|
| LBG 0%    | 2.83 ± 0.26  | 97.36 ± 14.93 | 3.90 ± 0.67  | 34.34 ± 8.49 |
| LBG 0.05% | 3.33 ± 0.41  | 92.03 ± 11.14 | 3.57 ± 0.82  | 49.69 ± 7.93 |
| LBG 0.1%  | 3.27 ± 0.31  | 76.04 ± 11.94 | 5.01 ± 0.68  | 36.16 ± 9.06 |
| LBG 0.2%  | 3.12 ± 0.43  | 75.96 ± 8.15  | 5.03 ± 0.68  | 26.52 ± 7.18 |
| LBG 0.3%  | 5.14 ± 0.64  | 61.05 ± 9.62  | 4.81 ± 0.87  | 33.28 ± 6.39 |
| LBG 0.4%  | 5.64 ± 0.73  | 57.82 ± 7.76  | 5.52 ± 0.74  | 42.44 ± 6.70 |

Means with the same capital letter, for the same response variable, in the same line did not differ significantly at p < 0.05 according to the Tukey test. Means with the same lower case letter, in the same column, did not differ significantly at p < 0.05 according to the Tukey test.

Tensile strength of SPC film was 27% smaller than TS of SPI film, however EB was almost 3 times higher. SPC has lower protein content than SPI and for this reason it forms less covalent disulfide intermolecular bonds among polypeptide chains (TOTOSAUS, MONTEJANO, SALAZAR & GUERRERO, 2002) which results in weaker films. Moreover, the high fat and moisture content presented in SPC can have contributed to greater flexibility of films, since these compounds can also act as plasticizer (ATARÉS et al, 2010).

A little LBG (0.05%) addition to SPC films increased 18% TS value of the films, however it did not change statistically TS in SPI films. Despite SPI have higher protein content and for this reason results in films with higher TS than SPC based film, a little concentration (0.05%) of LBG added in SPC films could provide SPC films as resistant as SPI films, but cheaper, once the additional processes required to manufacture the isolate protein increase the cost of product. Mechanical property of SPC and SPI films were improved as LBG concentration increased. When 0.4% LBG was added, TS of SPI and SPC film was 1.5 and 2 times, respectively, higher than film without gum.

The phase separation between SPI or SPC and LBG (Figure 1) promoted an enriched phase in polysaccharide that can have filled spaces within the polymer network, increasing the intermolecular interaction and the cohesion between polymer chain and, consequently, improving the...
resistance of the films. Aggregation of polysaccharide in composite films SPI-agar was also observed by Garrido et al., (2016) in SEM images, however the aggregation observed resulted in an increase of stiffness of the films, with reduction of TS and EB values. Mostafavi et al. (2016) studied the combination ratios of tragacanth gum/ LBG (100/0; 75/25; 50/50; 25/75; 0/100) and observed that tragacanth gum-LBG films composite presented higher TS and EB as LBG concentration increased and related this result to more cohesive structure of film, observed in SEM analysis.

Despite of LBG addition promotes the cohesion between polymer chain, other factors like gum concentration and kind of compounds that interact with this polysaccharide must be considered when mechanical property of films is analyzed. In Table 2, for instance, it is possible to observe that the interaction between LBG and SPI or SPC influenced in different way in deformation of both films. The 0.4% LBG addition reduced 40% of the EB of SPC film, but increased 24% EB of SPI film. Even so, SPC film was still 36% more elastics than SPI film. Silva et al. (2016) observed that 0.1% LGB addition did not change significantly tensile strength of the (5%) WPI films, but increased elasticity of them. The same LBG concentration (0.1%), when added in (5%) SPI solution improved considerably TS but did not influence significantly the elasticity of the films (Table 2).

3.5. OPTICAL PROPERTIES

Color parameters (L*, a* and b*) are presented in Figure 2 and absorbance measurements normalized by the thickness of SPI and SPC films with and without LBG at 600nm are presented in Table 4.

Table 4. Values of Opacity (absorbance at 600nm in relation to thickness) and film’s thickness for SPC (Soy Protein Concentrate) and SPI (Soy Protein Isolate) films, with glycerol, varying LBG (Locust Bean Gum) concentration.

| Treatment | Opacity  | thickness (mm) |
|-----------|----------|----------------|
| LBG 0%    | 4.26 ± 0.21<sup>a,A</sup> | 0.17 ± 0.01<sup>ab,A</sup> |
| LBG 0.05% | 4.28 ± 0.18<sup>a,A</sup> | 0.17 ± 0.01<sup>ab,A</sup> |
| LBG 0.1%  | 4.36 ± 0.24<sup>a,A</sup> | 0.17 ± 0.01<sup>ab,A</sup> |
| LBG 0.2%  | 4.38 ± 0.17<sup>a,A</sup> | 0.18 ± 0.01<sup>abc,A</sup> |
| LBG 0.3%  | 4.42 ± 0.09<sup>a,A</sup> | 0.18 ± 0.00<sup>b,A</sup> |
| LBG 0.4%  | 4.44 ± 0.07<sup>a,A</sup> | 0.19 ± 0.00<sup>c,A</sup> |

| Treatment | Opacity  | thickness (mm) |
|-----------|----------|----------------|
| LBG 0%    | 1.95 ± 0.03<sup>a,B</sup> | 0.16 ± 0.01<sup>a,A</sup> |
| LBG 0.05% | 2.10 ± 0.13<sup>a,B</sup> | 0.15 ± 0.01<sup>a,A</sup> |
| LBG 0.1%  | 2.25 ± 0.03<sup>a,B</sup> | 0.15 ± 0.01<sup>a,A</sup> |
| LBG 0.2%  | 2.91 ± 0.15<sup>b,B</sup> | 0.19 ± 0.01<sup>b,A</sup> |
| LBG 0.3%  | 3.83 ± 0.35<sup>c,B</sup> | 0.16 ± 0.01<sup>a,B</sup> |
| LBG 0.4%  | 3.44 ± 0.27<sup>b,c,B</sup> | 0.20 ± 0.01<sup>b,A</sup> |
SPC films were twice more opaque (higher absorbance/thickness) than SPI films (Table 4). LBG addition reduced significantly the transparency of SPI films but did not influence ($p \leq 0.05$) the opacity of SPC films. SPI films became significantly opaquer (49%) from 0.2% LBG addition. The increase of LBG concentration from 0.2% to 0.3% resulted films 96%, approximately, less transparent than SPI films without gum addition, but when polysaccharide concentration was increased to 0.4% none significant change was observed, indicating that the increase in concentration was not enough to change the opacity of the films. Despite LBG addition increases SPI films opacity, SPC films were still more opaque. In this way, SPC films present higher potential to protect food against deteriorative reactions promoted by light than SPI films (with or without LBG addition). However, if the less yellowish color of SPI films (Figure 2a) is a desirable characteristic, LBG addition can be an alternative to increase light barrier property of this film.

Figure 2. Color parameters ($L^*$, $a^*$ and $b^*$) of SPC and SPI films with and without LBG addition. Mean ± SD. Means with the same capital letter, for the same response variable, did not differ significantly at $p < 0.05$ according to the Tukey test. Means with the same lower case letter did not differ significantly at $p < 0.05$ according to the Tukey test.
SPC film was 29% more yellowish (higher b* value) than SPI film. It was observed a significant reduction of the lightness (L* value) and the little change in color (a* and b* value) in both films with increase of LBG concentration (Figure 2). Silva, Garcia, Amado & Mauro (2015) observed that 5% (w/w) WPI+0.05% (w/w) LBG edible coating preserved vitamin C of pineapple during drying at 60°C. SPI-LBG (0.3% LBG) and SPC-LBG (0.4% LBG) were 3 and 3.7 times, respectively, opaquer than formulation used as edible coating by Silva et al. (2015) (5% WPI + 0.1% LBG) and characterized by Silva et al. (2016) (Abs/mm = 1.19 ±0.13). The application of SPC or SPI+LBG films as edible coating prior drying can be an alternative to enhance nutritional properties of dried fruit.

The results obtained in this research reveal that the protein source can to influence transparency films. Silva et al. (2016) observed that 0.1% LBG addition in (5%) WPI films increased almost four times the opacity of the films. In our work, films manufactured with 5% SPI also become opaquer with polysaccharide addition, but not in the same proportion.

4. CONCLUSIONS

It was possible to manufacture composite films with soy protein (isolate or concentrate) plus locust bean gum. The increase of LBG concentration influenced in different way the characteristics of SPC and SPI films. The solubility and film barrier to the light was increased in SPI films with LBG addition, but was not changed in SPC films with gum addition. In addition, polysaccharide addition reduced elongation at break value of SPC films but increased elasticity of SPI films. Despite this, all composite films formed were superior to the simple films in tensile strength even with surface structure less homogeneous surface structure. The LBG addition in SPC films resulted in films as resistant as SPI-LBG films, but cheaper, since the additional processes required to manufacture the isolate protein increase the cost of product. The lightness of composite films was smaller than the simple one, however water vapor barrier was similar. SPC films (without or with gum addition) and SPI+0.4% LBG films was the best options presented in this study to manufacture soluble sachets of edible films, to be used as edible food wrap or to employ as edible coating in food due to high solubility in water.
SPI and SPC films are an eco-sustainable alternative in the application and replacement of food packaging due to its great physical and barrier properties, being an option for replacing primary packaging or even applying active compounds to obtain active packaging.

Acknowledgment

We would like to thank, CNPQ for their financial support (Proc. 405406/2016-9), Fundação Araucária for their financial support (Proc. 10884) and the COMCAP/FINEP for FEG-MEV facilities.

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