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Production and characterization of *pinhão* starch biofilms

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ABSTRACT

The aim of this work was to prepare and evaluate the properties of biofilms made from *pinhão* starch in association with xanthan gum and glycerol by casting technique. Tests were run to determine the moisture content, the morphology, the thickness, the water solubility, the glass transition temperature and the gas permeability of these biofilms. Results showed that the biofilms presented appropriate moisture content, high water resistance and uniform thickness. The Scanning Electron Microscopy (SEM) showed not only the presence of cracks on the surface of the biofilm but also some starch granules that did not gelatinize. By Differential Scanning Calorimetry (DSC) was detected the glass transition temperature (-4.8 ± 0.1 °C) and different values of melting temperature (182.3 ± 2.1 °C and 134.9 ± 0.6 °C). The permeability to gases, N₂ and CO₂, was significantly different.

Key words: *Araucaria angustifolia*, edible films, physical properties

Produção e caracterização de biofilmes de amido de pinhão

RESUMO

O objetivo deste trabalho foi preparar e avaliar as propriedades dos biofilmes feitos a partir de amido de pinhão em associação com goma xantana e glicerol, pela técnica de casting. Testes foram realizados para determinar o teor de umidade, a morfologia, a espessura, a solubilidade em água, a temperatura de transição vítrea e a permeabilidade a gases do biofilme, cujos resultados mostraram que os biofilmes apresentaram teor de umidade adequado, alta resistência à água e espessura uniforme. A microscopia eletrônica de varredura (MEV) indicou não apenas a presença de fissuras na superfície do biofilme mas também alguns grânulos de amido não gelatinizados. Por Calorimetria Diferencial de varredura (DSC) foram detectados a temperatura de transição vítrea ($-4,8 \pm 0,1$ °C) e diferentes valores de temperatura de fusão ($182,3 \pm 2,1$ °C e $134,9 \pm 0,6$ °C). A permeabilidade aos gases, N₂ e CO₂, foi significativamente diferente.

Palavras-chave: *Araucaria angustifolia*, filmes comestíveis, propriedades físicas

Introduction

Production and utilization of biodegradable films and coatings prepared from various biological polymers such as polysaccharides, proteins, lipids or combinations of those components have received great interest in recent years. The films can be utilized in the food industry, grafts biosynthesis, suture and wound dressing production (Weber, 2001).

Up to now, the common polysaccharides of interest for material production have been cellulose, starch, gums, chitosan, xanthan, pullulan and curdlan (Weber, 2001). Films developed from starch were the subject of numerous research studies (Santos et al., 2001; Chen & Lai, 2008; Mathew & Abraham, 2008; Talja et al., 2008; Al-Hassan & Norziah, 2012). Starch based films are described as odorless, tasteless, colorless and display very low permeability to oxygen at low relative humidity (RH). The moisture barrier properties of starch based films were found relatively poor compared to those of edible waxes or low-density polyethylene films (Mathew & Abraham, 2008). The development of edible films and coatings from water soluble polysaccharides has also brought the valorization of agricultural by-products.

The *pinhão* is the seed of *Araucaria angustifolia*, a pine tree distributed in southern Brazil and Argentina in native forests that nowadays represent around 1% of the original area. In the present study, it was chosen the *pinhão* starch as raw material, since there are few studies have reported the use this polymer (Cladera-Olivera et al., 2009; Thys et al., 2010; Spada et al., 2012). Film-forming properties of *pinhão* starch have not been studied up to now, so the main objective of this study was to investigate properties of films produced with this polysaccharide.

Materials and Methods

Starch extraction

Araucaria angustifolia seeds used in this study were purchased at a local market (Porto Alegre, RS, Brazil). Starch extraction protocol followed the methodology developed by Spada et al. (2012) and moisture content of starch was calculated in accordance to AOAC protocol n° 925.10 (AOAC, 1995).

Film preparation

Solutions with starch, glycerol, xantan gum were processed into films in accordance with the methodology of Talja et al. (2008) who used the following steps to prepare the biofilms: heating and stirring of the film forming solution, removing of the air bubbles, casting of solution and oven drying. Talja et al. (2008) used different materials to prepare the films, then it was necessary to adjust the process parameters as time and temperature. *Pinhão* starch film-forming solutions (3%, w/v) were prepared by dissolving 7.5 g *pinhão* starch in 250 ml distilled water under stirring for 10 min at 40°C, followed by heating at 95 ± 1 °C for 10 min in thermostatic bath (Q226M, Quimis, Diadema, Brazil) to obtain gelatinized starch. An appropriate amount of plasticizer (2.25g of glycerol and 0.001g of xantan gum) was added after the solution became clear.

After 10 min, the beaker remained into water bath at 70 °C for 10 min to prevent bubble formation in the films upon casting. Then, 20 g of the solution was pipetted into a petri dish (Ø 9.0 cm) that was placed in an oven at 40 °C for 48 h to obtain the starch-based films by evaporating water. After drying of material, the petri dishes were placed in a desiccator above the saturated salt solution of NaCl. The desiccator was placed at 21 °C during one week (BOD TE 381, Tecnal, Piracicaba, Brazil). This procedure was needed for removing the biofilms from the petri plates. The treatment was made in triplicate.

Moisture content

Moisture content of biofilm was calculated through of the weight loss of 4 cm² sample after heated in a conventional oven at 105 °C (A3 DG Temp, De Leo, Bento Gonçalves, Brazil) until constant weight (about 24 h).

Thickness determination

Thickness of the films was measured using a micrometer (Mitutoyo, no. 293-521-30, Japan). Measurements were taken at five different locations on the films and the mean value was calculated.

Water solubility

Water solubility (WS) of the films was determined in accordance with Phan et al. (2005). Films were cut in pieces sized 20 mm x 20 mm and dried in an oven at 105 °C to constant weight and then weighed to obtain the initial film dry weight. The piece of film was placed into a beaker with 80 ml distilled water under magnetic stirring at 25 °C for 24 h. Then, the films were again placed in an oven at 105 °C to constant weight and weighed to obtain the final film dry weight. Tests were performed in triplicate and the solubility was calculated as follows:

$$WS(\%) = \frac{\text{Initial dry weight} - \text{Final dry weight}}{\text{Initial dry weight}} \times 100$$

Glass transition temperature

The glass transition temperature (T_g) of the films was determined by Differential Scanning Calorimetry (DSC) (DSC 6000 Scanning Calorimeter, Perkin Elmer). Small pieces (6-9 mg) of the films were cut and placed into a hermetically sealed DSC sample pan. The reference was an empty pan. Both sample and reference pans were cooled to -20 °C. The T_g was measured at a heating scan rate of 10 °C/min from -20 to 200 °C and identified as the mid-point temperature of a step-down shift in baseline due to change in heat capacity upon glass transition. The T_g was determined in triplicate and the results averaged. The melting temperature was also determined.

Microstructure analysis

Surface and cross section morphology were investigated by Scanning Electron Microscopy (SEM) (Hitachi, modelo TM 3000, Japan). The films were cut and attached to the stubs of 1 cm diameter using a two-sided adhesive tape. The surface and the cross section film were examined at different magnifications. An acceleration potential of 5 kV was used.

Permeability to CO₂ and N₂

The quasi-isostatic method used was based on the measurement of the amount of gas diffusing through a film. The experiment was performed under conditions in which total pressure difference across the test material was zero and the partial pressure difference for the test gas was approximately 1 atm. Biofilms with 20 µm of thickness were produced in an acrylic plate and dried for 3 days at 40 °C in an oven (Tecnal, model TE-381, Brazil). Soon after, the films were cut in round shape with a total area of 13 cm² and inserted into the module of the plant of permeation gases. The permeability to CO₂ and N₂ was evaluated and calculated as follows:

$$P = \frac{22.414 V P_{amb}}{RT t A (P_2 - P_1)}$$

where, 22.414 is the molar volume of the gas (cm³ mol⁻¹), R is the gas constant (6.24 cmHg mol⁻¹.K⁻¹), A is the permeation area (cm²), T is the gas temperature (K), V/t is the gas flow (cm³ s⁻¹), P_{amb} is the ambient pressure (cmHg) and P₂ and P₁ corresponded to the feed pressure and permeate pressure (cmHg), respectively.

Results and Discussion

The yield of the starch isolation process described here was around 19.4 ± 0.3% and the value of moisture content was 10.4 ± 0.2 %.

Moisture content

The moisture content of the biofilm was 10.8 ± 0.4%, similar to found by Petersson & Stading (2005), between 11 and 15%, for biofilms of mixed starch -monoglycerides. According to Bertuzzi et al. (2007), the differences in moisture content is mainly related to the biofilm composition. The moisture content of pinhão starch based films was found to be lower than those reported (from 11.1 to 15.6%). Different from this work, the authors ranged the quantity of glycerol, starch, agar and span 80. The authors verified that high concentrations of plasticizer favor the adsorption of water molecules due to its hydrophilic nature, which retains water in the film matrix and form hydrogen bonds (O-H). On the other hand, the moisture content decreased for films with higher concentrations of span 80 because surfactant incorporation reduces the mobility of the polysaccharide matrix and decreases the moisture content of the films.

Measurement of thickness

Measurement of thickness of the films is important to evaluate the uniformity of these materials, since the variations in the thickness of a material could cause problems in their mechanical performance and fluctuations in the barrier properties.

The thickness found was 0.11 ± 0.03 mm, showing the uniformity of the biofilm. This value was similar to the reported by Laohakunjit & Noomhorm (2004), who developed films of rice starch and glycerol and found values ranged from 0.100 to 0.109 mm. Liu et al. (2005) developed pea starch

films by extrusion and found higher thickness values (0.329 to 0.422 mm). These differences can be related to the technique and formulation of the biofilms. To Maran et al. (2013), the film thickness increased from 0.029 to 0.045 mm due to the increased concentration of starch, glycerol and agar in the film forming solution.

Water solubility

Film water solubility is an important property for the film application. Potential applications may require water insolubility to enhance the integrity and resistance of the some products. However, in some cases like food coating, high film water solubility is required before of the consumption of the product (Perez-Gago & Krochta, 2000). The biofilms prepared in this study have not lost their integrity in aqueous solution at the end of 24 h and their solubility was 18.7 ± 0.4%.

Wang et al. (2007) reported that films from potato starch presented solubility of 31.8%, which is higher than those observed in this study. Tongdeesoontorn et al. (2011) also reported higher water solubility values (73%) for films prepared with cassava starch and glycerol. According to Matta et al. (2011), the plasticizer addition, in particular glycerol has a great influence on starch films solubility, due to its hydrophilic character. Moreover, Mehyar & Han (2004) concluded that starches with higher amylose content has higher interacting force with glycerol molecules as well as higher starch-starch intermolecular force, for this reason some biofilms can have greater water resistance when compared to other with the same amount of glycerol.

Glass transition temperature

The DSC analysis detected the glass transition temperature (T_g) and the melting temperature (T_f) of the biofilm that is related to a significant movement of molecular chains due to the disintegration of the crystals by increasing the temperature.

The T_g value of the film was -4.8 ± 0.1 °C, at this temperature the structure of the biofilm becomes elastomeric. Therefore the biofilm has good mobility at room temperature, desired characteristic for use as packaging. The T_g values can be attributed to strong interaction between amylose and amylopectin and to the low content of plasticizer (0.9%). The T_g of gellan films decreased linearly from -84 to -92 °C as glycerol concentration in the film-forming solution increased from 60 to 80%. This behavior is probably the result of the plasticizing effect of the glycerol molecules which generally increased the free volume of the polymer network and the segmental mobility of polymer chains, thus depressing the T_g (Sperling, 1992; Gontard & Guilbert, 1994).

It was observed distinct peaks regarding to T_f. These results that can be related to homogeneity of filmogenic solution, as the results of SEM showed starch granules. Some samples showed T_f of 182.3 ± 2.1 °C, while others had lower T_f (134.9 ± 0.6 °C). Both results showed that the biofilm has good resistance to high temperatures.

Microstructure analysis

Figure 1 shows the photomicrographs of the film surface. Starch granules were observed, as well as the presence of

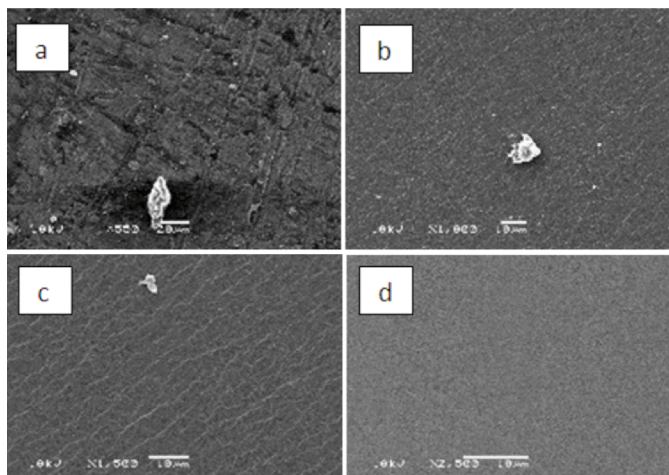


Figure 1. Photomicrographs of the surface of the biofilm at 550x (a), 1000x (b), 1500x (c) and 2500x (d)

cracks on the surface of the biofilm at 550 X. At 2500 X, it was observed homogeneity of the surface.

According to Zang & Han (2006), the starch biodegradable films are characterized by incomplete gelatinization even in excess water at high temperatures. The mixing process plays an important role in the morphology of these composites, therefore, the mechanical agitation used in this work did not produce appropriate shear force, which resulted in the presence of starch granules. Levya et al. (2008) and Zang & Han (2006) also observed the presence of fragments of granules in the micrographs of wheat starch films and yellow pea starch films, respectively.

Photomicrographs of the cross section of the biofilm were also obtained by SEM (Figure 2). The photomicrographs at 500, 600 and 700 X showed that the structure of the biofilm is dense; at 850 X it was seen small cracks in the structure that may be related to sample storage conditions or problems during drying by the use of forced convection or high temperature.

Permeability to CO₂ and N₂

ANOVA showed that CO₂ and N₂ permeabilities of films were significantly different ($p < 0.05$). Permeability to N₂

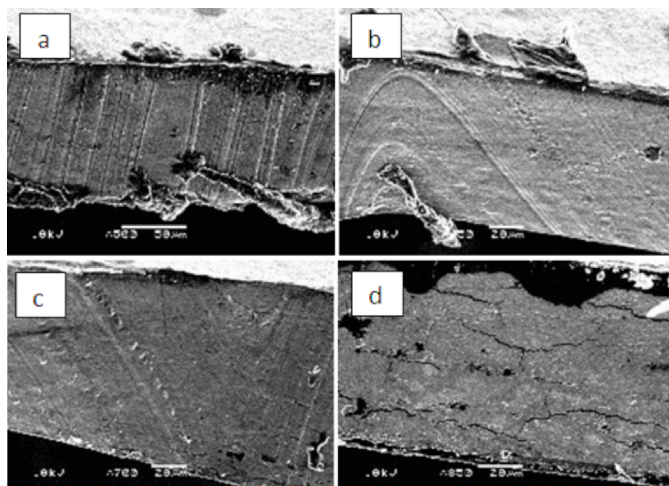


Figure 2. Photomicrographs of the cross section of the biofilm at 500x (a), 600x (b), 700x (c) and 850x (d)

and CO₂ were 42.7 ± 1.8 and 36.4 ± 1.6 barrer, respectively. Carbon dioxide (CO₂) permeability value of the films made with carboxymethylcellulose, glycerol and sunflower oil was 40.92 ± 0.95 barrer (Bifani et al, 2007), similar to reported in this work.

Lower values (between 3 and 29 barrer) regarding to CO₂ permeability were obtained for amylomaize and corn films either with sorbitol or glycerol (Garcia et. al., 2000). Permeability depends on the interaction between the polymer matrix and the permeating gas, as well as the environmental conditions such as temperature and relative humidity. Synthetic materials like LDPE (low-density polyethylene) also show lower CO₂ permeability (9.45 barrer).

It is noteworthy that environments with high levels of CO₂ and N₂ reduce the rates of respiration and ethylene production, promoting a delay in the deterioration of the food products such as fruits and vegetables. Therefore high permeability to these gases is required to the biofilms when used for this purpose. To verify the applicability of the biofilm in foods such as vegetables and fruits are needed permeability studies with other gases such as O₂ and ethylene.

Conclusions

It was possible to obtain a biofilm from *pinhão* starch that is a raw material renewable and biodegradable. The biofilms presented good water resistance when compared to other films of different sources of starch, uniform thickness and appropriate moisture content. The Scanning Electron Microscopy (SEM) showed the presence of cracks on the surface of the biofilm and some starch granules that did not gelatinize. The partial gelatinization process of starch granules was also observed by differential scanning calorimetry (DSC), since it was detected different values of melting temperature of the biofilm. Permeability studies require more attention, since other gases such as ethylene and O₂ should be evaluated. The permeability to N₂ and CO₂ was similar, but presented difference statistic. This paper presents results that can be used in future works aimed at optimization of the characteristics of the *pinhão* starch biofilm, as well as its use in food.

Literature Cited

- Al-Hassan, A. A.; Norziah, M. H. Starch-gelatin edible films: Water vapor permeability and mechanical properties as affected by plasticizers. *Food Hydrocolloids*, v.26, n.1, p.108-117, 2012.< <http://dx.doi.org/10.1016/j.foodhyd.2011.04.015>>.
- Association of Official Agricultural Chemists - AOAC. Official methods of analysis of the Association of Official Agricultural Chemists. 16.ed. Arlington: AOAC, 1995. 1025p.
- Bertuzzi, M. A.; Armada, M.; Gottifredi, J. C. Physicochemical characterization of starch based films. *Journal of Food Engineering*, v.82, n.1, p.17-25, 2007.<<http://dx.doi.org/10.1016/j.jfoodeng.2006.12.016>>.

- Bifani, V.; Ramírez, C.; Ihla, M.; Rubilara, M.; García, A.; Zaritzky, N. Effects of murta (*Ugni molinae* Turcz) extract on gas and water vapor permeability of carboxymethylcellulose-based edible films. *LWT - Food Science and Technology*, v.40, n.8, p.1473-1481, 2007. <<http://dx.doi.org/10.1016/j.lwt.2006.03.011>>.
- Chen, L. S.; Lai, C. H. Mechanical and water vapour barrier properties of tapioca starch/decolorized hsian-tsao leaf gum films in the presence of plasticizer. *Food Hydrocolloids*, v.22, n.8, p.1584-1595, 2008. <<http://dx.doi.org/10.1016/j.foodhyd.2007.11.006>>.
- Cladera-Olivera, F.; Marczak, L. D. F.; Noreña, C. P. Z.; Pettermann, A. C. Modeling water adsorption isotherms of pinhão (*Araucaria angustifolia* seeds) flour and thermodynamic analysis of the adsorption process. *Journal of Food Process Engineering*, v.34, n.3, p.826-843, 2009. <<http://dx.doi.org/10.1111/j.1745-4530.2009.00437.x>>.
- García, M. A.; Martino, M. N.; Zaritzky, N. E. Lipid addition to improve barrier properties of edible starch-based films and coatings. *Journal of Food Science*, v.65, n.6, p.941-947, 2000. <<http://dx.doi.org/10.1111/j.1365-2621.2000.tb09397.x>>.
- Gontard, N.; Guilbert, S. Biopackaging: technology and properties of edible and/or biodegradable material of agricultural origin. In Mathlouthi, M. (Ed.). *Food packaging and preservation*. London: Blackie Academic & Professional, 1994. P.159-181.
- Laohakunjit, N.; Noomhorm, A. Effect of plasticizer on mechanical and barrier properties of rice starch film. *Starch/Stärke*, v.56, n.8, p.348-356, 2004. <<http://dx.doi.org/10.1002/star.200300249>>.
- Leyva, M. B.; Chávez, P. T.; Wong, B. R.; Jatomea, M. P.; Bojórquez, F. B. (2008). Physical and mechanical properties of durum wheat (*triticum durum*) starch films prepared with a and b type granules. *Starch/Stärke*, v.60, n.10, p.559-567, 2008. <<http://dx.doi.org/10.1002/star.200800227>>.
- Liu, L.; Kerry, J. F.; Kerry, J. P. Selection of optimum extrusion technology parameters in the manufacture of edible/biodegradable packaging films derived from food-based polymers. *Journal of Food, Agriculture & Environment*, v.3, n.3-4, p.51-58, 2005. <<http://world-food.net/selection-of-optimum-extrusion-technology-parameters-in-the-manufacture-of-edible-biodegradable-packaging-films-derived-from-food-based-polymers/>>. 15 Feb. 2014.
- Maran, J. P.; Sivakumar, V.; Sridhar, R.; Thiruganasambandham, K. Development of model for barrier and optical properties of tapioca starch based edible films. *Carbohydrate Polymers*, v.92, n.2, p.1335-1347, 2013. <<http://dx.doi.org/10.1016/j.carbpol.2012.09.069>>.
- Mathew, S.; Abraham, T. E. Characterisation of ferulic acid incorporated starch-chitosan blend films. *Food Hydrocolloids*, v.22, n.5, p.826-835, 2008. <<http://dx.doi.org/10.1016/j.foodhyd.2007.03.012>>.
- Matta, M. D. Jr.; Sarmiento, S. B. S.; Sarantópoulos, C. I. G. L.; Zocchi, S. S. (2011). Barrier properties of films of pea starch associated with xanthan gum and glycerol. *Polímeros*, v.21 n.1, p.67-72, 2011. <<http://dx.doi.org/10.1590/S0104-14282011005000011>>.
- Mehyar, G. F.; Han, J. H. Physical and mechanical properties of high-amilose rice and pea starch films as affected by relative humidity and plasticizer. *Journal of Food Science*, v.69, n.9, p.449-454, 2004. <<http://dx.doi.org/10.1111/j.1365-2621.2004.tb09929.x>>.
- Perez-Gago, M. B.; Krochta, J. M. Drying temperature effect on water vapor permeability and mechanical properties of whey protein-lipid emulsion films. *Journal of Agricultural and Food Chemistry*, v.48, n.7, p.2687-2692, 2000. <<http://dx.doi.org/10.1021/jf0001583>>.
- Petersson, M.; Stading, M. Water vapour permeability and mechanical properties of mixed starch-monoglyceride films and effect of film forming conditions. *Food Hydrocolloids*, v.19, n.1, p.123-132, 2005. <<http://dx.doi.org/10.1016/j.foodhyd.2004.04.021>>.
- Phan, T. D.; Debeaufort, F.; Luu, D.; Voilley, A. Functional properties of edible agar-based and starch-based films for food quality preservation. *Journal of Agricultural and Food Chemistry*, v.53, n.4, p. 973-981, 2005. <<http://dx.doi.org/10.1021/jf040309s>>.
- Santos, A. E. O. dos; Assis, J. S. de; Berbert, P. A.; Santos, O. O. dos; Batista, P. F.; Gravina, G. de A. Influência de biofilmes de fécula de mandioca e amido de milho na qualidade pós-colheita de mangas 'Tommy Atkins'. *Revista Brasileira de Ciências Agrárias*, v.6, n.3, p.508-513, 2011. <<http://dx.doi.org/10.5039/agraria.v6i3a755>>.
- Spada, J. C.; Marczak, L. D. F.; Tessaro, I. C.; Noreña, C. P. Z. Microencapsulation of carotene using native pinhão starch, modified pinhão starch and gelatin by freeze-drying. *International Journal of Food Science and Technology*, v.47, n.1, p.186-194, 2012. <<http://dx.doi.org/10.1111/j.1365-2621.2011.02825.x>>.
- Sperling, L. H. Glass rubber transition behavior. In: Sperling, L. H. (Ed.). *Introduction to physical polymer science*. 2.ed. New York: John Wiley & Sons Inc, 1992. Chap.8, p.349-428.
- Talja, R. A.; Helen, H.; Roos, Y.; Joupplia, K. Effect of type and content of binary polyol mixtures on physical and mechanical properties of starch-based edible films. *Carbohydrate Polymers*, v.71, n.3, p.269-276, 2008. <<http://dx.doi.org/10.1016/j.carbpol.2006.05.019>>.
- Thys, R. C. S.; Noreña, C. P. Z.; Marczak, L. D. F.; Aires, A. G.; Cladera-Olivera, F. Adsorption isotherms of pinhão (*Araucaria angustifolia* seeds) starch and thermodynamic analysis. *Journal of Food Engineering*, v.100, n.3, p.468-473, 2010. <<http://dx.doi.org/10.1016/j.jfoodeng.2010.04.033>>.
- Tongdeesoontorn, W.; Mauer, L. J.; Wongruong, S.; Sriburi, P.; Rachtanapun, P. *Chemistry Central Journal*, v.5, n.6, p.1-8, 2011. <<http://dx.doi.org/10.1186/1752-153X-5-6>>.
- Wang, J.; Zhengyu, J.; Xiaoping, Y. Preparation of resistant starch from starch-guar gum extrudates and their properties. *Food Chemistry*, v.101, n.1, p.20-25, 2007. <<http://dx.doi.org/10.1016/j.foodchem.2006.01.005>>.
- Weber, C. Properties of biobased packaging materials. In: Weber, C. (Ed.), *Biobased packaging materials for the food industry*. Copenhagen: KVL Department of Dairy and Food Science, 2001. Chap.2, p. 13-39.
- Zhang, Y.; Han, J. H. Mechanical and thermal characteristics of pea starch films plasticized with monosaccharides and polyols. *Journal of Food Science*, v.71, n.2, p.109-118, 2006. <<http://dx.doi.org/10.1111/j.1365-2621.2006.tb08891.x>>.