

INCORPORATION OF ACTIVE COMPOUNDS FROM MANGO PEEL (*Mangifera indica* L. Cv. “Tommy Atkins”) INTO CORN STARCH–BASED ORAL DISINTEGRATING FILMS

A.P. GUERRA[†], C.M. BITENCOURT CERVI[‡],
V.A. DOS SANTOS GARCIA[†] and C. DA SILVA[†]

[†] Departamento de Tecnologia, Universidade Estadual de Maringá (UEM), 87506-370, Umuarama, PR, Brasil.

[‡] Departamento de Engenharia de Alimentos, Universidade Estadual de São Paulo (USP), 13635-900, Umuarama, PR, Brasil.

Email: anapaula-guerra2012@hotmail.com, camilabitencourt_@hotmail.com, garcia.vitoraugusto@gmail.com, camiladasilva.eq@gmail.com

Abstract— This study aimed to evaluate the incorporation of mango peel extract, as a source of natural active compounds, in oral disintegrating films (ODF). The starch-based ODF were produced using a casting technique with glycerol as a plasticizer. Four formulations were tested by varying the amount of extract in the films: control, ODF 20, ODF 40, and ODF 60 (0, 20, 40, and 60 g of extract per 100 g of filmogenic solution, respectively). The surface pH, disintegration time, total phenolic content, antioxidant activity, release of total phenolic content, and stability of the ODF was evaluated. All formulations studied could be classified as fast disintegrating films and source of phenolic compounds. ODF 60 showed the largest, most stable release of phenolic compounds under accelerated conditions (40 °C and 75% relative humidity). Thus, corn starch-based oral disintegrating films containing mango peel extract represent a useful vehicle for the delivery of phenolic compounds in the oral cavity.

Keywords— Natural extracts, phenolic compounds, polymers..

I. INTRODUCTION

Due to its warm climate and favorable planting conditions, Brazil is home to a wide variety of fruits, including the mango, which can be consumed in whole or in pulp, juice, nectar, candy and jam (Canuto, 2009). The processing of mangos generates large amounts of waste, including bark (12–15% of the fruit weight) and almonds (15–25%) (Arbos *et al.*, 2013). Several authors have reported the antioxidant activity of mango peel (Guo *et al.*, 2003; Huber *et al.*, 2012; Arbos *et al.*, 2013) mainly due to the presence of compounds such as glycosides, flavonols and xanthenes (Leontowicz *et al.*, 2003).

Oral disintegrating films (ODF) represent a potential route of administration of pharmaceutical phenolic compounds (Borges and Carvalho, 2015) since they can overcome common problems, including stability and difficulty in swallowing, which are associated with conventional oral dosage forms (Buanz *et al.*, 2015). ODF are thin films designed to dissolve in the buccal cavity and release the active component (Dixit and Puthli, 2009).

They can be produced from various polymers depending on the physicochemical properties of the active component of interest (Dixit and Puthli, 2009). Starch is considered to be an effective polymer for the production of ODF because it is nontoxic, biodegradable, renewable and inexpensive compared to other polymers (Da Róz, 2003).

The delivery of active compounds incorporated in films, through the buccal mucosa, has the advantage of increasing bioavailability, since the compounds do not suffer enzymatic degradation and pH variations in the gastrointestinal tract (Figueiras and Veiga, 2006). In the literature, there are few studies that report the addition of natural extracts to ODF; however, propolis (Juliano *et al.*, 2007; Borges and Carvalho, 2015; Borges *et al.*, 2016), and ginger (Daud *et al.*, 2011) have been previously studied for such usage.

In this context, this study aimed to develop and characterize starch-based ODF containing mango peel extract as a vehicle for the delivery of phenolic compounds.

II. METHODS

A. Material

Mangoes (*Mangifera indica* L. cv. “Tommy Atkins”) were obtained from the local market of Umuarama (Paraná, Brazil). The fruits were selected, washed, and peeled (with the aid of knives). The samples of mango peel were dried in an oven with circulation and air exchange (Marconi/MA035/5) at 60 °C for 17 h. Samples were then ground in knife mills (Cadence) and particle size separation was performed using a Tyler type sieve and mechanical stirrer (Marconi/MA750). Samples with an average diameter of 0.300 mm were selected for conducting the experiments.

B. Extraction of phenolics compounds

The extraction of phenolic compounds from mango peel was conducted in an ultrasound bath (Ultrasonic Q5. 9/40 A, frequency of 40 KHz, potency of 123 W) at 60°C for 60 min and 50% of ethyl alcohol (Anidrol).

C. Production of oral disintegration films

The films were produced using a casting technique with corn starch (Cargill Agrícola S/A) and glycerol as the polymer and plasticizer (Synth), respectively. The corn

starch, at a concentration of 2 g per 100 g of filmogenic solution (FS), was dispersed in distilled water under magnetic stirring for 30 min at ambient temperature. Posteriorly, the FS was kept in a thermostatic bath (Marconi/MA159) at 90 °C for 10 min. After this period, the glycerol (0.4 g 100 g⁻¹ of FS) and mango peel extract were added under magnetic stirring. The mango peel extract was added at different concentrations: 0 (control), 20 (ODF 20), 40 (ODF 40), and 60 (ODF 60) g 100 g⁻¹ FS. The solutions were placed into disposable polystyrene petri dishes and subjected to oven drying (Marconi/MA035/5) at 30 °C for 24 h.

D. Disintegration time

The disintegration time was determined according to Garsuch and Breitzkreutz (2010). Samples of ODF (2 x 3 cm) were placed into a petri dish, using a slide frame as a support. A fixed volume of distilled water (200 µL) was deposited on the film surface (Janßen *et al.*, 2013) and the time needed for the drop to dissolve and form an orifice was determined.

E. Surface pH

The surface pH was determined using phosphate buffered saline according to Föger *et al.* (2008) for saliva simulation (pH 6.8). The phosphate buffer solution was prepared using 8 g of sodium chloride (Synth), 0.2 g of potassium chloride, 0.2 g of potassium phosphate monobasic (Vetec Química Fina Ltda) and 1.536 g of sodium phosphate dibasic (Vetec Química Fina Ltda). Samples of ODF (2 x 1 cm) were placed in glass containers containing 15 mL of phosphate buffer solution (Prabhu *et al.*, 2015) and the pH was determined after 0, 0.5, 1.5, 2, 3, 4, 5, 7, 10, 12, and 15 min.

F. Total phenolic content

The total phenolic content was determined using the spectrophotometric method proposed by Singleton *et al.* (1999) with Folin-Ciocalteu reagent (Sigma Aldrich), anhydrous sodium carbonate (Synth) and a gallic acid standard (Sigma Aldrich). Samples of ODF (approximately 0.2 g) were dissolved in 10 mL of distilled water and maintained in an ultrasound bath for 10 min. Subsequently, the samples were centrifuged (3500 rpm/10 min). Aliquots of the solution (0.5 mL) were added to 2.5 mL of Folin's reagent (1:10, diluted in distilled water). After 5 min, 2.0 mL of calcium carbonate (7.5%) were added. The solution was homogenized and kept in the dark for 2 h. The absorbance at 740 nm was read on spectrophotometer (Kazuaki/IL-227), and the results were expressed in equivalent mg gallic acid equivalent g ODF⁻¹.

G. Iron reduction method (FRAP)

The antioxidant capacity by iron reduction method (FRAP) was determined according to Benzie and Strain (1996). FRAP reagent was prepared at the time of analysis from 0.3 M acetate buffer (25 mL), 10 mM TPTZ solution (Tris (2-pyridyl)-s-triazine, Sigma Aldrich) (2.5 mL), and an aqueous solution of 20 mM ferric chloride (Synth) (2.5 mL). Samples of ODF (~0.4 g) were solubilized in 10 mL of distilled water and kept in an ultrasound bath for 10 min, and then centrifuged (3500 rpm for 10

min). Posteriorly, 150 µL of this solution was added to 2850 µL FRAP reagent and the solution was incubated (37 °C for 30 min) in a thermostatic bath (Forlab, Dubnoff FL157/22R). The absorbance of the samples at 595 nm was read using a spectrophotometer (Kazuaki/IL-227) at 595 nm. Trolox (Sigma Aldrich) was used for an analytical curve and the results were expressed in µmol Trolox equivalent g ODF⁻¹.

H. Iron reduction method (FRAP)

To evaluate the release of total phenolic content, samples of ODF (~0.6 g) were placed in phosphate buffered saline solution (pH 6.8) (Wong *et al.*, 1999) at 37 °C and kept under stirring at 100 rpm (Perumal *et al.*, 2008). At different time intervals (0, 2, 3, 4, 5, 10, 15, and 20 min) aliquots of the solution (0.5 mL) were removed and the total phenolic content was determined using a spectrophotometric method (Singleton *et al.*, 1999). Kinetic data of the re-lease of total phenolic content was related to Eq. (1) (Korsmeyer and Peppas, 1981).

$$\frac{M_t}{M_\infty} = Kt^n + b \quad (1)$$

where M_t/M_∞ is the fraction of compounds released over time t , K is the kinetic constant, b is the initial concentration of phenolic compounds in ODF and n is the exponent of drug release.

I. Accelerated stability

The ODF that had the lowest disintegration time and greatest concentration of total phenolic content was stored at 40 °C in a relative humidity of 75% using a BOD incubator (Lucadema/Luca 161/04) to evaluate the stability of the film relative to the of total phenolic content over a period of 0, 3, 7, 10, 15, 20, and 30 days.

J. Statistical analysis

Statistical analysis was assessed using a mean Tukey's test using the SAS software (version 9.2, SAS, Inc) at the level of 95%.

III. RESULTS AND DISCUSSION

A. Disintegration time

ODF presented film-forming capacity, easy maneuverability (easy removal of the film of the plates), homogeneity and absence of insoluble particles, independent of the concentration of mango peel extract used. The disintegrating times of the films were 40.0 ± 1.8 seconds (control), 32.2 ± 2.7 seconds (ODF 20), 29.5 ± 3.6 seconds (ODF 40) and 22.4 ± 2.9 s (ODF 60), demonstrating statistically significant differences ($p < 0.05$) among different extract concentrations.

According to Jyoti *et al.* (2011), fast disintegrating films are characterized by a disintegration time of less than 60 seconds. All formulations evaluated in this study could be classified as rapidly disintegrating. This may be due to the high content, in mango peel extract, of phenolic compounds which have a high hydrophilicity due to the number of hydroxyl groups present in their structure (Angelo and Jorge, 2007).

Other studies have reported similar disintegration times for ODF containing starch or other natural poly-

mers. Garsuch and Breitreutz (2010) observed a disintegration time of 40 seconds for ODF composed of different polymers incorporated with caffeine and caffeine citrate. Liew *et al.* (2012) reported disintegration times between 39 and 47 seconds for ODF developed from hydroxypropyl methyl cellulose, corn starch and polyethylene glycol. Borges *et al.* (2016) showed that incorporation of hydrolyzed collagen and increasing concentrations of the propolis ethanol extract significantly reduced the disintegration time for gelatin–lecithin–based ODF, to between 18.9 and 25.3 seconds.

B. Surface pH

The surface pH of ODF was determined to identify possible side effects during the use of ODF by humans, since the acid or alkaline pH may cause irritation of the buccal mucosa (Bottenberg *et al.*, 1999). The surface pH of the ODF remained close to neutrality, with values between 6.65 and 7.65 after 15 min, independent of the mango peel extract concentration.

These results suggest that all the formulations studied here are suitable for human use, since they did not vary in pH over time and there were no significant differences between the pH of the control ODF and those containing mango peel extract. However, pH is not the only parameter that must be evaluated in order to ensure suitability for human use

C. Surface pH

Table 1 shows the power iron reducing and total phenolic content of ODF incorporated with mango peel extract. Increasing concentrations of mango peel extract in starch-based ODF favored the antioxidant activity of the films. This may be because the antioxidant capacity is related to the presence of antioxidant compounds in the mango extract, suggesting that the method used to produce ODF in this study did not cause degradation of the total phenolic compounds.

Consistent with our findings, Juliano *et al.* (2007) and Borges and Carvalho (2015) reported that ODF developed through incorporation of the propolis extract are sources of phenolic compounds, since ODF showed high levels of phenolic compounds even after the drying process.

D. Release of total phenolic content

Figure 1 shows the kinetics of phenolic compound release from starch-based ODF incorporated with mango peel extract. In all formulations, the maximum release of total phenolic content occurred at 3 min, with no significant difference ($p > 0.05$) after this period, while the ODF with the highest concentration peel of mango extract (ODF 60), showed higher release.

We suspect that greater concentrations of mango peel extract favoured the solubility of the film in aqueous media and consequently the release of phenolic compounds. These results corroborate those observed for the disintegration time. Borges and Carvalho (2015) found that the addition of a higher concentration of ethanol extract of propolis also caused increased release of phenolic compounds and shorter disintegration times.

Table 1. Total phenolic content (TPC) and power iron reducing (FRAP) of oral disintegrating films with addition of different peel of mango extract concentrations.

Formulations	TPC*	FRAP**
	mg GAE g ODF ⁻¹	μmol TE g ODF ⁻¹
ODF 20	0.41±0.02 ^a	436.4±3.28 ^a
ODF 40	0.56±0.02 ^b	549.2±22.46 ^b
ODF 60	1.36±0.02 ^c	726.4±14.06 ^c

Means followed by same lowercase letters (in the same column) did not differ statistically ($p > 0.05$).

* mg gallic acid equivalent g oral disintegrating film⁻¹

** μmol Trolox equivalent g oral disintegrating film⁻¹

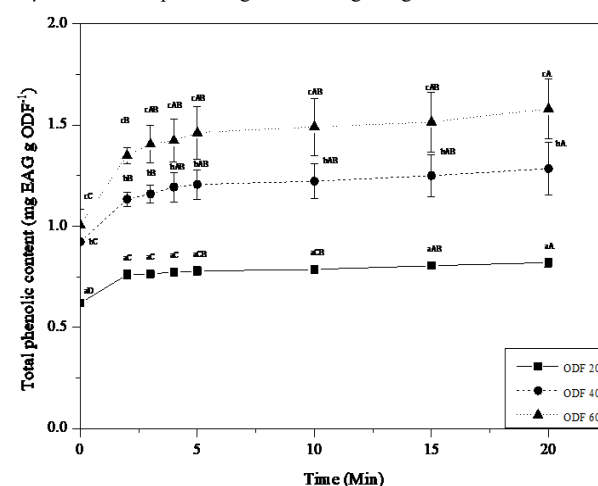


Figure 1. Release of total phenolic content in the oral disintegrating films with addition of different peel of mango extract concentrations a function of time.

Means followed by same lowercase letters (in the different formulations) and uppercase letters (in the same formulations) did not differ statistically ($p > 0.05$).

For all formulations studied, the data release of compound phenolics set the mathematical model Korsmeyer and Peppas (1981) depending on the R² values of 0.89, 0.96 and 0.95 for ODF 20, ODF 40 and ODF 60, respectively. The ODF containing the highest concentration of mango peel extract (ODF 60) showed a higher K value (1.30), indicating a faster release of total phenolic content compared to ODF 20 and ODF 40, which showed K values of 1.13 and 1.24, respectively.

E. Accelerated stability of oral disintegration films

Figure 2 shows the accelerated stability of the ODF 60, measured from the concentration of phenolic compounds as a function of time. This value was measured for ODF 60 because this formulation showed the lowest disintegration time (22.4 seconds) and antioxidant activity (Table 1).

Figure 2 demonstrates that, in the first three days of storage, there was no significant difference in the concentration of phenolics ($p > 0.05$), however, from the third day a significant reduction in the concentration of total phenolic content was observed. At the end of the storage period (30 days), there was a 42% reduction in the total phenolic content. Factors such as temperature can accelerate the loss of antioxidant compounds from ODF and the use of polymers can minimize this loss by aiding in the preservation of antioxidants.

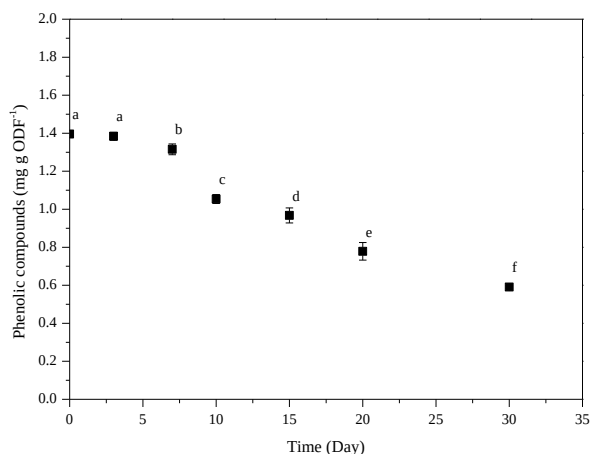


Figure 2. Effect of storage time (days) total phenolic content in the based starch ODF with 60 g of peel of mango extract 100 g-1 of filmogenic solution.

Same lowercase letters did not differ statistically ($p > 0.05$).

According to Bordenave *et al.* (2014) the interactions between starch and phenolic compounds are non-covalent bonds, involving hydrogen bonds, hydrophobic interaction, and electrostatic and ionic interactions, being strongly affected according to storage conditions, mainly by temperature (Zhu, 2015), thus the temperature (40°C) and relative humidity (75%), possibly influenced the bonds formed between the starch and phenolic compounds, reducing their concentration according to time.

Chandrasekhar *et al.* (2009) reported that the development of ODF with matrices that make them stable active compounds minimizes the cost of packaging to protect the embedded compounds and Schmidt *et al.* (2013) reported that starch-based films have good barrier properties against oxygen, but are sensitive to moisture. Thus, the use of ODF developed from starch can minimize the degradation of total phenolic content, especially in the first days of storage.

IV. CONCLUSIONS

This study demonstrated the possibility of producing starch-based ODF incorporated with the extract from mango peels. The developed formulations showed reduced disintegration time, a surface pH close to neutral and antioxidant activity. In general, ODF incorporated with mango peel extract can be classified as a novel vehicle for the release of active natural compounds.

REFERENCES

Angelo, P.M. and N. Jorge, "Compostos fenólicos em alimentos – Uma breve revisão," *Revista Instituto Adolfo Lutz*, **66**, 232-240 (2007).

Arbos, K.A., P.A. Stevani and R.F. Castanha, "Atividade antimicrobiana, antioxidante e teor de compostos fenólicos em casca e amêndoa de frutos de manga," *Revista Ceres.*, **60**, 161-165 (2015).

Benzie, I.F.F. and J.J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay," *Analytical bio-chemistry*, **239**, 70-76 (1999).

Bordenave, N., B.R. Hamaker and M.G. Ferruzzi, "Nature and consequences of non-covalent interactions between flavonoids and macronutrients in foods," *Food & Function*, **N**, 18-34 (2014).

Borges, J.G. and R.A. Carvalho, "Orally Disintegrating Films Containing Propolis: Properties and Release Profile," *Pharmaceutics, Drug Delivery and Pharmaceutical Technology*, **104**, 1431-1439 (2015).

Borges, J.G., A.G. Silva, C.M. Cervi-Bitencourt, F.M. Vanin and R.A. Carvalho, "Lecithin, gelatin and hydrolyzed collagen orally disintegrating films: functional properties," *International Journal of Biological Macromolecules*, **86**, 907-916 (2016).

Bottenberg, P., R. Cleymaet, C. Muynck, J.P. Remon, D. Coomans, Y. Michotte and D. Slop, "Development and testing of bioadhesive, fluoride-containing slow release tablets for oral use," *Journal of Pharmaceutical Pharmacology*, **43**, 457-464 (1999).

Buanz, A.B., C.C. Belaunde, N. Soutari, C. Tuleu, M.O. Gul and S. Gaisford, "Ink-jet printing versus solvent casting to prepare oral films: effect on mechanical properties and physical stability," *International Journal Pharmaceutics*, **28**, 2386-2392 (2015).

Canuto, K.M., "Propriedades Químicas e Farmacológicas de Mangiferina: um composto bioativo de manga (*Mangifera indica* L.)," *Documentos online Embrapa*, **21**, 6-27 (2009).

Chandrasekhar, R., Z. Hassan, F. Alhusban, A.M. Smith and A.R. Mohammed, "The role of formulation excipients in the development of lyophilised fast-disintegrating tablets," *European Journal of Pharmaceutics and Biopharmaceutics*, **72**, 119-129 (2009).

Da Róz, A.I., "Prepared biodegradable plastics from starch," *Polymers*, **13**, 4 (2003).

Daud, A.S., N.P. Sapkal and M.N. Bonde, "Development of *Zingiber officinale* in oral dissolving films: effect of polymers on *in vitro*, *in vivo* parameters and clinical efficacy," *Asian Journal Pharmaceutical*, **5**, 183-189 (2011).

Dixit, R. P. and S.P. Puthli, "Oral strip technology: Overview and future potential," *Journal of Control Release*, **139**, 94-107 (2009).

Figueiras, A. and F. Veiga, "A mucosa bucal como uma via de administração alternativa: Potencialidades, limitações e formas farmacêuticas," *Revista Lusófona de Ciências e Tecnologia da Saúde*, **3**, 87-102 (2006).

Föger, F.A., A. Kopf, B. Loretz, K. Albrecht and A. Bernkop-Schnürch, "Correlation of *in vitro* and *in vivo* models for the oral absorption of peptide drugs," *Amino Acids*, **35**, 333-241 (2008).

Garsuch, V. and J. Breitreutz, "Comparative investigations on different polymers for the preparation of fast-dissolving oral films," *Journal of Pharmaceutical Pharmacology*, **62**, 539-545 (2010).

Guo, C., J. Yang, J. Wei, Y. Li, J. Xu and Y. Jiang, "Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay," *Nutrition Research*, **23**, 1719-1726 (2003).

- Huber, K., J.H. Queiroz, A.V.B. Moreira and S.M.R. Ribeiro, "Caracterização química do resíduo agroindustrial da Manga Ubá (*Mangifera indica* L.): uma perspectiva para a obtenção de antioxidantes naturais," *Revista Brasileira de Tecnologia Industrial*, **6**, 640-650 (2012).
 - Janßen, E.M., R. Schliephacke, A. Breitenbach and J. Breitreutz, "Drug-printing by flexographic printing technology - A new manufacturing process for orodispersible films," *International Journal Pharmaceutics*, **441**, 818-825 (2013).
 - Jyoti, A., S. Gurpreet, S. Seema and A. Rana, "Fast dissolving films: a novel approach to oral drug delivery," *International Research Journal of Pharmacy*, **2**, 69-74 (2011).
 - Juliano, C., C.L. Pala and M. Cossu, M. "Preparation and characterization of polymeric films containing propolis," *Journal of Drug Delivery Science and Technology*, **17**, 177-181 (2007).
 - Korsmeyer, R.W. and N.A. Peppas, "Effect of the morphology of hydrophilic polymeric matrices on the diffusion and release of water soluble drugs," *Journal of Membrane Science*, **9**, 211-227 (1981).
 - Leontowicz, M., S. Gorinstein, H. Leontowicz, R. Krzeminski, A. Lojek, E. Katrich, M. Cíz, O. Martin-Belloso, R. Soliva-Fortuny, R. Haruenkit and S. Trakhtenberg, "Apple and pear and pulp and their influence on plasma lipids and antioxidant potentials in rats fed cholesterol-containing diets," *Journal of Agricultural and Food Chemistry*, **51**, 5780-5785 (2003).
 - Liew, K. B., Y.T. Tan and K.K. Peh, "Characterization of oral disintegrating film containing donepezil for Alzheimer disease," *AAPS PharmSciTechn*, **13**, 134-142 (2012).
 - Prabhu, P., A. Dubey and K. Kamath, "Formulation and evaluation of fast-dissolving films of Lisinopril," *Egyptian Pharmaceutical Journal*, **14**, 56-64 (2015).
 - Perumal, V.A., D. Lutchamn, I. Mackraj and T. Govender, "Formulation of monolayer films with drug and polymers of opposing solubilities," *International Journal Pharmaceutics*, **358**, 184-191 (2008).
 - Schmidt, V.C.R., M.L. Porto, J.B. Laurindo and F.C: Menegalli, "Water vapor barrier and mechanical properties of starch films containing stearic acid," *Industrial Crops and Products*, **41**, 227-234 (2013).
 - Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventós, "Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent," *Methods in Enzymology*, **299**, 152-178 (1999).
 - Wong, C.F., K.H. Yuen and K.K. Peh, "Formulation and evaluation of controlled release Eudragit buccal patches," *International Journal of Pharmaceutics*, **178**, 11-22 (1999).
 - Zhu, F., "Interactions between starch and phenolic compound," *Trends in Food Science & Technology*, **43**, 129-143 (2015).
- Received: August 20, 2017.**
Sent to Subject Editor: February 01, 2018.
Accepted: August 15, 2018.
Recommended by Subject Editor María L. Ferreira.