



## Development and application of edible film of active potato starch to extend mini panettone shelf life



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### ABSTRACT

Physicochemical properties may determine quality attributes and consumer acceptability of mini panettones. To reduce the conservatives added in food, this study developed active edible films of potato starch, inverted sugar and sucrose to coat mini panettones. All three variables significantly affected ( $p < 0.05$ ) moisture, aw, hardness and elasticity of panettones. The selected formulation (46 g/kg starch, 14 g/kg inverted sugar and 7 g/kg sucrose) was added to potassium sorbate (1 g/kg), citric acid (10 g/kg) and both additives (1 g/kg sorbate + 10 g/kg citric acid and 0.5 g/kg sorbate + 5 g/kg citric acid). From 16 to 24 days (35 °C/60% RH), panettones without coating and without additives (controls) showed growth of mold/yeast; while with both additives coating, fungal growth was observed from 40 days. When using potassium sorbate, mold/yeast was not detected until 48 days. During storage, there was reduced water activity, moisture, elasticity and cohesiveness of panettones with additives, while the reverse occurred in controls. The incorporation of food-graded antimicrobial compounds in the packaging films of potato starch coatings in concentrations lower than those normally used for mini panettones increased up to 130% their shelf life and may contribute to product loss reduction during storage.

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## 1. Introduction

Consumers are increasingly interested in the quality of products consumed. Such quality depends on characteristics that can be altered during storage and selling processes (Guilbert, Gontard, & Gorris, 1996). Food manufacturers hold packages as allies for having a protective function, providing greater shelf life and ensuring the quality of purchased products (Wen-Xian, Avena-Bustillos, Sui Sheng & Tara, 2011).

Although panettones are seasonal products, the mini panettone is a choice for addressing the current desires of consumers coming

in single doses or individual portions.

In the bread industry, several additives are used to improve dough properties, bread quality and tolerance processes, in particular the optimization of shelf life (Benejam, Steffolani, & León, 2009). Thus, new alternatives to increase the shelf life of products, while keeping characteristics similar to a freshly prepared product, are of great interest to industries.

In bread-making products, the growth of certain microorganisms begins or is more intense at the surface, what makes the use of edible films and coats a choice for maintaining the quality of these products (Appendini & Hotchkiss, 2002).

The development and the application of films and active edible coatings in bread-making products becomes a viable alternative to meet the demands of the market by offering products with quality

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and safety, maintaining the desired sensorial characteristics and reducing loss caused by growth of molds and yeasts (Devlieghere, Vermeiren, & Debevere, 2004; Padgett, Han, & Dawson, 1998; Soares, Rutishauser, Melo, Cruz, & Andrade, 2002).

To reduce the total amount of conservatives added to food, edible coatings are used as surface of active agents retention to maintain a high effective preservative concentration where such microorganisms tend to contaminate and/or grow; i.e. on the coating surface. The advantage is requiring a lower amount of conservative agents. In response to the consumers' demand for conservative-free food, as well as more natural, disposable, biodegradable and recyclable food-packaging materials, the objective of this study was to develop and characterize edible coatings of potato starch, inverted sugar and sucrose to coat mini panettones and select the formulation to incorporate synthetic active additives in the conservation of this product during storage.

## 2. Materials and methods

Edible coatings were prepared with potato starch (Yoki<sup>®</sup>, São Paulo – SP, Brazil – 220 g/kg amylose and 780 g/kg amylopectin), inverted sugar (INVEX 60 Guarani<sup>®</sup>, São Paulo – SP, Brazil – 76–78% BRIX, 90% inversion and pH 4.5–5), sucrose (União, Sertãozinho – SP, Brazil), potassium sorbate and citric acid (Pantec, São Paulo – SP, Brazil).

The mini panettones were prepared with wheat flour, margarine, water, sugar, egg yolks, whole milk powder, glucose, sodium chloride, yeast, emulsifier (lecithin), chocolate chips and essence of panettone; all purchased on the local market (Salvador – Bahia, Brazil). They were baked at 180 °C for 35 min, coated with edible coatings and stored using bi-axially oriented polypropylene metal packaging (BOPP) (Polo Films, Montenegro – RS, Brazil).

### 2.1. Experimental design

Seventeen formulations of filmogenic solutions with variations in potato starch, inverted sugar and sucrose concentrations (Table 1) were developed following a rotatable central composite design (RCCD) with a model of order 2<sup>3</sup>. Solutions were applied as coats in mini panettones and compared with mini panettones without coating (control). From the results, a Pareto chart was designed to determine the influence of independent variables on sensorial, physicochemical and physical parameters of mini panettones. Out of the 17 formulations, one was selected for the additive to be added. Results that showed significant influence from Pareto charts were evaluated by ANOVA considering pure error and a 95% confidence interval.

### 2.2. Preparation of filmogenic solutions and application of coatings on mini panettones

The filmogenic solutions were prepared by dissolving potato starch (46–73 g/kg), plasticizers invert sugar (9–19 g/kg) and sucrose (4–10 g/kg) in distilled water. Dispersions were shaken at 68 ± 2 °C with constant stirring under water bath, followed by cooling at room temperature (25 ± 2 °C). Filmogenic solutions were applied to the mini panettones with the aid of a confectionery brush. Four layers were brushed at all the surface of baked mini panettones with three-minute intervals between applications. Thereafter, coated panettones were brought to the oven at 180 °C for five minutes to dry the coating. For comparison, the control panettones (without coating) were also heated to 180 °C for 5 min.

### 2.3. Sensorial, physical and physicochemical characterization of mini panettones with edible coatings

#### 2.3.1. Sensory evaluation

The flavor, texture and dissolution of mini panettones were evaluated by using an acceptability test. A total of 60 untrained tasters were recruited. The evaluations were carried out in individual booths under artificial daylight at temperature between 22 and 24 °C and air circulation. The samples were evaluated in a monadic way. Each taster evaluated the eighteen samples of mini panettones in six sessions, according to an experimental design of complete balanced and randomized blocks. The samples (1 unit) were served on disposable plates, coded with random 3 – digit numbers.

A 9-cm non-structured hedonic scale was used in the acceptability test with extremes of “(1) extremely disliked” and “(9) extremely liked” (Stone & Sidel, 2004).

The study plan was evaluated by the Ethics in Research Committee of College of Nursing, Federal University of Bahia, Salvador, Bahia, Brazil, resolution number 01 of 13/06/1988-CNS, who presented favorable opinion for the development of this study (number 921.004).

#### 2.3.2. Water activity ( $a_w$ ) and moisture ( $M$ )

The  $a_w$  was measured using a decagon (Lab Masster, Novasina – TECNAL, SP/Brazil), with a CM-2 electrolyte measuring cell and pre-packaged samples at 60% RH and 25 °C (Veiga-Santos, Oliveira, Cereda, Alves, & Scamparini, 2005). Moisture content (g/kg) was determined by infrared drying (Mettler LTJ) (IAL, 2005).

#### 2.3.3. Texture

The hardness, cohesiveness, elasticity and chewiness were evaluated using a CT3 Texture Analyzer (Brookfield, Middleboro/USA) according to the AACC 74 – 09 method (AACC, 2000).

### 2.4. Incorporating of synthetic additives to the selected edible coating

To the selected the base formulation four concentrations of additives were incorporated in isolation and in the following combinations: ASF<sub>1</sub> (1 g/kg potassium sorbate), ASF<sub>2</sub> (10 g/kg citric acid), ASF<sub>3</sub> (1 g/kg potassium sorbate + 10 g/kg citric acid) and ASF<sub>4</sub> (0.5 g/kg potassium sorbate + 5 g/kg citric acid). After preparing the filmogenic solution (see item 2.2), additives were solubilized, followed by cooling at room temperature (25 ± 2 °C).

### 2.5. Monitoring of the microbiological, physical and physicochemical stability of mini panettones with additive edible coatings

After packaged in foil BOPP metallic packages and stored in a climatic chamber (TECNAL) for 48 days (60% RH, 35 °C), mini panettones with active edible coatings were monitored every 8 days by evaluating  $a_w$ , moisture, texture and count of molds and yeasts. Mini panettones without coating and edible coating without additives were also packaged under the same conditions for results comparison.

#### 2.5.1. Microbiological analysis

Analysis was performed from the homogenization of a 25 g sample with 225 mL of 1 mg/L peptone water in stomacher for 2 min. Afterwards, decimal serial dilutions were carried out in triplicate, and the plating was performed on a Dichloran Rose-Bengal Chloramphenicol (DRBC) surface for researching yeasts and molds. The plates were incubated at 25 °C for 5 days (King,

**Table 1**

Average values obtained in the mini panettone acceptance test with edible coatings (17 formulations) and control (C – mini panettone without coating).

F	Potato starch (g/kg)	Invert sugar (g/kg)	Sucrose (g/kg)	Flavor	Texture	Dissolution
C	–	–	–	6.6	5.5	7.2
F1	52	12	5	6.9	6.0	5.9
F2	52	12	9	7.2	6.3	6.0
F3	52	18	5	6.7	5.7	5.9
F4	52	18	9	7.4	6.8	5.0
F5	68	12	5	7.0	6.0	4.5
F6	68	12	9	6.7	6.0	4.2
F7	68	18	5	7.1	6.6	4.4
F8	68	18	9	7.3	6.9	6.7
F9	46	14	7	7.2	7.2	7.3
F10	73	14	7	7.4	7.2	6.5
F11	60	09	7	7.5	7.0	6.5
F12	60	19	7	7.0	6.3	6.4
F13	60	14	4	7.4	7.0	6.4
F14	60	14	10	7.4	6.9	6.0
F15 <sup>a</sup>	60	14	7	6.7	5.7	5.9
F16 <sup>a</sup>	60	14	7	6.2	5.6	6.1
F17 <sup>a</sup>	60	14	7	6.4	5.1	6.1
Break	46–73	9–19	4–10	6.2–7.5	5.1–7.2	4.2–7.3

<sup>a</sup> Central points. C = Control. F = Formulations.

Hocking, &amp; Pitt, 1979).

## 2.6. Shelf life

Besides storage under accelerated conditions, mini panettones were stored at different temperatures (25 °C and 35 °C) with microbiological and  $a_w$  evaluation every 8 days. Data on  $a_w$  evaluation of samples were used to determine the reaction order of degradation and to calculate the respective reaction rate constant ( $K_{T25\text{ °C}}$  and  $K_{T35\text{ °C}}$ ) at the different temperatures studied. The temperature effect was evaluated using the Arrhenius equation (Equation (1)):

$$K_T = Ae^{Ea/RT} \quad (1)$$

where  $K_T$  is the reaction rate constant ( $K_{T25\text{ °C}}$  and  $K_{T35\text{ °C}}$ ),  $R$  is the molar gas constant (8.31 J/K/mol),  $T$  is the absolute temperature,  $Ea$  is the activation energy (J/mol) and  $A$  is the pre-exponential factor.

Rate constants ( $K_{T25\text{ °C}}$  and  $K_{T35\text{ °C}}$ ) were calculated from the slopes of  $a_w$  curves plotted against storage time using a 0-order kinetic model (Farhoosh, Niazmand, Rezaei, & Sarabi, 2008; Trezza & Krochta, 2000). The slope of each line was determined using the general linear regression model (Equation (2)).

$$Y = Ax + B \quad (2)$$

Activation energies ( $Ea/R$ ) and pre-exponential or frequency factors ( $A$ ) were determined from the slopes and intercepts of the lines generated by regression (Equation (2)). Where  $A = \ln A$  and  $B = Ea/R$  using the Arrhenius Equation (1):

The  $Q_{10}$  factor was calculated to express the reaction rate temperature dependence.  $Q_{10}$  factor also is adopted as the indicator to evaluate the quality change during storage and transportation, which is a measure of the change rate of a biological or chemical system as a consequence of increasing temperature by 10 °C to describe the relationship between temperature and reaction rate.  $Q_{10}$  is defined as the ratio of the shelf life (s) at two storage temperatures differing by 10 °C. The  $Q_{10}$  factor may be related to the following temperature dependence equation:

$$Q_{10} = \frac{K_T}{K_{T-10}} = \frac{K_{T35\text{ °C}}}{K_{T25\text{ °C}}} \quad (3)$$

Thus, the  $Q_{10}$  was obtained by the Equation (3), which multiplied by the shelf life (storage time) of mini panettones under accelerated conditions will determine the shelf life of these products under current conditions.

## 3. Results and discussion

### 3.1. Sensory evaluation of mini panettone with edible coatings

The results obtained for the parameters of flavor, texture and dissolution of mini panettones are shown in Table 1.

In the evaluation of flavor and texture attributes, it became clear that the results showed no significant difference ( $p > 0.05$ ) by suggesting that mini panettones did not change these attributes when coated with edible coatings containing different potato starch, sucrose and inverted sugar concentrations (Fig. 1).

When evaluating the dissolution, mini panettones coated with the F9 and F6 formulations, with the lowest and the second highest potato starch concentration, had the highest and lowest average, respectively, showing a tendency for negative influence of starch on this attribute, which was corroborated by the Pareto chart (Fig. 1C).

Despite showing that a significant representation of the independent variables effect (potato starch, inverted sugar and sucrose) on the evaluated attributes is not possible, the results of analysis of variance (ANOVA) from the sensorial evaluation are clear that different concentrations, especially of the polymer matrix, may affect the dissolution in particular, but not flavor (Fig. 1).

### 3.2. Physical and physicochemical characterization of mini panettones with edible coatings

Results of the physical and physicochemical analyses of control mini panettones and 17 edible coatings are shown in Table 2. The increased starch concentration tends to increment the product's ability to bind water, reducing the value of  $a_w$  (Osawa, Fontes, Walter, Chang, & Steel, 2009). However, Pareto chart (Fig. 2B) generated for this parameter shows the significant positive interference ( $p < 0.05$ ) of the three independent variables, both on the  $a_w$  and moisture results. By the analysis of variance (ANOVA), a significant representation of the variables effects on  $a_w$  and moisture could not be demonstrated.

When comparing means by the Tukey test at 95% confidence

(Table 2), the F8 was the only sample in which the  $a_w$  significantly differed ( $p < 0.05$ ) from the control, representing one of the highest  $a_w$  levels (0.8) among samples analyzed. In this parameter, as well as moisture, the samples with the lowest values were formulations of central points. There was a direct correlation between  $a_w$  and moisture ( $R^2 = 0.61$ ) (Table 2, Fig. 3A).

The products hardness may be influenced by moisture loss or gain, usually with inversely proportional relation (Szczesniak, 2002). Mini panettones with edible coatings that had higher hardness and cohesiveness values (F15, F16 and F17) were those that had the lowest moisture and  $a_w$  content (Fig. 3C). The Pareto chart shows a significant negative interference ( $p < 0.05$ ) of the three independent variables on hardness (Fig. 2C).

For elasticity parameter, the Pareto chart (Fig. 2E) shows the significant positive effect ( $p < 0.05$ ) of the three independent variables, which is desirable for bakery products since increased elasticity tends to reduce chewiness values, resulting in softer products.

Another important parameter used to evaluate the texture of foods is chewiness (Szczesniak, 2002), and it may suffer a directly proportional interference of hardness and cohesiveness (Osawa et al., 2009). Such a fact was observed in this study, where chewiness had direct correlation with cohesiveness ( $R^2 = 0.63$ ) (Fig. 3D) and hardness ( $R^2 = 0.97$ ) (Fig. 3E), and inversely proportional relation to moisture and  $a_w$  ( $R^2 = 0.60$  and  $R^2 = 0.75$ , respectively) (Fig. 3F and G).

### 3.3. Formulation selection for incorporation of synthetic additives in mini panettones coating

Despite analysis of variance (ANOVA) not demonstrating the significant effect of independent variables on the evaluated parameters, Pareto charts (Figs. 1 and 2) show certain tendencies. In the dissolution, the negative influence of potato starch was observed. The F9 formulation with lower concentrations (46 g/kg) was the only one to present values higher than the control in the sensorial evaluation (Table 1). The potato starch was the variable that most influenced the evaluation of products with a desirable positive influence on parameters such as elasticity and moisture, and negative desirable influences on parameters such as hardness and chewiness (Table 2). Thus, from the set of desirable outcomes for sensorial, physical and physicochemical evaluations, F9 was selected as base formulation for incorporation of active additives (potassium sorbate and citric acid).

### 3.4. Monitoring of microbiological, physical and physicochemical stability of mini panettones with additive edible coatings active during storage

Since microorganisms such as molds and yeasts tend to contaminate and grow on the coating surface, the surface concentration is essential to evaluate the coat efficacy in reducing microbial growth. Mini panettones with edible coatings containing potassium sorbate (ASF<sub>1</sub>), citric acid (ASF<sub>2</sub>) or both (ASF<sub>3</sub> and ASF<sub>4</sub>) showed no growth of molds and yeasts during 32 days of storage (Table 3). Control samples (CS: mini panettones without coating) and samples without additives (SF: mini panettones with edible coating without additives) had growth of these microorganisms from the 16th and 24th days, respectively, which became more evident after the 24th and 32 days, with high growth of mold, countless in microbiological analysis (Table 3). This microbial growth may have been favored by the moisture content of these samples and the absence of antimicrobial additives (Tables 3 and 4). It is notable the positive effect of non-active edible coating (SF) on microbial growth, with increased microbiological stability when

compared to the control (CF), and 33% increase in the development time of these microorganisms under accelerated conditions. A similar result was achieved in the evaluation of pistachios with growth of mold after 48 h without coating, and only after 10 days when coated, even without antimicrobial additives (Sayanjali, Ghanbarzadeh, & Ghiassifar, 2011).

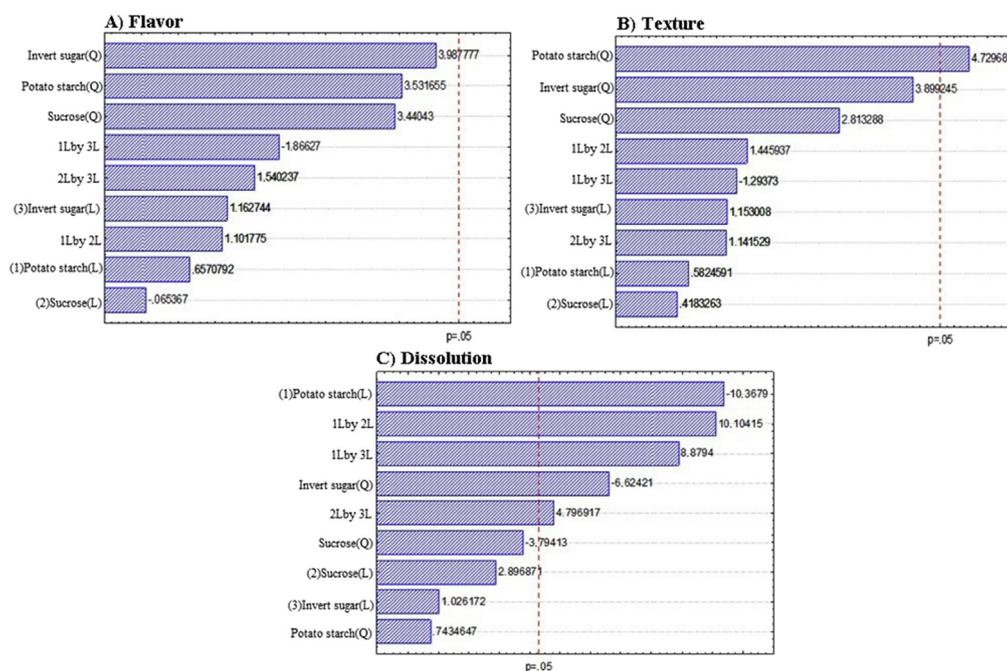
In mini panettones coated with active coating (ASF<sub>2</sub>, ASF<sub>3</sub> and ASF<sub>4</sub>), the growth of molds and yeasts occurred from the 40th day, indicating a similar antimicrobial efficacy in all three coatings. Only the ASF<sub>1</sub> formulation, containing 1 g/kg potassium sorbate, remained viable up to the 40th day of storage under accelerated conditions, with microbial growth only on the 48th day. This shows higher antimicrobial efficacy of this coating in comparison to others and no synergistic effect of potassium sorbate and citric acid on developed coatings, as shown in Table 4. In this context, the developed active coatings caused inhibitory effect on microbial growth, with up to 50% increase in mold and yeast development time under accelerated conditions when compared to mini panettones without active coating. They also showed up to 100% increase compared to mini panettones without coating, representing reduced loss by deterioration related to increased shelf life (Table 3).

The use of edible coatings with potassium sorbate (2.5, 5 and 10 g/kg) was also effective in the growth of control pistachio molds during storage (Sayanjali et al., 2011). In fried dough, the interleave use of films containing 30 g/kg and 60 g/kg of sorbic acid significantly reduced microbiological growth, proving effectiveness of the incorporation of this additive in films when compared to the sample without conservatives (Silveira et al., 2007), acting as active barrier to the microorganisms growth (Ozdemir & Floros, 2001).

The increase of  $a_w$  in bakery products may reduce the antifungal effect of ingredients such as potassium sorbate, sodium benzoate and calcium propionate, the latter being the least effective among them (Suhr & Nielsen, 2004). In this study, there was reduced growth of microorganisms caused by the additives of coatings (Table 3) simultaneously with  $a_w$  decrease, although no significant differences had been found in the evaluation of this parameter for most samples (Table 4).

On days 0 and 8, ASF<sub>3</sub> and ASF<sub>4</sub> samples with both additives were those that had the highest moisture content compared to the others. Samples containing only potassium sorbate and citric acid (ASF<sub>1</sub> and ASF<sub>2</sub>) were those with the lowest moisture content during the storage period (Table 4). During storage, all samples with additives had moisture reduction, indicating possible migration of product water to the films of the four coatings. A result different from that obtained for control formulations (CF) and without additives (SF), where moisture content gradually increased, indicating water absorption by samples (Table 4). In active films of cellulose acetate, there may be increased moisture content after 15 days of storage depending on the packaging's permeability to water vapor (Soares et al., 2002).

In this study, all samples that showed reduced moisture content during the storage period (active samples) had higher hardness and chewiness values. It seems that in the storage period, which could be viewed as apparent "maturation", could even help to accentuate the product flavor, thus being beneficial to the final product (Benejam et al., 2009). Similar results were found for chocolate cake without coating and with various types of edible coatings (based on gelatin, stearic acid, modified starch and carnaúba wax), in which, hardness of cakes generally increased during storage and the highest value for cake without coating was due to moisture loss (Osawa et al., 2009). The apparent permeability constants for potassium sorbate of an edible film composed of methylcellulose and palmitic acid (weighted ratio 3:1), which is a crucial factor for microorganisms growth on the surface, decreased from  $2.0 \times 10^{-8}$



**Fig. 1.** Pareto charts of flavor (A), texture (B) and dissolution (C) attributes of mini panettones coated with edible coatings of potato starch (46–73 g/kg), inverted sugar (9–19 g/kg) and sucrose (4–10 g/kg).

**Table 2**

Average values ( $\pm$ sd) of the levels of  $a_w$ , moisture and texture parameters of mini panettones with edible coatings and control (C = without coating).

F	$a_w$	Moisture (g/kg)	Texture			
			Hardness (N)	Cohesiveness	Elasticity (mm)	Chewiness (mj)
C	0.75 $\pm$ 0 <sup>bcdef</sup>	153 $\pm$ 2 <sup>abcde</sup>	61 $\pm$ 11 <sup>de</sup>	0.54 $\pm$ 0.07 <sup>d</sup>	8 $\pm$ 0.6 <sup>b</sup>	275 $\pm$ 101 <sup>e</sup>
F1	0.79 $\pm$ 0.03 <sup>abc</sup>	135 $\pm$ 8 <sup>def</sup>	44 $\pm$ 3 <sup>ef</sup>	0.58 $\pm$ 0.01 <sup>cd</sup>	9 $\pm$ 0.1 <sup>a</sup>	226 $\pm$ 18 <sup>e</sup>
F2	0.77 $\pm$ 0.02 <sup>abcde</sup>	149 $\pm$ 15 <sup>abcdef</sup>	34 $\pm$ 2 <sup>f</sup>	0.58 $\pm$ 0.04 <sup>cd</sup>	9 $\pm$ 0 <sup>a</sup>	176 $\pm$ 21 <sup>e</sup>
F3	0.78 $\pm$ 0.03 <sup>abcd</sup>	135 $\pm$ 20 <sup>cdef</sup>	39 $\pm$ 0 <sup>f</sup>	0.58 $\pm$ 0.01 <sup>cd</sup>	9 $\pm$ 0.1 <sup>a</sup>	202 $\pm$ 4 <sup>e</sup>
F4	0.80 $\pm$ 0.04 <sup>abcd</sup>	161 $\pm$ 9 <sup>abc</sup>	39 $\pm$ 2 <sup>f</sup>	0.59 $\pm$ 0.02 <sup>cd</sup>	9 $\pm$ 0 <sup>a</sup>	210 $\pm$ 15 <sup>e</sup>
F5	0.78 $\pm$ 0.02 <sup>abc</sup>	165 $\pm$ 2 <sup>a</sup>	38 $\pm$ 5 <sup>f</sup>	0.59 $\pm$ 0.02 <sup>cd</sup>	8.8 $\pm$ 0 <sup>a</sup>	200 $\pm$ 31 <sup>e</sup>
F6	0.80 $\pm$ 0 <sup>ab</sup>	163 $\pm$ 5 <sup>ab</sup>	37 $\pm$ 4 <sup>f</sup>	0.59 $\pm$ 0.02 <sup>cd</sup>	8.9 $\pm$ 0.1 <sup>a</sup>	195 $\pm$ 29 <sup>e</sup>
F7	0.77 $\pm$ 0.01 <sup>abcd</sup>	152 $\pm$ 1 <sup>abcde</sup>	39 $\pm$ 0 <sup>f</sup>	0.61 $\pm$ 0.02 <sup>bc</sup>	9 $\pm$ 0.2 <sup>a</sup>	212 $\pm$ 10 <sup>e</sup>
F8	0.80 $\pm$ 0.01 <sup>a</sup>	161 $\pm$ 2 <sup>abcd</sup>	44 $\pm$ 1 <sup>ef</sup>	0.60 $\pm$ 0.03 <sup>cd</sup>	9 $\pm$ 0 <sup>a</sup>	239 $\pm$ 17 <sup>e</sup>
F9	0.80 $\pm$ 0.02 <sup>ab</sup>	153 $\pm$ 10 <sup>abcde</sup>	45 $\pm$ 1 <sup>ef</sup>	0.59 $\pm$ 0 <sup>cd</sup>	8.8 $\pm$ 0 <sup>a</sup>	232 $\pm$ 7 <sup>e</sup>
F10	0.80 $\pm$ 0 <sup>ab</sup>	161 $\pm$ 10 <sup>abcd</sup>	40 $\pm$ 1 <sup>f</sup>	0.60 $\pm$ 0.01 <sup>cd</sup>	8.9 $\pm$ 0.1 <sup>a</sup>	213 $\pm$ 11 <sup>e</sup>
F11	0.75 $\pm$ 0.01 <sup>bcdef</sup>	137 $\pm$ 6 <sup>bcdef</sup>	77 $\pm$ 10 <sup>cd</sup>	0.59 $\pm$ 0.01 <sup>cd</sup>	8.7 $\pm$ 0 <sup>ab</sup>	398 $\pm$ 59 <sup>d</sup>
F12	0.78 $\pm$ 0.01 <sup>abc</sup>	162 $\pm$ 1 <sup>abc</sup>	34 $\pm$ 0 <sup>f</sup>	0.58 $\pm$ 0.01 <sup>cd</sup>	8.6 $\pm$ 0.4 <sup>ab</sup>	168 $\pm$ 9 <sup>e</sup>
F13	0.78 $\pm$ 0.01 <sup>abcd</sup>	151 $\pm$ 10 <sup>abcdef</sup>	42 $\pm$ 13 <sup>f</sup>	0.55 $\pm$ 0.01 <sup>cd</sup>	8.8 $\pm$ 0.3 <sup>a</sup>	200 $\pm$ 54 <sup>e</sup>
F14	0.73 $\pm$ 0.03 <sup>cdef</sup>	130 $\pm$ 13 <sup>ef</sup>	92 $\pm$ 6 <sup>bc</sup>	0.59 $\pm$ 0.01 <sup>cd</sup>	8.5 $\pm$ 0.1 <sup>b</sup>	464 $\pm$ 27 <sup>cd</sup>
F15	0.71 $\pm$ 0.01 <sup>f</sup>	129 $\pm$ 5 <sup>ef</sup>	101 $\pm$ 9 <sup>ab</sup>	0.61 $\pm$ 0.01 <sup>abc</sup>	8.7 $\pm$ 0.3 <sup>ab</sup>	535 $\pm$ 14 <sup>bc</sup>
F16	0.73 $\pm$ 0 <sup>def</sup>	128 $\pm$ 3 <sup>ef</sup>	114 $\pm$ 0 <sup>a</sup>	0.75 $\pm$ 0.02 <sup>ab</sup>	8.6 $\pm$ 0.1 <sup>a</sup>	731 $\pm$ 23 <sup>a</sup>
F17	0.72 $\pm$ 0 <sup>ef</sup>	125 $\pm$ 2 <sup>f</sup>	103 $\pm$ 4 <sup>ab</sup>	0.68 $\pm$ 0.01 <sup>ab</sup>	8.7 $\pm$ 0.1 <sup>ab</sup>	633 $\pm$ 18 <sup>ab</sup>
Break	0.71–0.80	125–165	34–114	0.54–0.75	8–9	168–731

Lower case letters different in the same column, significant differences ( $p < 0.05$ ) by Tukey test at 95% confidence. Averages of triplicates.

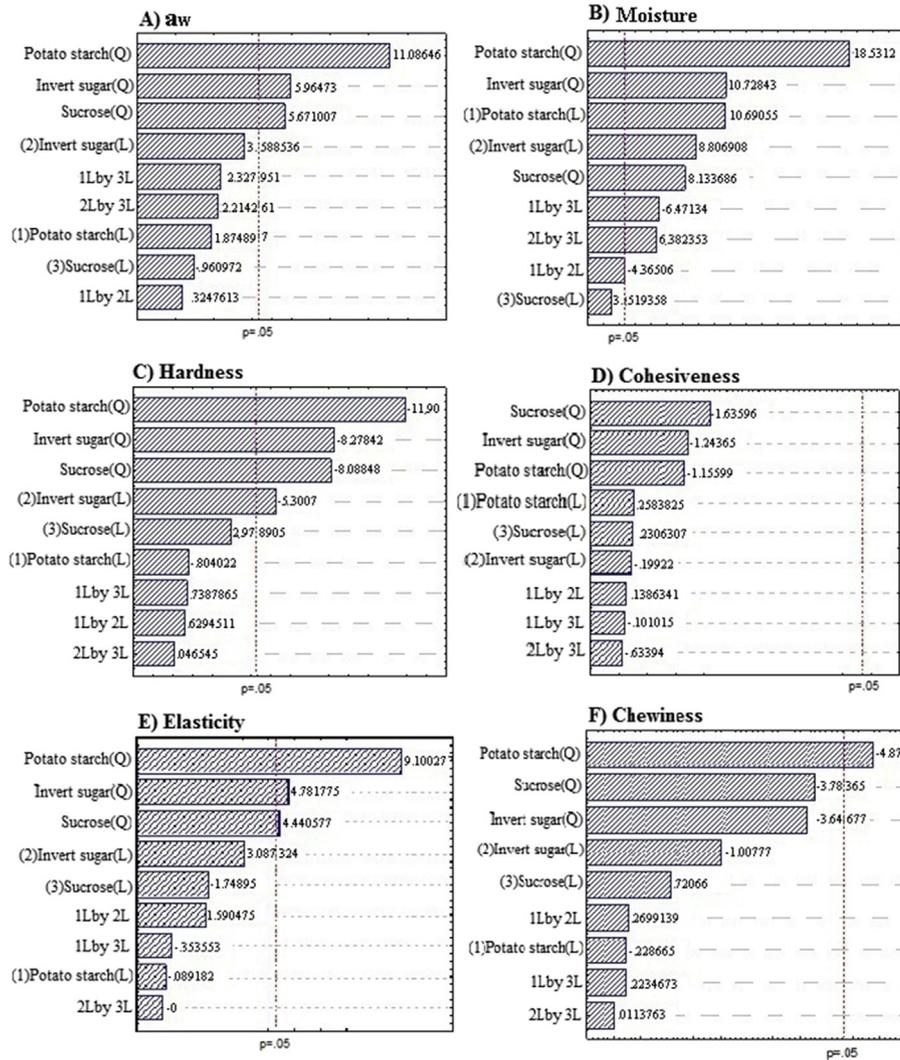
to  $2.3 \times 10^{-10}$  (mg/sec cm<sup>2</sup>) (cm)/(mg/mL), while  $a_w$  decreased from 0.80 to 0.65 (Rico-Peña & Torres, 1991).

During storage, only the formulation with additives (SF) showed decrease in hardness and chewiness values. The control (CF) and additive formulations (ASF<sub>1</sub>, ASF<sub>2</sub>, ASF<sub>3</sub> and ASF<sub>4</sub>) had increasing values for these parameters during the period, which can be explained by the reduction of moisture (except CF) (Table 4). It may suffer interference of a starch retrogradation process, in which there is water loss (Esteller & Lannes, 2005).

In general, cohesiveness and elasticity data show that the samples had no significant differences ( $p > 0.05$ ) during the storage period, although there was a gradual increase in these parameters

in samples with additives and in the control, while, in all mini panettones packaged in coatings with additives, these parameters showed an opposite behavior (Table 4). The decrease in these parameters may occur due to the drying of products during storage (Esteller, Yoshimoto, Amaral, & Lannes, 2004), which makes them more prone to disintegration (Osawa et al., 2009).

From the results, it is clear that the isolated or joint incorporation of additives such as potassium sorbate and citric acid in edible coatings influenced attributes such as hardness, cohesiveness, elasticity, chewiness,  $a_w$ , moisture and microbial count of mini panettones.



**Fig. 2.** Pareto chart for  $a_w$  (A), moisture (B), hardness (C), cohesiveness (D), elasticity (E) and chewiness (F) of mini panettones with edible potato starch coatings (46–73 g/kg), inverted sugar (9–19 g/kg) and sucrose (4–10 g/kg).

### 3.5. Shelf life of mini panettones stored with active edible coatings

The Fig. 4 show the activity water plotted against time for two different temperatures (25 °C and 35 °C) of study. The  $a_w$  of mini panettones without a coating and with non-active and active coatings (ASF<sub>1</sub>, ASF<sub>2</sub>, ASF<sub>3</sub> and ASF<sub>4</sub>) under accelerated conditions (35 °C) and at normal storage temperature (25 °C) were monitored every 8 days. The CF formulation (without coating) kept the mini panettones viable for a shorter time (16 days) under accelerated conditions was, which also showed shelf life of 16 days under normal storage conditions (25 °C) (Table 5).

Despite having shown shelf life inferior to that one using formulations with additives, mini panettones with non-active edible coatings had approximately 187% increased in their expected life-time if compared to the formulation without coating, demonstrating that even without additives incorporation, the application of edible coatings seems to be effective for preserving these products. The ASF<sub>1</sub>, ASF<sub>2</sub>, ASF<sub>3</sub> and ASF<sub>4</sub> formulations with potassium sorbate and citric acid incorporation combined or in isolation presented 41% increase in shelf life comparing to SF, proving the

effectiveness of additives in conservation and increased shelf life of mini panettones (Table 5).

The formulation that remained viable for a longer period (40 days) under accelerated conditions was ASF<sub>1</sub>, with coating containing 1 g/kg of potassium sorbate without interaction with citric acid and shelf life of approximately 106 days under normal storage conditions (25 °C). This represents approximately 130% and 560% increase in the shelf life of products in relation to the non-additive formulation and to the control formulation (without coating), respectively (Table 5). The ASF<sub>3</sub> formulation with associated incorporation of the highest concentrations of potassium sorbate and citric acid additives in the coating had shelf life of 71 days under normal storage conditions, about 33% lower than ASF<sub>1</sub>, thus proving the absence of synergistic effects of these additives.

Commercial panettone brands have shelf life of around 90–120 days and use calcium propionate and sorbic acid additives incorporated into the dough of formulations or spray solutions with boundaries of 2 g/kg (BRAZIL, 1999). The result of the shelf life of mini panettones coated with ASF<sub>1</sub> formulation

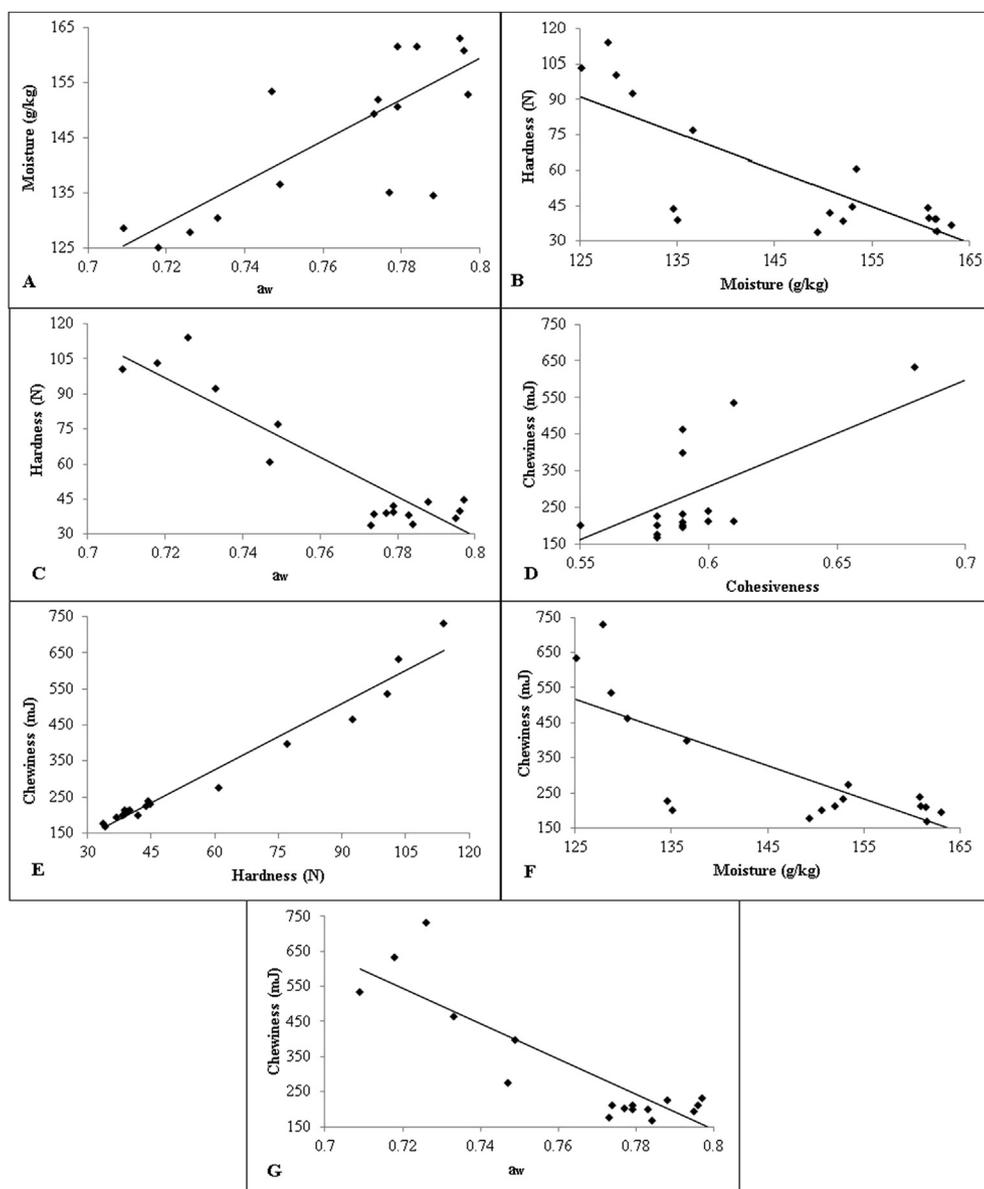


Fig. 3. Linear correlations among different parameters (moisture,  $a_w$ , hardness, chewiness and cohesiveness) of mini panettones with edible coatings.

Table 3

Count of molds and yeasts (CFU/g) of mini panettones without coating and with non-active and active edible coatings stored in biaxially oriented polypropylene (BOPP) packaging under accelerated conditions (60% RH/35 °C).

Days	CF	SF	ASF <sub>1</sub>	ASF <sub>2</sub>	ASF <sub>3</sub>	ASF <sub>4</sub>
0	<1	<1	<1	<1	<1	<1
8	<1	<1	<1	<1	<1	<1
16	<10	<1	<1	<1	<1	<1
24	Countless	<10	<1	<1	<1	<1
32	—	Countless	<1	<1	<1	<1
40	—	—	<10	$2.0 \times 10^2$	$2.0 \times 10^2$	$3.0 \times 10^2$
48	—	—	$2.0 \times 10^2$	—	—	—

CF = Control Formulation (mini panettone without coating). SF = Selected Formulation non-additive (46 g/kg potato starch/14 g/kg invert sugar/7 g/kg sucrose). ASF<sub>1</sub> = Selected Formulation additive (1 g/kg potassium sorbate). ASF<sub>2</sub> = Selected Formulation additive (10 g/kg citric acid). ASF<sub>3</sub> = Selected Formulation additive (1 g/kg potassium sorbate + 10 g/kg citric acid). ASF<sub>4</sub> = Selected Formulation additive (0.5 g/kg potassium sorbate + 5 g/kg citric acid). Averages of triplicates.

corresponds to the shelf life of such commercial brands, but with 50% less additives, where in the potassium sorbate conservative was incorporated only to the coating and not to the dough of products.

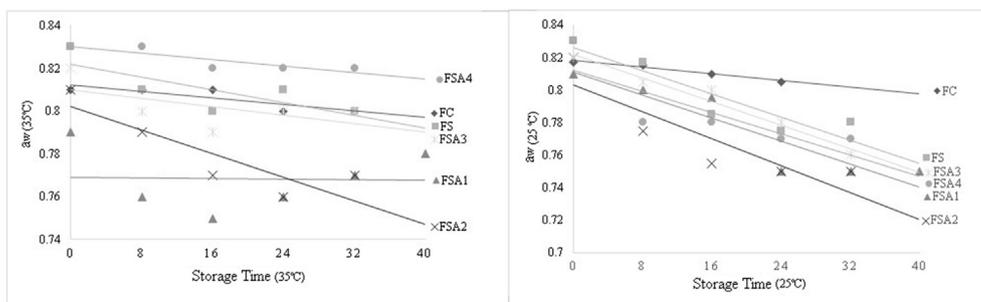
#### 4. Conclusion

The results obtained in the present work point the potential advantages of using edible coatings consisting of potato starch, inverted sugar and sucrose to improve the safety and quality of mini panettones. Experimental data suggest that the incorporation of sorbate potassium and citric acid as additives isolated or in combination inhibited the growth of yeasts and molds in stored products under accelerated conditions, with at least 3 times increase of mini panettone storage time when compared to the control (without coating). It is a competitive and economically viable alternative to reduce the loss by undesirable alterations in

**Table 4**  
Average values ( $\pm$ sd) of physical and physical-chemical parameters of mini panettones without coating (FC) and with non-active and active edible coatings stored in BOPP packaging under accelerated conditions (60% RH/35 °C) for 48 days.

Parameters	Days	CF	SF	ASF <sub>1</sub>	ASF <sub>2</sub>	ASF <sub>3</sub>	ASF <sub>4</sub>
Moisture (g/kg)	0	148 $\pm$ 0 <sup>bc</sup>	163 $\pm$ 16 <sup>abA</sup>	159 $\pm$ 9 <sup>abA</sup>	149 $\pm$ 3 <sup>ba</sup>	183 $\pm$ 15 <sup>aA</sup>	185 $\pm$ 18 <sup>aA</sup>
	8	164 $\pm$ 4 <sup>abB</sup>	170 $\pm$ 1 <sup>abA</sup>	153 $\pm$ 14 <sup>abA</sup>	146 $\pm$ 8 <sup>ba</sup>	173 $\pm$ 14 <sup>abAB</sup>	181 $\pm$ 14 <sup>aA</sup>
	16	182 $\pm$ 1 <sup>aA</sup>	176 $\pm$ 13 <sup>ba</sup>	135 $\pm$ 13 <sup>ba</sup>	144 $\pm$ 8 <sup>ba</sup>	163 $\pm$ 13 <sup>abAB</sup>	176 $\pm$ 12 <sup>aA</sup>
	24	183 $\pm$ 0 <sup>aA</sup>	178 $\pm$ 3 <sup>aA</sup>	135 $\pm$ 2 <sup>cA</sup>	140 $\pm$ 18 <sup>bcA</sup>	150 $\pm$ 4 <sup>bcB</sup>	162 $\pm$ 10 <sup>abA</sup>
	32	*	178 $\pm$ 8 <sup>aA</sup>	135 $\pm$ 13 <sup>cA</sup>	139 $\pm$ 6 <sup>bcA</sup>	148 $\pm$ 3 <sup>bcB</sup>	156 $\pm$ 5 <sup>ba</sup>
	40	*	*	134 $\pm$ 11 <sup>ba</sup>	137 $\pm$ 4 <sup>abA</sup>	147 $\pm$ 6 <sup>abB</sup>	156 $\pm$ 6 <sup>aA</sup>
	48	*	*	133 $\pm$ 13 <sup>A</sup>	*	*	*
a <sub>w</sub>	0	0.81 $\pm$ 0 <sup>aA</sup>	0.83 $\pm$ 0.01 <sup>aA</sup>	0.79 $\pm$ 0.01 <sup>aA</sup>	0.81 $\pm$ 0.03 <sup>aA</sup>	0.82 $\pm$ 0.01 <sup>aA</sup>	0.83 $\pm$ 0.02 <sup>aA</sup>
	8	0.81 $\pm$ 0.01 <sup>aA</sup>	0.81 $\pm$ 0.01 <sup>aAB</sup>	0.76 $\pm$ 0.02 <sup>ba</sup>	0.79 $\pm$ 0.02 <sup>abA</sup>	0.80 $\pm$ 0.01 <sup>aA</sup>	0.83 $\pm$ 0.01 <sup>aA</sup>
	16	0.81 $\pm$ 0.01 <sup>aA</sup>	0.80 $\pm$ 0 <sup>abB</sup>	0.75 $\pm$ 0.02 <sup>ba</sup>	0.77 $\pm$ 0.04 <sup>abA</sup>	0.79 $\pm$ 0.01 <sup>abA</sup>	0.82 $\pm$ 0.01 <sup>aA</sup>
	24	0.80 $\pm$ 0.01 <sup>abA</sup>	0.81 $\pm$ 0.01 <sup>abAB</sup>	0.76 $\pm$ 0.01 <sup>ba</sup>	0.76 $\pm$ 0.04 <sup>abA</sup>	0.80 $\pm$ 0.01 <sup>abA</sup>	0.82 $\pm$ 0.02 <sup>aA</sup>
	32	*	0.80 $\pm$ 0.01 <sup>ab</sup>	0.77 $\pm$ 0.02 <sup>aA</sup>	0.77 $\pm$ 0.01 <sup>aA</sup>	0.80 $\pm$ 0.03 <sup>aA</sup>	0.82 $\pm$ 0.03 <sup>aA</sup>
	40	*	*	0.78 $\pm$ 0.02 <sup>aA</sup>	0.80 $\pm$ 0.03 <sup>aA</sup>	0.78 $\pm$ 0.02 <sup>aA</sup>	0.79 $\pm$ 0.01 <sup>aA</sup>
	48	*	*	0.77 $\pm$ 0.01 <sup>A</sup>	*	*	*
Hardness (N)	0	16 $\pm$ 0 <sup>bcC</sup>	24 $\pm$ 3 <sup>aA</sup>	22 $\pm$ 1 <sup>abA</sup>	21 $\pm$ 1 <sup>abC</sup>	14 $\pm$ 3 <sup>cC</sup>	17 $\pm$ 4 <sup>abcB</sup>
	8	17 $\pm$ 0 <sup>abB</sup>	23 $\pm$ 4 <sup>aA</sup>	24 $\pm$ 2 <sup>aA</sup>	22 $\pm$ 2 <sup>aC</sup>	18 $\pm$ 4 <sup>aC</sup>	19 $\pm$ 3 <sup>aAB</sup>
	16	19 $\pm$ 0 <sup>aA</sup>	21 $\pm$ 2 <sup>aA</sup>	25 $\pm$ 5 <sup>aA</sup>	26 $\pm$ 2 <sup>aC</sup>	21 $\pm$ 1 <sup>abC</sup>	24 $\pm$ 2 <sup>aAB</sup>
	24	20 $\pm$ 0 <sup>ba</sup>	20 $\pm$ 2 <sup>abA</sup>	28 $\pm$ 4 <sup>abA</sup>	28 $\pm$ 3 <sup>abBC</sup>	28 $\pm$ 4 <sup>aAB</sup>	24 $\pm$ 4 <sup>abAB</sup>
	32	*	20 $\pm$ 2 <sup>ba</sup>	28 $\pm$ 4 <sup>abA</sup>	33 $\pm$ 4 <sup>aAB</sup>	31 $\pm$ 2 <sup>aA</sup>	26 $\pm$ 3 <sup>abA</sup>
	40	*	*	28 $\pm$ 2 <sup>ba</sup>	37 $\pm$ 2 <sup>aA</sup>	32 $\pm$ 4 <sup>ba</sup>	26 $\pm$ 3 <sup>ba</sup>
	48	*	*	31 $\pm$ 3 <sup>A</sup>	*	*	*
Cohesiveness	0	0.52 $\pm$ 0.03 <sup>aA</sup>	0.67 $\pm$ 0.02 <sup>aA</sup>	0.77 $\pm$ 0.34 <sup>aA</sup>	0.63 $\pm$ 0.06 <sup>aA</sup>	0.73 $\pm$ 0.03 <sup>aA</sup>	0.74 $\pm$ 0.04 <sup>aA</sup>
	8	0.54 $\pm$ 0.01 <sup>ba</sup>	0.72 $\pm$ 0.03 <sup>aA</sup>	0.68 $\pm$ 0.08 <sup>abA</sup>	0.66 $\pm$ 0.03 <sup>abA</sup>	0.76 $\pm$ 0.10 <sup>aA</sup>	0.63 $\pm$ 0.04 <sup>abAB</sup>
	16	0.54 $\pm$ 0.03 <sup>aA</sup>	0.68 $\pm$ 0.01 <sup>aA</sup>	0.64 $\pm$ 0.08 <sup>aA</sup>	0.61 $\pm$ 0.02 <sup>aA</sup>	0.60 $\pm$ 0.09 <sup>aA</sup>	0.62 $\pm$ 0.04 <sup>aAB</sup>
	24	0.54 $\pm$ 0.02 <sup>aA</sup>	0.70 $\pm$ 0.15 <sup>aA</sup>	0.59 $\pm$ 0.08 <sup>aA</sup>	0.59 $\pm$ 0.05 <sup>aA</sup>	0.63 $\pm$ 0.05 <sup>aA</sup>	0.63 $\pm$ 0.06 <sup>aAB</sup>
	32	*	0.94 $\pm$ 0.35 <sup>aA</sup>	0.59 $\pm$ 0.09 <sup>aA</sup>	0.58 $\pm$ 0.03 <sup>aA</sup>	0.66 $\pm$ 0.06 <sup>aA</sup>	0.62 $\pm$ 0.06 <sup>aAB</sup>
	40	*	*	0.62 $\pm$ 0.05 <sup>aA</sup>	0.64 $\pm$ 0.05 <sup>aA</sup>	0.63 $\pm$ 0.05 <sup>aA</sup>	0.60 $\pm$ 0.04 <sup>abB</sup>
	48	*	*	0.55 $\pm$ 0.03 <sup>A</sup>	*	*	*
Elasticity (mm)	0	8.2 $\pm$ 0.5 <sup>aA</sup>	8.6 $\pm$ 0.8 <sup>aA</sup>	9.4 $\pm$ 0.7 <sup>aA</sup>	8.9 $\pm$ 0.2 <sup>aA</sup>	9.4 $\pm$ 0.2 <sup>aA</sup>	8.7 $\pm$ 0 <sup>aA</sup>
	8	8.0 $\pm$ 0.2 <sup>ba</sup>	8.7 $\pm$ 0.5 <sup>abA</sup>	9.0 $\pm$ 0.1 <sup>aA</sup>	8.8 $\pm$ 0.2 <sup>aA</sup>	8.7 $\pm$ 0.2 <sup>aB</sup>	8.8 $\pm$ 0.1 <sup>aA</sup>
	16	7.4 $\pm$ 0.6 <sup>ba</sup>	8.7 $\pm$ 0.2 <sup>aA</sup>	8.9 $\pm$ 0.8 <sup>aA</sup>	8.6 $\pm$ 0.2 <sup>abA</sup>	9.0 $\pm$ 0.1 <sup>aAB</sup>	8.5 $\pm$ 0.6 <sup>abA</sup>
	24	7.6 $\pm$ 0.1 <sup>aA</sup>	8.9 $\pm$ 0.3 <sup>aA</sup>	8.9 $\pm$ 0.3 <sup>aA</sup>	8.6 $\pm$ 0.1 <sup>aA</sup>	8.9 $\pm$ 0.3 <sup>aAB</sup>	8.5 $\pm$ 1.1 <sup>aA</sup>
	32	*	9.9 $\pm$ 1.3 <sup>aA</sup>	8.7 $\pm$ 0.4 <sup>aA</sup>	8.4 $\pm$ 0.4 <sup>aA</sup>	8.9 $\pm$ 0.1 <sup>aAB</sup>	8.2 $\pm$ 0.5 <sup>aA</sup>
	40	*	*	8.6 $\pm$ 0.4 <sup>aA</sup>	8.6 $\pm$ 0.4 <sup>aA</sup>	8.4 $\pm$ 0.3 <sup>aB</sup>	8.6 $\pm$ 0.6 <sup>aA</sup>
	48	*	*	8.7 $\pm$ 0.4 <sup>A</sup>	*	*	*
Chewiness (mj)	0	62 $\pm$ 2 <sup>bc</sup>	204 $\pm$ 66 <sup>aA</sup>	133 $\pm$ 47 <sup>abA</sup>	107 $\pm$ 6 <sup>abA</sup>	93 $\pm$ 20 <sup>ba</sup>	130 $\pm$ 44 <sup>abA</sup>
	8	71 $\pm$ 2 <sup>bb</sup>	169 $\pm$ 61 <sup>aA</sup>	142 $\pm$ 32 <sup>abA</sup>	102 $\pm$ 7 <sup>abA</sup>	131 $\pm$ 44 <sup>abA</sup>	141 $\pm$ 27 <sup>abA</sup>
	16	74 $\pm$ 2 <sup>ab</sup>	124 $\pm$ 33 <sup>aA</sup>	164 $\pm$ 50 <sup>abA</sup>	130 $\pm$ 53 <sup>aA</sup>	112 $\pm$ 19 <sup>aA</sup>	144 $\pm$ 41 <sup>aA</sup>
	24	81 $\pm$ 1 <sup>ba</sup>	122 $\pm$ 25 <sup>abA</sup>	161 $\pm$ 7 <sup>aA</sup>	129 $\pm$ 39 <sup>abA</sup>	160 $\pm$ 29 <sup>aA</sup>	162 $\pm$ 32 <sup>aA</sup>
	32	*	111 $\pm$ 15 <sup>aA</sup>	158 $\pm$ 21 <sup>aA</sup>	142 $\pm$ 52 <sup>aA</sup>	156 $\pm$ 49 <sup>aA</sup>	145 $\pm$ 29 <sup>aA</sup>
	40	*	*	163 $\pm$ 28 <sup>aA</sup>	196 $\pm$ 59 <sup>aA</sup>	163 $\pm$ 33 <sup>aA</sup>	145 $\pm$ 46 <sup>aA</sup>
	48	*	*	205 $\pm$ 13 <sup>A</sup>	*	*	*

Lower case letters or capital letters different in the same row or column, respectively, significant differences ( $p < 0.05$ ) by Tukey test at 95% confidence. CF = Control Formulation (mini panettone without coating). SF = Selected Formulation non-additive (46 g/kg potato starch/14 g/kg invert sugar/7 g/kg sucrose). ASF<sub>1</sub> = SF + 1 g/kg potassium sorbate. ASF<sub>2</sub> = SF + 10 g/kg citric acid. ASF<sub>3</sub> = SF + 1 g/kg potassium sorbate + 10 g/kg citric acid. ASF<sub>4</sub> = SF + 0.5 g/kg potassium sorbate + 5 g/kg citric acid. \*Discarded formulations due to mold growth in the previous period. Averages of triplicates.



**Fig. 4.** Behavior of the activity water values of mini panettone stored at 25 °C and 35 °C.

physical, physicochemical and microbiological attributes. The benefits to be afforded to food processors and consumers by effective edible coating formulations justify further research in this field. Thus, the current work opens a new perspective to edible

coatings application to assure safety and quality of bakery products; however, more research is needed in order to prove the significance of such results in other food products.

**Table 5**

Shelf life of mini panettones without coating (CF) with non-active (SF) and active coating (ASF<sub>1</sub>, ASF<sub>2</sub>, ASF<sub>3</sub> and ASF<sub>4</sub>) under accelerated conditions (35 °C) and storage at normal temperature (25 °C).

Formulations	Shelf life (days)		
	Accelerated conditions (35 °C)	Q <sub>10</sub>	Normal conditions (25 °C)
CF	16	1.0	16
SF	24	1.93	46
ASF <sub>1</sub>	40	2.67	106
ASF <sub>2</sub>	32	2.03	65
ASF <sub>3</sub>	32	2.22	71
ASF <sub>4</sub>	32	2.04	65

CF = Control Formulation (mini panettone without coating). SF = Selected Formulation (46 g/kg potato starch/14 g/kg invert sugar/7 g/kg sucrose). ASF<sub>1</sub> = SF + 1 g/kg potassium sorbate. ASF<sub>2</sub> = SF + 10 g/kg citric acid. ASF<sub>3</sub> = SF + 1 g/kg potassium sorbate + 10 g/kg citric acid. ASF<sub>4</sub> = SF + 0.5 g/kg potassium sorbate + 5 g/kg citric acid.

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