

Full Length Research Paper

Physicochemical and microbiological stability of muffins packed in actives edible coatings from cassava starch: Inverted sugar/sucrose and natural additives

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Received 31 December, 2018; Accepted 4 February, 2019

The aim of this study was to develop edible coatings containing natural additives incorporated to the matrix of cassava starch, for use in muffins, as well as to evaluate the efficacy of its use in the increase of shelf life and maintenance of the products characteristics. Seventeen film forming formulations were developed following a central composite experimental design (CCD). The influence of independent variables (soluble coffee, cocoa powder and propolis extract) was determined on the sensorial (colour, taste, texture and dissolution) and physical-chemical parameters (water activity, humidity, hardness, chewiness, cohesiveness and elasticity) of the muffins with edible active coatings. Two new formulations were selected for the study of microbiological and physical-chemical stability during the storage. In the sensorial evaluation, it was verified that the variables studied exerted a significant effect ($p < 0.05$) for colour, taste and solubility of the coated product. Regarding the texture, none of the variables showed a significant effect ($p > 0.05$). For colour, the concentrations of soluble coffee and cocoa powder were significantly influenced ($p < 0.05$) both by the tasters and by instrument (L^* , a^* , and b^*). In the shelf life test, the active coating containing soluble coffee (0.76%), cocoa powder (0.22%) and propolis extract (0.82%) increased the shelf life of the muffins in up to six times, when compared with the control. The result was approximately 87 days shelf life in normal storage conditions (25°C). It was evidenced that the additives tested have an antimicrobial action, associated to the preservation of the other stability properties of the product.

Key words: Additives, stability, shelf life, edible coatings.

INTRODUCTION

Cakes are confectionary products much appreciated as desert or snacks, coming in different formats, tastes and textures, which vary with formulation or the method used

in manufacturing (Jan et al., 2018). It is a product created through the mixture, homogenization and convenient cooking of the dough prepared with fermented or non-

fermented flours and other food items such as milk, eggs and fat (Gutkoski et al., 2009). A strong tendency of the industrialized cakes market is the snacks, small cakes wrapped in individual packaging, also called muffins (Channaiah et al., 2017; Kaur and Kaur, 2018). It is noted that one of the main factors associated with poor quality in cakes is the low specific volume and the non-uniform structure of the centre, besides hardness, loss of humidity and microbial development during shelf life (Abdou et al., 2008; Liu et al., 2018).

The minimization of humidity migration during storage could be reached with the use of films and coatings, giving special attention to edible ones (Fakhouri et al., 2015; Ollé Resa et al., 2016). Edible coating could be defined as a fine layer of biodegradable material, deposited on a food item. Its purpose is to inhibit or minimize the migration of humidity, oxygen, carbon dioxide and aromas, working as a semi-permeable barrier and flavouring, antioxidants and antimicrobial carrier, promoting the improvement of the product's texture and colouring and the increase of the products shelf life (Razavi et al., 2015; Antoniou et al., 2015; Treviño-Garza et al., 2015; Galus and Kadzińska, 2015; Pagno et al., 2016).

Novel food packaging technologies arose as a result of consumer's desire for convenient, ready to eat, tasty and mild processed food products with extended shelf life and maintained quality (Majid et al., 2018). However, currently, researchers have been exploring novel and reliable alternatives in order to delay bacterial growth and also contribute to preserving the freshness and quality of food products (Tian et al., 2018). Edible films are an example of these new products preservation methods. Edible coatings have been manufactured with incorporated antimicrobial and antioxidant agents, which can reduce spoilage events by enhancing the shelf life of food products (Ramírez-Guerra et al., 2018; Resende et al., 2018).

The biopolymers mostly used in the manufacturing of these coatings are the proteins (gelatines, casein, egg albumin, wheat gluten, zein and myofibrillar proteins), the polysaccharides (starch and its derivatives, pectin, cellulose and its derivatives, alginate and carrageenan) and the lipids (acetylated glycerides, stearic acid, wax and fatty acid ester) or their combination (Oriani et al., 2014; Antoniou et al., 2015; Gutiérrez et al., 2015; Ban et al., 2015; Barba et al., 2015; Aydogdu et al., 2018).

The interest of the food industry on edible films and coatings for the control of humidity transference in food is justified by the need to maintain the quality throughout the whole shelf life of the product. Besides, there is also a need to decrease the volume of disposed synthetic packaging (Vilela et al., 2018; Vital et al., 2018; Rangel-

Marrón et al., 2019). Another aspect is the possibility of introducing additives to films and coatings, such as antioxidants (Ganiari et al., 2017), aromas and antimicrobial agents, improving the product's integrity (Sun et al., 2014; Gutiérrez et al., 2016).

Due to its availability, biodegradability, renewability, film-forming ability, and low cost, starch from different botanical sources (cassava, corn, wheat, rice, potato, pea, and others) is one of the most promising natural polymers for packaging applications (Sapper et al., 2019). Studies have shown that the use of cassava starch as raw material to manufacture edible films and coatings provides a good aspect and an intense shine, making the food items more commercially attractive due to the more resistant, transparent and efficient biodegradable packaging, acting as barriers against water loss (Farris et al., 2014; Da Silva et al., 2015; Abreu et al., 2015). Due to the food grade of the cassava starch film, it can be ingested as a whole packaged product (Veiga-Santos et al., 2007; Fakhouri et al., 2015; Gutiérrez et al., 2016). In addition, there is the concern of consumers on food safety, increasing the search for natural additives with antimicrobial action (Bajpai et al., 2018; Karmaus et al., 2018), among other functions, to be used in substitution to the synthetic additives normally used with this end in bakery, such as potassium sorbate, citric acid and calcium propionate (Valerini et al., 2018). The sources of bioactive compounds are cocoa powder, coffee and propolis extract, which can be used as sources of natural antimicrobial compounds (Sorour et al., 2014; Femi-Adepoju and Olufemi Adepoju, 2014; Bonilla and Sobral, 2016).

In this context, the aim of this study was to develop edible coatings containing different natural additives (soluble coffee, cocoa powder and propolis extract) incorporated to the matrix of cassava starch, plasticised with sucrose and inverted sugar for use in muffins, as well as to evaluate the efficacy of its use in the increase of shelf life and maintenance of the products characteristics.

MATERIALS AND METHODS

Raw material

In the development of edible active coatings, cassava starch (Cargill Agrícola S/A), inverted sugar (Guarany S/A), sucrose (União, Brasil), cocoa powder (Garoto, Brasil), soluble coffee (Nescafé – Brasil), propolis extract (Prodapys, Brasil) and BOPP (Bioriented Polypropylene) metallic packaging (Doces Sabor da Bahia, Brasil) were used. To prepare the muffins, products were used as a wheat flour, refined sugar, pasteurized liquid eggs, liquid milk, lectin and glycerine P.A., from the local market in Salvador, Bahia, Brazil.

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Table 1. Actual values of each natural additive (independent variables) for the seventeen formulations (active edible coatings) studied.

Formulation	Independent variables		
	Soluble coffee (X1)	Cocoa powder (X2)	Propolis extract (X3)
F1	0.40	0.40	0.40
F2	0.40	0.40	1.60
F3	0.40	1.60	0.40
F4	0.40	1.60	1.60
F5	1.60	0.40	0.40
F6	1.60	0.40	1.60
F7	1.60	1.60	0.40
F8	1.60	1.60	1.60
F9	0.00	1.00	1.00
F10	2.00	1.00	1.00
F11	1.00	0.00	1.00
F12	1.00	2.00	1.00
F13	1.00	1.00	0.00
F14	1.00	1.00	2.00
F15*	1.00	1.00	1.00
F16*	1.00	1.00	1.00
F17*	1.00	1.00	1.00

*Central points.

Experimental planning

Seventeen film forming formulations were developed following a central composite experimental design (CCD), with an order model 2³, containing 4 axial points, 10 orthogonal points and three central points (Table 1). The solutions were applied as edible coatings for muffins, and evaluated alongside the control (without coating).

With the results of the parameters of muffins coated with the seventeen formulations, Pareto graphs were built to determine the influence of independent variables (soluble coffee, cocoa powder and propolis extract) on the sensorial (colour, taste, texture and dissolution) and physical-chemical parameters (water activity, humidity, hardness, chewiness, cohesiveness and elasticity) of the muffins with edible active coatings, for selection of two new formulations. The results that showed a significant influence on the Pareto graphs were evaluated through the ANOVA test, at a confidence level of 95%. Two new formulations were selected for the study of microbiological and physical-chemical stability during the storage, as subsequently shown.

Preparation of films solutions and application of edible active coatings on muffins

A solution of cassava starch (5.0%), inverted sugar (1.4%), sucrose (0.7%) and natural additives, such as soluble coffee (0.0-2.0%), cocoa powder (0.0-2.0%) and propolis extract (0.0-2.0%) (Table 1), dissolved in distilled water, warmed at 70°C under constant agitation and cooled at room temperature (25±2°C) was prepared for the formulations of the film forming solutions. Afterwards, the solutions were applied in two layers in the muffins samples, using a silicon brush. After the application, the muffins were put in the oven

at 190 to 200°C temperature for 5 min, to completely dry the coating (Figure 1).

Characterization of muffins with edible active coatings sensorial evaluation

After coating, samples of all formulations were submitted to a sensorial evaluation of reaction to colour, taste, texture and dissolution. In order to determine the scores given to the products, a hedonic scale structured in nine points and a team of 60 tasters were used (IAL - Instituto Adolfo Lutz, 2008).

Water activity (Wa) and humidity (RH)

The Wa was determined as a decagon (Lab Master, Novasina - TECNAL, SP/Brazil), with an electrolytic measurement cell CM-2. The samples were pre-stored at 60% RH at 25°C (Veiga-Santos et al., 2005). The humidity content (%) was determined by infrared drying (Mettler LTJ) (IAL - Instituto Adolfo Lutz, 2008).

Instrumental analysis of texture and colour

The hardness, cohesiveness, elasticity and chewiness were evaluated on the texture analyser CT3 Texture Analyzer (Brookfield), according to method AACC 74-09 (American Association of Cereal Chemists, 2000). The colour was determined on Konica Minolta colorimeter - TECNAL, through the following parameters: L* (luminosity), a* and b* (chromaticity coordinates). In this system, L* indicates luminosity (0 = black and 100 = white) and

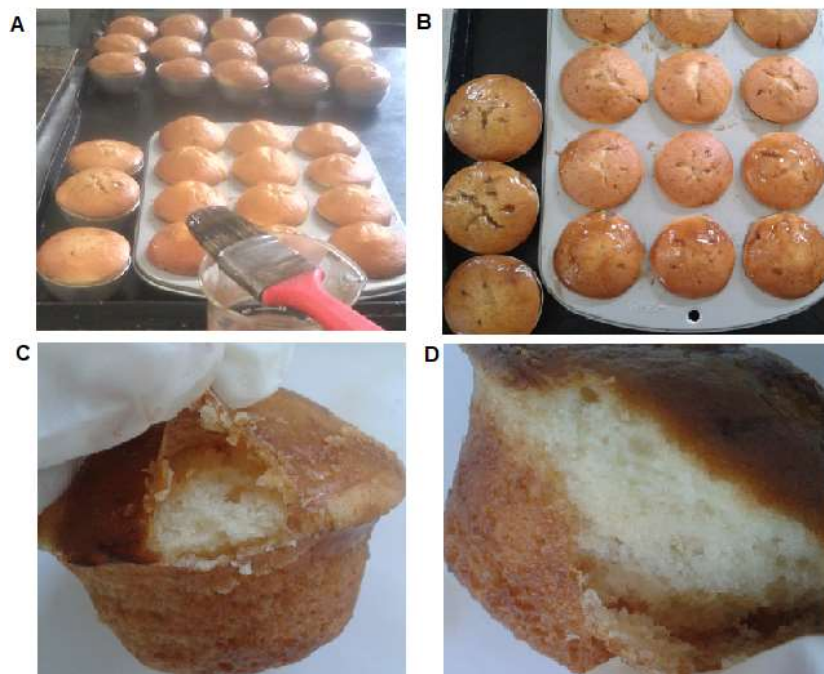


Figure 1. Application of edible films on muffins (A), coating completely dry (B), and muffins with edible films (C and D).

a^* and b^* indicate the directions that the colour could assume ($+a^*$ = red and $-a^*$ = green; $+b^*$ = yellow and $-b^*$ = blue) (Lopes, 2005).

Microbiological analysis

The mould and yeast counts were evaluated in the muffins during storage through the surface plating method using the Agar Dicloran Rose Bengal Chloramphenicol (DRBC) medium (King et al., 1979).

Monitoring of muffins stability with edible active coatings selected during storage

The best concentrations defined by the central composite experimental design for the independent variables (soluble coffee, cocoa powder and propolis extract), in the evaluation of sensorial attributes of colour, taste, texture and dissolution were used on the development of two new formulations selected for coating the muffins.

The muffins with active edible coatings and the control (without coating), stored in a bio-oriented polypropylene (BOPP) commercial packaging were monitored in each 8 days for 48 days of storage in climatic chamber (TECNAL) on accelerated conditions of oxidation (60% UR, 35°C), by analysing W_a , humidity, texture, colour and microbiological evaluation.

Shelf life

The Arrhenius mathematical model was used to determine the reaction rate constant (K), activation energy (Ea) and temperature acceleration factor (Q10), in order to determine the shelf life of the products in normal and accelerated conditions. Besides the storage in accelerated conditions, the muffins were stored in room

temperature (25°C) with W_a and microbiological evaluation performed at each eight days.

The reaction rate constant (K) was calculated using Equation 1. A graphic representation of the W_a values versus storage period for temperatures 25 and 35°C produced a linear regression equation, which enabled the determination of K in the temperatures of 25 and 35°C (K25 and K35).

$$K_T = e^{\ln A - \frac{E_a}{R} \frac{1}{T}} \quad (1)$$

For the calculation of the temperature acceleration factor (Q10), Equation 2 was used, which is multiplied by the shelf life of the muffins in accelerated conditions, determining the shelf life of these products in real conditions.

$$Q_{10} = \frac{K_T}{K_{T-10}} \quad (2)$$

where Ea = activation energy in cal.mL⁻¹, R = universal constant for gases (1.987 cal.mL⁻¹K⁻¹), T = temperature in absolute scale (Kelvin). K_T = reaction rate coefficient in a determined temperature, and K_{T-10} = reaction rate coefficient in 10°C lower temperature.

RESULTS AND DISCUSSION

Sensorial evaluation

Edible films and coatings are innovations within biodegradable active packaging concept, which can

Table 2. Average values found for the acceptance test (Hedonic Scale) for the 17 formulations of muffins with active edible coatings and the control (without coating).

Formulation	Colour	Flavour	Texture	Dissolution
C	8.10	7.97	8.00	8.13
F1	6.83	7.40	7.13	6.30
F2	6.20	5.03	6.90	6.40
F3	4.57	6.70	6.67	6.73
F4	6.27	6.03	5.87	6.33
F5	6.77	6.70	6.70	6.83
F6	6.17	6.07	5.87	6.17
F7	4.77	5.87	5.83	5.73
F8	4.40	4.93	5.53	5.70
F9	6.17	6.77	6.90	6.80
F10	4.93	5.47	5.67	6.23
F11	6.60	5.93	6.20	6.50
F12	4.57	6.00	5.87	5.93
F13	5.83	7.33	6.30	6.73
F14	5.63	6.37	6.27	5.70
F15*	6.50	6.63	6.57	6.73
F16*	6.67	6.70	7.27	6.80
F17*	6.53	6.90	6.67	6.47

*Central points; Muffins without coating (Control, C); Muffins coated with formulations of different composition (F).

improve safety and/or functional or sensory properties while maintaining the quality of food packaging (Parra et al., 2004; Chinma et al., 2014). The average values of the sensorial parameters colour, taste, texture and dissolution of the muffins coated with the 17 formulations are shown in Table 2. The control treatment resulted in parameters with better acceptance by the tasters, understanding, at first, that the natural additives (soluble coffee, cocoa powder and propolis extract) interfere in order to reduce the acceptance of the muffins.

In the sensorial evaluation of the attribute colour, when compared with the control, only the average scores for the muffins coated with formulations F3 and F12 were less accepted by the tasters. The statistical analysis presented that the results obtained showed a significant negative effect ($p < 0.05$), indicating that the increase in the concentration of variables (soluble coffee, cocoa powder and propolis extract) results in the decrease of these attributes average, which was corroborated by the surface response graphs obtained (Figure 2A and B).

The average values obtained for the attribute taste of coated muffins are adjusted to the model, and can represent the effect of the concentration of the independent variables (soluble coffee, cocoa powder and propolis extract) in a significant ($p < 0.05$) and predictive manner. The response surfaces obtained for this parameter allow the visualization of the results for the sensorial analysis of attribute taste (Figure 2C, D and E). Among the tested variables, propolis contributes more

significantly for decrease of the attribute taste (Figure 2D and E).

The averages reached in the sensorial evaluation of the attribute colour of the muffins with active edible coatings resulted in a low correlation with the attribute taste ($R^2 = 0.38$). These results mean that only 38% of the formulations with the best evaluations for colour tend to show the best evaluation for the attribute taste. For the texture evaluation, the formulations F1 and F16 obtained the higher average in the acceptance (Table 2). The statistical analysis shows that the tested variables did not exert an effect with significant difference ($p > 0.05$) on the parameter. This indicates that the muffins were not altered for this attribute, independently of the concentrations tested on the coatings (soluble coffee, cocoa powder and propolis extract), which indicate a relative robustness in the formulations (Table 2 and Figure 2C).

In the sensorial evaluation of the attribute dissolution, it was noted that, in the same way identified for taste, the variable propolis extract exerts a stronger significant effect ($p < 0.05$) on the decrease of this parameter (Figure 2D). Through the response surfaces (Figure 2F, G and H), it can be seen that the variables cocoa powder and soluble coffee compensate this lower acceptance even more pronounced in relation to dissolution than on the taste attribute. That way, the increase in concentration of propolis extract, as well as the interaction variables, results in a lower dissolution of the product during the

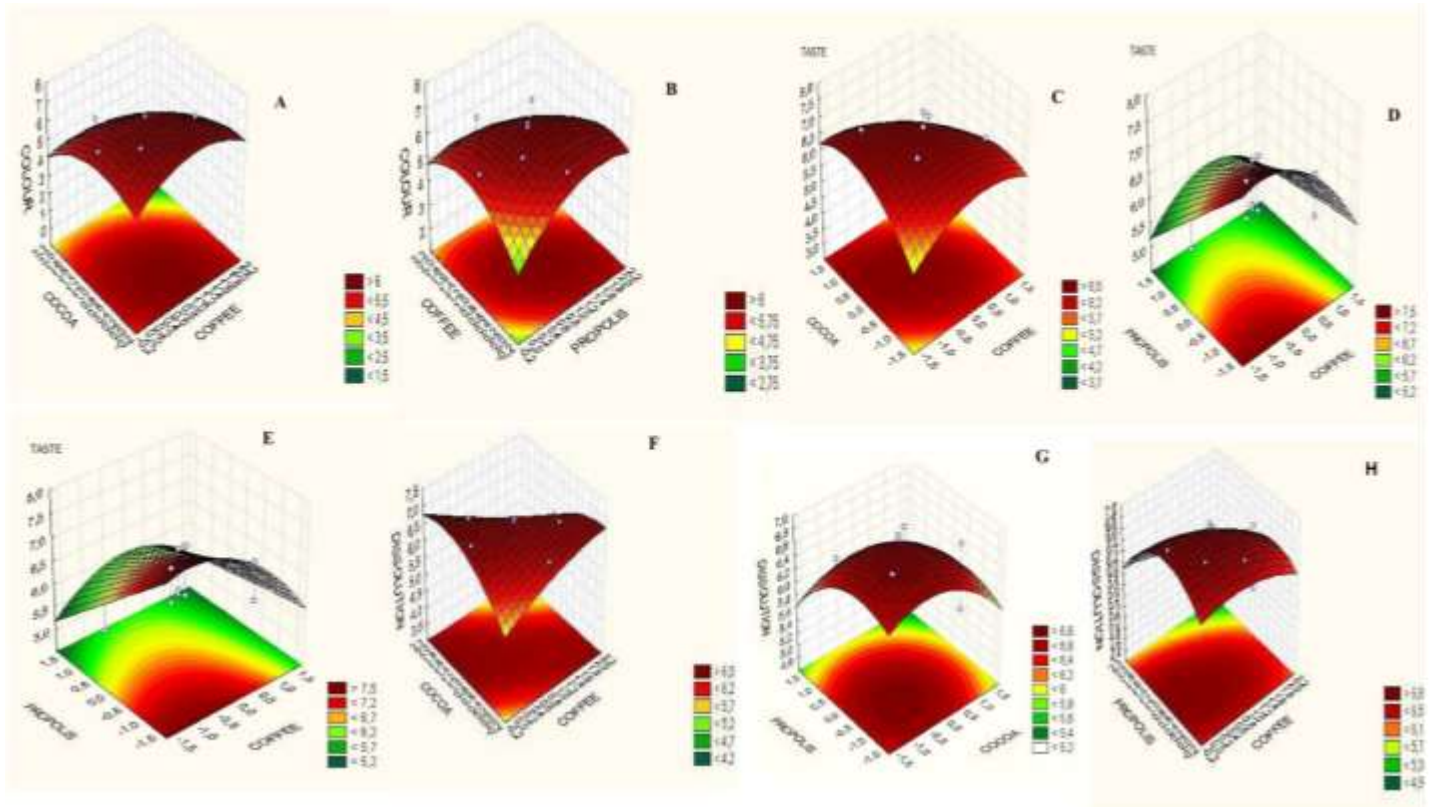


Figure 2. Response surface for sensorial analysis of the colour (A, B), taste (C, D, E) and dissolution (F, G, H) attributes of the muffins with active edible coatings, compared to the concentration of the independent variables (soluble coffee, cocoa powder and propolis extract).

muffins during chewing. This undesirable effect can be noted in the averages evaluation of the attribute dissolution, where all muffins samples coated with the 17 formulations obtained inferior marks when compared with the control (without coating) (Table 2).

These results are in accordance with the observations reported in the literature. For example, chitosan has also been widely used as an edible coating for extending the shelf life of foods, and chitosan was applied to Cavendish banana (Suseno et al., 2014). Sensory analyses were conducted to monitor the changes in color, texture, and aroma. The uncoated bananas showed unsatisfactory appearance after four days of storage, whereas the coated bananas still demonstrated an acceptable appearance up to seven day storage. The results showed that coated banana fruit demonstrated delayed ripening processes compared to the uncoated banana.

In this context, from the results of the sensorial evaluation it was possible to verify that the different concentrations, especially of the variable propolis extract, show a significant influence on the attributes taste, colour and texture, but not on the texture of the muffins. The results of the variance analysis (ANOVA) demonstrate the possibility of a significant representation of the effects of the independent variables (soluble coffee, cocoa powder and propolis extract) on the evaluated attributes.

Physical-chemical characterization of the muffins packed with edible active coatings

Table 3 shows the results of the averages for the physical-chemical characterization (W_a , humidity and texture) of the muffins covered with edible active coatings and the control.

The values of W_a of the different formulations of muffins with edible active coatings varied between 0.620 and 0.710, being therefore considered products of intermediate humidity. Comparing with the control ($W_a = 0.700$), it is noted that, generally, the incorporation of active compounds to the coatings contribute to the decrease in W_a of the products.

The formulation F10 containing soluble coffee, cocoa powder and propolis extract showed the lower result for W_a (0.620), associated to a lower humidity percentage (9.88%). The humidity values varied from 9.88 to 12.16% (Table 3). In the comparison between the samples of the averages by the Tukey test at 95% confidence level, for both A_w and humidity, only the sample F10 showed a significant difference ($p < 0.05$) among the formulations. The statistical analysis showed that these two parameters are not significantly different ($p > 0.05$) of the independent variables in the tested concentrations. The hardness values of the muffins with the different edible

Table 3. Average values (\pm standard deviation) for the Wa content, humidity and texture of the muffins with edible active coatings and control (without coating).

Formulation	Wa	Humidity (%)	Texture			
			Hardness (g)	Cohesiveness	Elasticity (mm)	Chewiness (mJ)
C	0.700 \pm 0.01 ^{ab}	12.47 \pm 0.53 ^a	227.63 \pm 404.3 ^e	0.50 \pm 0.01 ^b	8.51 \pm 0.16 ^{bc}	94.43 \pm 17.12 ^g
F1	0.690 \pm 0.00 ^{ab}	12.16 \pm 0.32 ^a	295.33 \pm 413.6 ^{de}	0.51 \pm 0.00 ^{ab}	8.74 \pm 0.04 ^{abc}	129.50 \pm 18.14 ^{efg}
F2	0.680 \pm 0.01 ^{bc}	11.81 \pm 0.15 ^a	280.53 \pm 164.9 ^{de}	0.51 \pm 0.02 ^{ab}	8.78 \pm 0.08 ^{abc}	122.60 \pm 10.33 ^{fg}
F3	0.690 \pm 0.00 ^{ab}	11.34 \pm 0.56 ^{ab}	355.03 \pm 742.1 ^{cde}	0.54 \pm 0.03 ^{ab}	8.70 \pm 0.17 ^{abc}	165.33 \pm 42.48 ^{cdefg}
F4	0.680 \pm 0.00 ^{abc}	11.16 \pm 0.17 ^{abc}	395.70 \pm 446.5 ^{bcdde}	0.52 \pm 0.01 ^{ab}	8.70 \pm 0.04 ^{abc}	174.80 \pm 21.94 ^{bcddefg}
F5	0.680 \pm 0.02 ^{abc}	11.78 \pm 0.17 ^a	327.33 \pm 702.9 ^{de}	0.55 \pm 0.02 ^{ab}	8.96 \pm 0.20 ^{ab}	155.50 \pm 28.52 ^{defg}
F6	0.690 \pm 0.00 ^{ab}	11.99 \pm 0.11 ^a	281.63 \pm 495.0 ^{de}	0.54 \pm 0.02 ^{ab}	8.51 \pm 0.37 ^{abc}	126.23 \pm 19.27 ^{fg}
F7	0.690 \pm 0.01 ^{ab}	11.52 \pm 0.31 ^{ab}	365.43 \pm 1162.7 ^{cde}	0.51 \pm 0.02 ^{ab}	8.69 \pm 0.09 ^{abc}	156.93 \pm 45.03 ^{defg}
F8	0.700 \pm 0.00 ^{ab}	11.59 \pm 0.21 ^{ab}	329.06 \pm 884.8 ^{de}	0.52 \pm 0.01 ^{ab}	8.73 \pm 0.14 ^{abc}	146.50 \pm 40.09 ^{defg}
F9	0.700 \pm 0.01 ^{ab}	11.84 \pm 0.43 ^a	392.40 \pm 346.7 ^{bcdde}	0.60 \pm 0.11 ^a	9.05 \pm 0.18 ^a	209.40 \pm 27.82 ^{bcddef}
F10	0.620 \pm 0.00 ^d	9.88 \pm 0.22 ^c	1053.33 \pm 592.0 ^a	0.47 \pm 0.02 ^b	8.39 \pm 0.09 ^c	410.43 \pm 29.90 ^a
F11	0.650 \pm 0.00 ^c	10.79 \pm 0.46 ^{abc}	585.40 \pm 1323.7 ^{bc}	0.52 \pm 0.02 ^{ab}	8.80 \pm 0.10 ^{abc}	261.47 \pm 45.66 ^{bc}
F12	0.710 \pm 0.01 ^{ab}	12.09 \pm 0.44 ^a	375.96 \pm 795.7 ^{cde}	0.55 \pm 0.01 ^{ab}	8.87 \pm 0.18 ^{abc}	179.47 \pm 42.00 ^{cdefg}
F13	0.690 \pm 0.01 ^{ab}	11.54 \pm 0.64 ^{ab}	475.93 \pm 1231.0 ^{bcd}	0.55 \pm 0.02 ^{ab}	8.96 \pm 0.04 ^{abc}	230.20 \pm 54.55 ^{bcd}
F14	0.680 \pm 0.01 ^{abc}	10.31 \pm 0.32 ^{bc}	624.33 \pm 1236.5 ^b	0.53 \pm 0.05 ^{ab}	8.79 \pm 0.18 ^{abc}	281.43 \pm 28.30 ^b
F15*	0.690 \pm 0.01 ^{ab}	11.09 \pm 0.60 ^{abc}	475.23 \pm 748.0 ^{bcd}	0.55 \pm 0.03 ^{ab}	8.96 \pm 0.18 ^{abc}	226.03 \pm 32.91 ^{bcdde}
F16*	0.710 \pm 0.01 ^a	10.87 \pm 0.79 ^{abc}	302.03 \pm 487.9 ^{de}	0.55 \pm 0.02 ^{ab}	8.82 \pm 0.04 ^{abc}	138.47 \pm 15.20 ^{defg}
F17*	0.700 \pm 0.01 ^{ab}	10.98 \pm 0.64 ^{abc}	388.63 \pm 586.4 ^{bcdde}	0.54 \pm 0.02 ^{ab}	8.89 \pm 0.09 ^{abc}	182.25 \pm 23.53 ^{bcddefg}

*Central points; Control (C); Formulations (F); Values showing the same letter, on the same column, show significant differences ($p < 0.05$) by the Tukey Test at 95% confidence.

active coatings oscillated between 280.53 and 1053.33 g (control 227.63 g), depending on the concentration of the incorporated additive soluble coffee (Table 3). The only sample that showed a significant difference ($p < 0.05$) compared to control was the formulation F10 with a higher concentration of soluble coffee (2.0%). The higher hardness values were the same that showed the lower humidity levels and Wa (F10, F11 and F14), which was corroborated by the existence of moderate correlations inversely proportional between hardness and humidity ($R^2 = 0.66$) and between hardness and Wa ($R^2 = 0.69$).

According to Table 3, the values of cohesiveness of the muffins varied between 0.47 and 0.60, with no significant differences ($p > 0.05$) between the 17 formulations and the control. The average values found for the parameter elasticity varied from 8.39 to 9.05 mm, whereas the control showed a value of 8.51 mm. Chewiness is a parameter of texture easily related to the sensorial analysis through trained panels (Esteller and Lannes, 2005) and can be interfered directly proportional to the hardness and cohesiveness, elasticity different from elasticity (Osawa et al., 2009). This can be verified in the evaluation of the variables correlation, where it was possible to note the existence of a directly proportional correlation of this variable with hardness ($R^2 = 0.96$), and

inversely proportional with humidity and Wa ($R^2 = 0.58$ and 0.65, respectively).

The comparison of the averages for the instrumental colour indices by the Tukey test identified a significant difference ($p < 0.05$) of the value for chromaticity a^* on the 17 formulations and control, which can be visualized on the Pareto graphs (Figure 3), demonstrating that soluble coffee and cocoa powder significantly interfere ($p < 0.05$) linearly and negatively on the colour indices of the coated muffins.

There are no significant effects ($p > 0.05$) of the independent variables (soluble coffee, cocoa powder and propolis extract) on the parameters Wa, humidity, hardness, cohesiveness, elasticity and chewiness of the muffins coated with the 17 formulations, indicating that the formulations present solidity, a desirable characteristic regarding the process in relation to these parameters.

Due to the effects observed on the response surfaces relative to the evaluation of the sensorial analysis for acceptance of the attributes for colour, taste, texture and dissolution, two formulations were selected for the study of microbiological stability and physical-chemical throughout storage. The selected formulations were FS1 (0.7% of soluble coffee, 0.88% cocoa powder and 0.0% propolis extract) and FS2 (0.76% soluble coffee, 0.22%

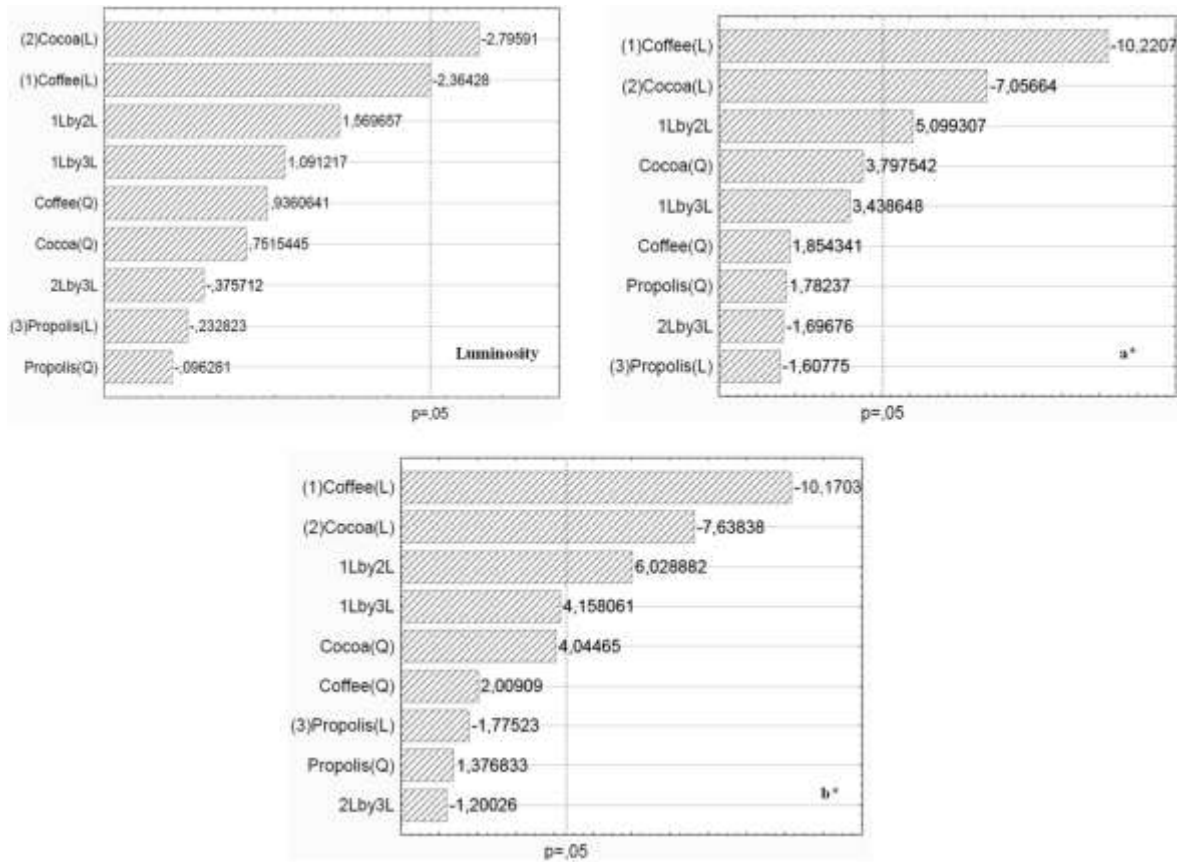


Figure 3. Pareto graph for instrumental analysis of luminosity, a* and b* of the muffins with cassava starch edible active coating (5.0%, p/p), inverted sugar (1.4%, p/p) and sucrose (0.7%, p/p), soluble coffee (0.0-2.0%, p/p), cocoa powder (0.0-2.0%, p/p) and propolis extract (0.0-2.0%, p/p).

cocoa powder and 0.82% propolis extract).

Monitoring of the microbiological and physical-chemical stability of the muffins with edible active coating selected during storage

It is important to highlight that, as the samples presented a deteriorating aspect visible to the naked eye on the coating surface, supposedly due to the growth of mould and/or yeast, they were disposed of, which occurred from 8 and 24 days, respectively, for the muffins control (no coating) and coated with the formulation (FS1). For the muffins coated with formulation FS2, this alteration was not detected until 48 days of storage, maximum limit of study, showing the most pronounced effect of propolis extract which can be strengthened by soluble coffee and cocoa powder on the coatings (Table 4). Costa et al. (2014) report a similar result on the evaluation of the antimicrobial efficacy of the active film with alcoholic extract of red propolis used to pack curdled cheese, which performed an important role in inhibiting the development of the microorganism *Staphylococcus*,

proving therefore an antimicrobial effect of the active film.

Edible film coatings represent a viable preservation technology, they often constitute a barrier that delays deterioration and maintain the product structural integrity (Osorio et al., 2011). In addition, edible film coatings is a food covering film which can be eaten as part of the food, as was showed in this work. In this context, the edible active coating developed caused an inhibitory effect in the microbial growth, with an increase of at least 200% throughout the period of development of mould and yeast in accelerated conditions, when compared with muffins without coating. This represents a reduction on the losses by deterioration of the product due to the increase of its shelf life.

The contents of Wa on day zero and on the 16th day of storage shown by the muffins coated on formulations FS1 and FS2 reached a similar performance to that shown by the muffins control, with no significant difference ($p > 0.05$) by the Tukey test. On the 8th day of storage, these parameters of the muffins coated by formulations FS1 and FS2 showed significant difference ($p < 0.05$) in relation to the muffin control. On the 32nd day of storage it was not possible to analyse it statistically, since the

Table 4. Values of mould and yeast count of control (C) and muffins coated with formulations FS1 and FS2 throughout 48 days storage.

Days	C (UFC.g ⁻¹)	FS ₁ (UFC.g ⁻¹)	FS ₂ (UFC.g ⁻¹)
0	<10	<10	<10
8	<100	<10	<10
16	-*	<10	<10
24	-*	<100	<10
32	-*	-*	<10
40	-*	-*	<10
48	-*	-*	<10

Control (muffin with no edible active coating), FS1 = (5% cassava starch, 1,4% inverted sugar, 0,7% sucrose, 0,7% soluble coffee, 0,88% cocoa powder and 0,0% propolis extract). FS2 = (5% cassava starch, 1,4% inverted sugar, 0,7% sucrose, 0,76% soluble coffee, 0,22% cocoa powder and 0,82% propolis extract). -*Formulation presented mould visible to the naked eye.

formulation FS1 also showed a visible growth of microorganisms, and the monitoring was interrupted for this formulation. But it can be observed that the value found for the muffins coated with formulation FS2 (0.74) was similar to 16 and 24th days (0.73) (Table 5). The values found in this study are inferior to those obtained by Osawa et al. (2009) who studied the effect of edible coatings made of gelatine, stearic acid or carnauba wax on the physical-chemical properties of the chocolate cakes.

Other authors have demonstrated similar results in foods coated with edible film, because of the antimicrobial capacity of natural additives or the polymer matrix, especially against the fungi and yeast spoilage (Chiumarelli et al., 2010; Romanazzi et al., 2013). Fungal contamination on the surface of foods is a main reason that consumers do not purchase and consume these foods (fruits, breads, cheeses), and coating the surface of these foods with an edible film-forming solution can decrease this type of spoilage and increase the shelf life (Valenzuela et al., 2015).

The humidity values of the muffins coated with formulations FS1 and FS2 on day zero were similar (14.74 and 14.40%, respectively), differently from the control muffins (18.58%). So, the muffins produced with the edible active coatings (FS1 and FS2) are significantly different ($p < 0.05$) of the muffins control. On the 8th day of storage, the humidity content of the muffins coated with FS1 and FS2 did not differ statistically among themselves ($p > 0.05$), when compared with the value on day zero (Table 5). These results confirmed that the samples with edible active coatings are capable of interacting with the environment and with the packaged product, stopping the increase of humidity, which could damage the texture of the product, especially its smoothness.

The humidity contents on the 16th day of storage reached similar results, showing no significant difference amongst them ($p > 0.05$) by the Tukey test (Table 5). During this period, a decrease in the humidity of the

product coated with formulations FS1 and FS2 were observed, which could be explained by the downgrading of the starch induced by the thermal processing, causing undesirable effects such as the migration of water to the surface. These effects induce major changes in consistency and texture of the product (Martínez-Cervera et al., 2014).

On the comparison of the averages by the Tukey test at 95% confidence level, it was noted that the lower values for hardness were found for the control, which significantly differs from the muffins coated with formulations FS1 and FS2 on the 8th day of storage ($p < 0.05$). The muffins coated with formulation FS2 showed less hardness between the 8 and 24th days when compared with formulation FS1 (13 to 31% lower). This effect is caused by the difference in composition of the variables on coatings (Table 5).

The hardness of the muffins coated with formulation FS1 did not differ statistically between themselves until the 16th day ($p > 0.05$), but they are different statistically from the muffins with 24 days of storage ($p < 0.05$, Table 4), which is approximately twice higher than the initial. Studies report double the initial hardness in cakes stored at 21°C for 21 days (Gélinas et al., 1999). An inappropriate barrier, packaging or edible coating leads to loss of humidity of the muffin. The effect of the starch downgrading should also be considered (Sluimer, 2005).

The values of hardness and chewiness of the muffins coated with formulations FS1 and FS2 progressively increased between days 0 and 24 ($R^2 = 0.84$) or 0 and 48 ($R^2 = 0.92$), respectively, whereas cohesiveness and elasticity showed a variable behaviour throughout storage.

The inversely proportional correlations between W_a and hardness of the muffins are low ($R^2 = 0.2$ FS1, $R^2 = 0.3$ FS2). However, between humidity and hardness they were higher ($R^2 = 0.76$ FS1, $R^2 = 0.94$ FS2). The same behaviour was found for the correlations between chewiness and W_a ($R^2 = 0.49$ FS1, $R^2 = 0.26$ FS2) and

Table 5. Average values (\pm standard deviation) Wa, humidity, hardness, cohesiveness, elasticity and chewiness of control (C) and the muffins coated with formulations FS1 and FS2 throughout the 48 days of storage.

Parameter	Days	C	FS ₁	FS ₂
Wa	0	0.82 \pm 0.00 ^{aA}	0.79 \pm 0.04 ^{Aa}	0.73 \pm 0.02 ^{aBC}
	8	0.83 \pm 0.02 ^{*aA}	0.77 \pm 0.01 ^{bAB}	0.77 \pm 0.01 ^{bA}
	16	*	0.73 \pm 0.02 ^{aB}	0.73 \pm 0.01 ^{aBC}
	24	*	0.78 \pm 0.00 ^{*aAB}	0.73 \pm 0.01 ^{bBC}
	32	*	*	0.74 \pm 0.02 ^{BC}
	40	*	*	0.76 \pm 0.01 ^{AB}
	48	*	*	0.71 \pm 0.01 ^C
Humidity	0	18.58 \pm 1.31 ^{aA}	14.74 \pm 0.73 ^{bA}	14.40 \pm 0.75 ^{bA}
	8	20.37 \pm 0.35 ^{*aA}	14.08 \pm 0.69 ^{bA}	14.34 \pm 0.28 ^{bA}
	16	*	13.68 \pm 0.71 ^{aA}	13.48 \pm 0.47 ^{aAB}
	24	*	11.30 \pm 0.32 ^{*bB}	13.43 \pm 0.43 ^{aAB}
	32	*	*	12.94 \pm 0.31 ^B
	40	*	*	12.52 \pm 0.44 ^B
	48	*	*	10.58 \pm 0.32 ^C
Hardness (g $\times 10^{-1}$)	0	145.67 \pm 9.96 ^{aA}	344.53 \pm 126.06 ^{aB}	385.27 \pm 144.18 ^{aB}
	8	139.23 \pm 40.60 ^{*bA}	423.67 \pm 16.75 ^{aAB}	368.90 \pm 38.93 ^{aB}
	16	*	558.27 \pm 52.04 ^{aAB}	444.40 \pm 74.56 ^{aB}
	24	*	615.17 \pm 137.69 ^{*aA}	423.80 \pm 66.37 ^{aB}
	32	*	*	461.77 \pm 52.25 ^B
	40	*	*	527.63 \pm 123.75 ^B
	48	*	*	837.77 \pm 100.63 ^A
Cohesiveness	0	0.60 \pm 0.00 ^{aA}	0.50 \pm 0.10 ^{aA}	0.40 \pm 0.10 ^{aA}
	8	0.60 \pm 0.00 ^{aA}	0.60 \pm 0.00 ^{aA}	0.60 \pm 0.00 ^{aA}
	16	*	0.50 \pm 0.00 ^{aA}	0.60 \pm 0.00 ^{aA}
	24	*	0.50 \pm 0.00 ^{aA}	0.60 \pm 0.10 ^{aA}
	32	*	*	0.50 \pm 0.00 ^A
	40	*	*	0.50 \pm 0.10 ^A
	48	*	*	0.50 \pm 0.00 ^A
Elasticity (mm)	0	8.50 \pm 0.30 ^{aB}	8.80 \pm 0.30 ^{aB}	8.80 \pm 0.40 ^{aA}
	8	8.60 \pm 0.10 ^{aA}	8.70 \pm 0.30 ^{aA}	8.80 \pm 0.10 ^{aA}
	16	*	8.90 \pm 0.30 ^{aAB}	8.70 \pm 0.10 ^{aA}
	24	*	8.70 \pm 0.20 ^{aAB}	9.10 \pm 0.80 ^{aA}
	32	*	*	8.70 \pm 0.10 ^A
	40	*	*	8.80 \pm 0.10 ^A
	48	*	*	8.60 \pm 0.10 ^A
Chewiness (mJ)	0	68.70 \pm 5.80 ^{aA}	138.9 \pm 49.90 ^{aB}	142.0 \pm 65.10 ^{aB}
	8	65.70 \pm 23.60 ^{bA}	214.9 \pm 15.70 ^{aAB}	181.6 \pm 07.70 ^{aB}
	16	*	268.70 \pm 39.90 ^{aA}	223.8 \pm 39.10 ^{aB}
	24	*	255.20 \pm 39.70 ^{aA}	229.0 \pm 79.40 ^{aB}
	32	*	*	196.3 \pm 21.00 ^B
	40	*	*	251.80 \pm 14.60 ^B
	48	*	*	390.9 \pm 6.10 ^A

Values showing different lower case or upper case letters on the same line or column, respectively, show significant differences ($p < 0.05$) by the Tukey Test at 95% confidence level. C = Control (muffin without active edible coating). FS1 = (5% cassava starch; 1.4% inverted sugar; 0.7% sucrose; 0.7% soluble coffee; 0.88% cocoa powder; and 0.0% propolis extract). FS2 = (5% cassava starch; 1.4% inverted sugar; 0.7% sucrose; 0.76% soluble coffee; 0.22% cocoa powder; and 0.82% propolis extract). *Formulation that showed mould and yeast on the product surface.

Table 6. Average values (\pm standard deviation) of the luminosity analyses (L), a* and b* of the crust and core of the control (C) and muffins coated on formulations FS1 and FS2 throughout 48 days storage.

Color	Days	Treatments					
		Crust Muffin			Crumb Muffin		
		C	FS ₁	FS ₂	C	FS ₁	FS ₂
L*	0	51.03 \pm 0.65 ^{aA}	28.86 \pm 0.89 ^{bA}	29.76 \pm 0.26 ^{bA}	74.65 \pm 0.53 ^{aB}	70.39 \pm 0.35 ^{bC}	70.61 \pm 0.30 ^{bC}
	8	49.75 \pm 0.00 ^{aB}	27.69 \pm 0.83 ^{cA}	29.46 \pm 0.34 ^{bA}	80.91 \pm 0.00 ^{aA}	71.55 \pm 4.38 ^{bB}	71.56 \pm 0.53 ^{bB}
	16	*	28.01 \pm 0.40 ^{bA}	29.65 \pm 0.59 ^{aA}	*	72.27 \pm 0.21 ^{aA}	72.74 \pm 0.32 ^{aA}
	24	*	27.37 \pm 0.31 ^{bA}	28.80 \pm 0.47 ^{aAB}	*	72.70 \pm 0.27 ^{aA}	71.63 \pm 0.19 ^{bB}
	32	*	*	27.56 \pm 0.65 ^{BC}	*	*	70.27 \pm 0.23 ^C
	40	*	*	27.47 \pm 0.42 ^{BC}	*	*	70.56 \pm 0.37 ^C
	48	*	*	27.23 \pm 0.74 ^C	*	*	71.61 \pm 0.16 ^B
a*	0	15.66 \pm 0.00 ^{aA}	13.32 \pm 0.61 ^{bA}	13.15 \pm 0.53 ^{bA}	-0.45 \pm 1.74 ^{bA}	-2.47 \pm 3.06 ^{aB}	-0.17 \pm 0.02 ^{aD}
	8	15.29 \pm 0.98 ^{aA}	11.23 \pm 0.42 ^{bB}	12.41 \pm 0.37 ^{bAB}	-1.93 \pm 0.00 ^{bA}	0.78 \pm 0.02 ^{aA}	0.33 \pm 0.03 ^{aC}
	16	*	9.77 \pm 0.04 ^{bC}	12.38 \pm 0.27 ^{aAB}	*	1.30 \pm 0.17 ^{aA}	0.53 \pm 0.06 ^{aBC}
	24	*	7.89 \pm 0.07 ^{bD}	13.18 \pm 0.18 ^{aA}	*	3.24 \pm 0.50 ^{aA}	0.64 \pm 0.12 ^{aC}
	32	*	*	11.69 \pm 0.08 ^{aBC}	*	*	0.71 \pm 0.07 ^{BC}
	40	*	*	11.45 \pm 0.37 ^{BC}	*	*	1.07 \pm 0.08 ^{AB}
	48	*	*	11.38 \pm 0.43 ^C	*	*	1.48 \pm 0.14 ^A
bb*	0	39.04 \pm 1.07 ^{aA}	10.24 \pm 0.20 ^{cA}	12.91 \pm 0.74 ^{bA}	25.99 \pm 0.40 ^{bB}	29.25 \pm 0.16 ^{aB}	29.67 \pm 0.69 ^{aD}
	8	38.13 \pm 0.00 ^{aA}	9.29 \pm 1.16 ^{cB}	11.34 \pm 0.49 ^{bB}	28.79 \pm 0.00 ^{bA}	32.12 \pm 1.38 ^{aA}	31.79 \pm 0.31 ^{aC}
	16	*	5.95 \pm 0.29 ^{bC}	11.23 \pm 0.40 ^{aBC}	*	32.57 \pm 0.23 ^{aA}	32.80 \pm 0.48 ^{aBC}
	24	*	5.67 \pm 0.48 ^{bC}	11.36 \pm 0.16 ^{aB}	*	32.61 \pm 0.40 ^{aA}	32.24 \pm 0.18 ^{aC}
	32	*	*	10.42 \pm 0.29 ^{BC}	*	*	32.74 \pm 0.09 ^{BC}
	40	*	*	10.29 \pm 0.31 ^{BC}	*	*	33.94 \pm 0.60 ^{AB}
	48	*	*	10.14 \pm 0.15 ^C	*	*	34.48 \pm 0.36 ^A

Values showing different lower case or upper case letters on the same line or column, respectively, show significant differences ($p < 0.05$) by the Tukey test at 95% confidence level. C = Control (muffin without edible active coating). FS₁ = (5% cassava starch; 1.4% inverted sugar; 0.7% sucrose; 0.7% soluble coffee; 0.88% cocoa powder; and 0.0% propolis extract). FS₂ = (5% cassava starch; 1.4% inverted sugar; 0.7% sucrose; 0.76% soluble coffee; 0.22% cocoa powder; and 0.82% propolis extract). *Formulation showing mould and yeast on the surface of the product.

humidity ($R^2=0.43$ FS₁, $R^2=0.91$ FS₂). That way, as the humidity of the muffins coated with formulations FS₁ and FS₂ decreased during storage (Table 5), hardness and chewiness increased.

On the analysis of cohesiveness by the Tukey test, it was possible to observe that the control muffins and those coated with formulations FS₂ did not show significant difference between them ($p > 0.05$) during storage. In terms of elasticity, the values varied according to the formulation of each edible coating and in face of the respective hardness values. It was noted that the samples of muffins control and coated with formulation FS₁ differ statistically among themselves ($p < 0.05$) throughout the storage period (Table 5). It has been reported that the value of elasticity for bakery products generally decreases during storage periods (Baik et al., 2000), and cakes with higher alteration also show higher elasticity (Baixauli et al., 2008).

Throughout storage the values for the colour parameter L* of the control muffins (no coating) and coated with

formulations FS₂ differed significantly ($p < 0.05$). Whereas the muffins coated with formulations FS₁ did not show significant difference ($p > 0.05$, Table 6). The similarity in colour on formulations FS₁ and FS₂ are confirmed by the chromaticity a*, which indicated low values for the samples on crust and near the zero on the crumb. According to Baixauli et al., (2008) the higher the concentration of resistant starch in the dough, the lower the parameter a*.

On the analysis of the chromaticity parameter b*, it was noted that, regarding the crumb characteristics, the formulations FS₁ and FS₂ did not differ statistically between themselves ($p > 0.05$). That way, it was noted that during storage for up to 48 days, there was significant difference ($p < 0.05$) for the parameters L*, a* and b*, both for crust and for the crumb. This was expected, since the incorporation of the natural additives on the edible coating altered the colour of the muffins, due to the migration of the pigments derived from the soluble coffee and cocoa powder, which could have

Table 7. Shelf life of the control (no coating) and the muffins coated with the selected formulations (FS1 and FS2) in accelerated conditions (35°C) and in normal storage temperature (25°C).

Formulation	Shelf life (days)		
	Accelerated conditions (35°C)	Q ₁₀	Normal conditions (25°C)
C	8	1.0	8
FS ₁	24	2.18	33
FS ₂	40	2.04	87

altered the volatile compounds, and therefore justified the preference of the tasters on the sensorial analysis (Table 2).

Shelf life of the muffins with edible active coatings

Controlled delivery of active agents into the food via packaging films for extended periods of storage and distribution restricts the development of undesirable flavours produced as a result of directly incorporating additives into the food (Majid et al., 2018). In addition, the use of edible films and coatings can enhance the process of preservation of food as shown in this work.

The counting of values for the mould and yeast and *Wa* of the control (no coating) and that of the muffins with selected active coatings (FS1 and FS2), under accelerated conditions (35°C) and normal storage temperature (25°C) were monitored at each 8 days. The values of the shelf life and the Q₁₀ of the muffins coated with formulations FS1 and FS2 in accelerated conditions (35°C) and in normal storage temperature (25°C) are shown in Table 7. The formulation showing the shorter shelf life (8 days) in accelerated conditions was the control (no coating), which did not have any type of coating, also showing a shelf life of 8 days under normal storage conditions (25°C).

The coating that kept the muffins for long (40 days) in accelerated conditions was FS2 (0.76% soluble coffee, 0.22% cocoa powder and 0.82% propolis extract), with a shelf life of approximately 87 days in normal conditions of storage (25°C). This represents an increase of approximately 990 and 160% on the shelf life of products in relation to the control and formulation FS1, respectively.

The commercial brands of muffins show a best before date around 90 to 120 days, using additives such as potassium sorbate and sorbic acid incorporated to the formulations dough, with a maximum limit of 1.000 mg.kg⁻¹ (Codex Alimentarius, 2015). The result of the shelf life of muffins coated with formulation FS2 corresponds to the shelf life of the commercial brands, however without the use of these additives on its dough. This shows that the use of natural additives incorporated to the coating, in the tested concentrations, can exert the same function, representing an alternative to decrease the ingestion of synthetic preservatives and protect the consumer health.

In a recent study, muffins were developed from flax seed germinated and not germinated (Kaur et al., 2018). The prepared muffins were packed in linear low density polyethylene (LLDPE) and stored under ambient conditions and refrigerated for 1 month to evaluate the shelf life of the best levels. Even using LLDPE, only the muffins stored for 15 days under ambient conditions and for 1 month under refrigerated conditions had consumption conditions, demonstrating the efficiency of the material developed in this study for this type of food product.

In other work, cassava starch (and soy protein concentrate) edible coatings containing 20% glycerol was used in extending the shelf life of toasted groundnut (Chinma et al., 2014). Chemical indices of oxidative rancidity and sensory parameters were evaluated. The use of 50:50 (cassava starch:soy protein concentrate) edible coatings on toasted groundnut extended the shelf life of toasted groundnuts for 14 days compared to uncoated toasted groundnuts which developed objectionable taste after second day of storage at ambient (27 ± 1°C) condition.

The results obtained indicate that the edible active coatings based on cassava starch, plasticised with inverted sugar and sucrose, containing the natural active additives soluble coffee, cocoa powder and propolis extract, can be a competitive alternative to reduce the synthetic additives on the composition and preservation of muffins. The concentrations of the natural additives tested for the edible coatings reduce the water activity and humidity, fundamental properties for the storage of bakery products.

Conclusions

The edible active coating containing 0.76% soluble coffee, 0.22% cocoa powder and 0.82% propolis, effectively controlled the development of mould and yeast on the muffins without synthetic additives for 40 days in accelerated conditions. That resulted in a shelf life of 87 days in normal conditions, due to the antimicrobial action associated to the maintenance of the other properties of stability of the product. The shelf life of the commercial muffins containing synthetic additives is 90 days. The natural additives in the concentration used in this study

can exert the same function, representing an alternative to decrease the ingestion of synthetic preservatives. However, there is a need for additional studies to evaluate the action of these coatings in other bakery products, where the storage conditions, together with the products characteristics and its possible interactions with the coatings can demonstrate the real efficacy of the active coating.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors would like to thank the Serviço Nacional de Aprendizagem Industrial - Departamento Nacional (SENAI DN) (BA-13111), the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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