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Zein-based blend coatings: Impact on the quality of a model cheese of short ripening period

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ABSTRACT

Two biodegradable zein-based blend coatings were evaluated according to the impact on the quality of the "Minas Padrão" cheese throughout a storage period of 56 days. Throughout the storage, the biodegradable-coated cheese samples showed similar physicochemical characteristics in comparison to unpackaged and plastic-packaged cheese samples in terms of chlorides, ash, protein and acidity. Besides that, cheese samples with biodegradable coatings exhibited ca. 30% lower weight loss and avoided microbiological contamination for more than 50 days when compared to unpackaged cheese samples that exhibited contamination after 21 days. Although cheese samples with biodegradable coatings exposed a positive behavior in comparison to unpackaged cheeses, some challenges remain and require further studies. For instance, as a consequence of the moisture loss, after 28 days of storage, the biodegradable-coated cheeses were 124% harder, displayed 30% less proteolysis and more than 50% change in color surface than cheese samples with polyethylene packaging.

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1. Introduction

"Minas Padrão" is a semi-hard cheese and one of the most important cheeses in Brazil. It is a traditional cheese, widely consumed and prepared from cow's milk in few Brazilian states. According to *Laticínio Balkis*, the cheese supplier, "Minas Padrão" ripening is carried out after the cheese is salted in brine and packaged in a polyethylene packaging that undergoes thermal shrinkage. Cheese is ripened for 25 days under controlled conditions, 3.8 ± 0.5 °C and 85% relative humidity; thereafter, the cheese is distributed to the supermarkets across the country.

The commercial "Minas Padrão" cheese is distributed and commercialized in plastic packaging, in order to avoid external chemical or microbiological contamination and to help preserving the cheese quality. Plastics are the most frequently used food packaging materials (Lagaron and López, 2011; Siracusa et al., 2008); nonetheless, due to environmental concern and their low recycling rate (Siracusa et al., 2008; Salarbashi et al., 2014), new biodegradable and edible materials have been and are developed to reduce the plastic packaging usage (Auras et al., 2006; Fajardo et al., 2010; Salarbashi et al., 2014).

One material that has been used to produce biodegradable and edible films and coatings is zein, the main corn protein (Zhang et al., 2011; Wu et al., 2012), which is recognized by the U.S. Food and Drug Administration (FDA) as a GRAS (Generally Recognized as Safe) food ingredient (Scramin et al., 2011; U.S. Department of Health and Human Services (2015)). Zein films have exhibited glossy appearance, toughness, low water solubility, resistance to microbial attack and high hydrophobicity (Del Nobile, Conte, Incoronato & Panza, 2008). In spite of that, several authors have demonstrated that blend films, i.e. a combination of zein with other biomolecules, mainly lipids (Cuq et al., 1995; Wang and Padua, 2006; Arcan and Yemenicioğlu, 2013), allows for improving the functional properties of zein-based films.

In this regard, several studies have been focused on the development of new biodegradable and edible films, and the evaluation of their functional properties; nevertheless, few researches have explored their application to food (Gennadios et al., 1997; Fajardo et al., 2010).

For instance, some biodegradable materials that have been reported as cheese coatings are mainly polysaccharide-based, e.g. chitosan (Fajardo et al., 2010; Zhong et al., 2014), galactomannan (Cerqueira et al., 2010), alginate (Zhong et al., 2014), carrageenan, gellan (Kampf and Nussinovitch, 2000), and few protein-based

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coating such as whey protein isolate-WPI (Ramos et al., 2012) and soy protein isolate-SPI (Zhong et al., 2014). In all the previously referenced cases, cheeses with biodegradable coatings exhibited better preservation of cheese physicochemical characteristics than the uncoated counterparts. Moreover, to the best of our knowledge, Ramos et al. (2012) reported the uniquely available study that has compared synthetic and biodegradable cheese packaging. According to Ramos et al. (2012), the synthetic nonedible coating made of polyvinyl acetate (PVA) and the WPI biodegradable coating displayed similar performance on cheese quality preservation; therefore, the WPI coating is a suitable alternative for cheese packaging.

Hereby, the current study aims to evaluate the impact of two zein-based blend coatings on the physicochemical characteristics of the "Minas Padrão" cheese, as a model for short ripened cheeses, in comparison to polyethylene packaged and unpackaged cheese samples.

2. Materials and methods

2.1. Materials

A commercial brand of the "Minas Padrão" cheese was obtained from *Laticínios Balkis* (Santo Antônio do Aracanguá, Brazil) on the day of production. For the preparation of the zein-oleic acid (Z-OA) and zein-oleic acid-xanthan gum (Z-OA-XG) coatings, the following components were used: corn-zein (Sigma, São Paulo, Brazil), 99.5% ethanol (Synth, São Paulo, Brazil), oleic acid (Synth, São Paulo, Brazil), glycerol (Dinamica, Diadema, Brazil), xanthan gum (ADM, Chicago, USA) and Emustab emulsifier (Duas Rodas, Jaraguá do Sul, Brazil) composed by water, distilled monoglycerides of fatty acids, sorbitan monostearate and polyoxyethylene sorbitan monostearate.

2.2. Cheese sample preparation

After production, 84 semicircular cheeses with an approx. net weight of 0.4 kg each, were packaged in the regular polyethylene (commercial) packaging and transported under refrigeration from the dairy company to São Paulo State University in São Jose do Rio Preto, Brazil. The amount of cheeses was randomly divided into four groups that were named as unpackaged cheese (negative control), commercially packaged cheese (positive control), Z-OA coated cheese and Z-OA-XG coated cheese; thus, each group was composed by 21 semicircular cheeses.

After 5 h from cheese production, cheeses were cut, in order to obtain experimental samples (that will be referred to as samples in the remainder of the document). Cheese cutting was made first in four similar pieces along the radius of the semicircular shaped cheese and then each piece was further cut transversally providing eight similar samples of approx. 0.05 kg.

2.3. Coating solution preparation

The two zein-based coating solutions were prepared by adding 20 g zein to 100 mL 95% ethanol, and mechanically stirred for 5 min at 65 ± 0.5 °C. Glycerol (2 g), emulsifier (1 g) and oleic acid (14 g) were added to both solutions. Xanthan gum (0.05 g) was also added to the Z-OA-XG solution; afterwards, Z-OA and Z-OA-XG solutions were stirred for 10 min and these were allowed to reach room temperature before applying on the cheese surface.

2.4. Cheese coating procedure

After the cheese cutting, Z-OA and Z-OA-XG experimental samples were coated with a three-layer coating, as follows. One layer of the coating solution was brushed on the surface of the cheese sample and after 1 h of drying; a second layer was applied, repeating the procedure until completing three layers. Once the three layers were applied, the coating dried during 4 h at 24 °C and 50% relative humidity (%RH). Thereafter, the samples of the four cheese groups were organized in plastic trays and stored under controlled conditions (3.8 °C, 85%RH). The brushing method was used in order to avoid contamination of the coating solutions with cheese residues (such as whey or cheese fragments) that might interfere with the formation of the coating. Furthermore, the application of the three layers of the coating solution to the cheese surface allowed for producing a homogeneous coating with similar thickness in comparison to the commercial polyethylene packaging.

2.5. Cheese chemical composition and physicochemical analyses

Throughout 56 days of storage time, the "Minas Padrão" cheese samples were tested for physicochemical characteristics such as titratable acidity, moisture, total ash, chlorides, lipids, total nitrogen and proteolysis rate. The results of the tests were expressed on dry basis. Three replicates of 200 g each were used to perform the physicochemical analyses; based on that, each replicate was composed by four samples (4 × 0.05 kg) belonging to each cheese group. The three replicates were taken at 0, 7, 14, 21, 28, 42 and 56 days of coating application. Furthermore, weight loss analysis was performed twice a week during the storage period using eight samples (replicates) of each cheese group.

Titratable acidity was assayed and expressed as lactic acid (Instituto Adolfo Lutz, 1985); moisture content was determined according to the 926.12 AOAC method by drying the cheese samples to constant weight at 70 °C in vacuum oven (AOAC, 1997); total ash content was analyzed by incineration at 550 °C according to the 935.42 AOAC method (AOAC, 1997); chloride content was measured through an argentometric method using the total amount of ash previously obtained (Instituto Adolfo Lutz, 1985); lipid content was tested by the Gerber-Van Gulik method (Instituto Adolfo Lutz, 1985)); weight loss was determined by weighing samples at the beginning (W_0) and throughout the storage period (W_i), the relative weight loss (ΔW) was calculated as follows $\Delta W = (W_0 - W_i)^*100/W_0$; total nitrogen content was assessed by the micro-Kjeldahl method in accordance with the 960.52 AOAC method (AOAC, 1997); furthermore, the total protein content was calculated multiplying the nitrogen content by the conversion factor 6.38; cheese proteolysis was analyzed by separating the nitrogenous compounds into solvent-soluble and solvent-insoluble nitrogen, followed by the fractionation of the soluble components with discriminatory precipitants and quantification with the micro-Kjeldahl method. Thereby, soluble nitrogen at pH 4.6 (pH 4.6-SN) was obtained by precipitation with hydrochloric acid 1.41 N and trichloroacetic acid soluble nitrogen (TCA-SN) was obtained by precipitation with TCA 12% (Merheb-Dini et al., 2012).

2.6. Cheese surface color analysis

The color surface change of cheese samples was analyzed throughout the storage period with a Chroma meter ColorFlex EZ (HunterLab Inc., Reston, USA) using the CIELab color scale (where L = lightness, a = red-yellow color, and b = blue-green color) under daylight ($D65$ illuminant). The total color difference (ΔE) was calculated as follows $\Delta E = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{1/2}$, where L_0, a_0, and b_0 were the initial values (1 day of coating application) obtained for the samples of each cheese group, and L, a, b were the values measured during the storage period (Cerqueira et al., 2010;

Ramos et al., 2012). Three samples (replicates) belonging to each cheese group were used for the analysis and four-color readings were made on each one.

2.7. Cheese texture profile analysis (TPA)

The cheese texture profile was analyzed during the storage period using cylindrical cheese samples (25 mm diameter and 30 mm height). The analyses were made with a texture analyser TA. XT *plus* (Stable Micro Systems Ltd., Surrey, UK) equipped with 5 kg load cell and the 36R probe. The texture analyser was operated with two compression–decompression cycles and 2 mm s^{-1} of cross-head speed (Diamantino et al., 2014). Cheese textural parameters such as hardness, cohesiveness and gumminess were obtained using the texture profile function of the *Exponent* software. Nine samples (replicates) belonging to each cheese group were used for the measurements.

2.8. Experimental design and statistical analysis

A randomized repeated measures design was applied using the cheese packaging type as the factor under study with four different levels (unpackaged, Z-OA, Z-OA-XG and plastic). The statistical analysis was performed with Statgraphics centurion *v. 16.1* software (Statpoint Technologies, Inc., Warrenton, USA), performing ANOVA and Tukey multiple comparison test functions to evaluate the significant mean difference ($p < 0.05$) between packaging.

3. Results and discussion

The impact of two biodegradable zein-based coatings on the physicochemical characteristics of “Minas Padrão” cheese was evaluated throughout 56 days of storage. Both coatings, Z-OA and Z-OA-XG, exhibited the similar effect on cheese physicochemical characteristics ($p > 0.05$).

The visual examination of the cheese samples allows for detecting microbiological contamination on cheese surface (Fig. 1); nonetheless, due to the microbiological analyses were not the scope of this study, the identification and characterization of the contaminant microorganisms is subject of further studies. After 21 days of storage, 33% of unpackaged cheese samples displayed microbiological contamination on the “Minas Padrão” cheese surface whereas Z-OA and Z-OA-XG coated samples exhibited microbiological contamination after 50 and 53 days respectively. Therefore, the use of the Z-OA and Z-OA-XG biodegradable coatings allowed for extending cheese quality during the storage.

Physicochemical characteristics such as acidity (Fig. 2a), ash (Fig. 2b), chloride (Fig. 2c) and total protein (Fig. 2d) were similarly and stably preserved along the storage by the four-tested type of packaging ($p > 0.05$). The biodegradable coatings helped to decrease weight (Fig. 3a) and moisture (Fig. 3b) loss in comparison to the unpackaged cheese, due to the better water barrier property of the biodegradable coatings. Similarly, Ramos et al. (2012) reported lower moisture loss for “Saloio” cheese with WPI coating than uncoated samples.

Although the Z-OA and Z-OA-XG coatings showed a positive behavior when compared to unpackaged cheeses, the water vapor permeability of the coatings requires further improvement, in order to achieve the performance of the polyethylene packaging. The higher water vapor transmission (WVT) of the biodegradable materials ($WVT = 6.3$ g d^{-1} m^{-2} with thickness (x) of 64 μm, reported by Pena Serna and Lopes Filho, 2015) compared to polyethylene ($WVT = 6$ g d^{-1} m^{-2}, $x = 61$ μm) caused the greater moisture loss of the coated samples (Fig. 3b).

In comparison to unpackaged and plastic-packaged samples, cheese samples with biodegradable coatings exhibited an initial moisture loss ($t = 0$) of ca. 10% likely due to whey loss during the drying of the coating. Therefore, in order to overcome such problem further studies shall be conducted, aiming to test the use of Z-OA and Z-OA-XG biodegradable films instead of Z-OA and Z-OA-XG biodegradable coatings.

The mozzarella cheese with SPI or chitosan coatings (Zhong et al., 2014) and the “Saloio” cheese coated with galactomannan (Cerqueira et al., 2010) or WPI (Ramos et al., 2012) exhibited more than 15% weight loss during 20 days of storage. Considering the same storage period, the “Minas Padrão” cheese with Z-OA and Z-OA-XG coatings displayed lower weight loss (ca. 9%), possibly as a consequence of the lower water vapor permeability of the zein-based materials in comparison to other biodegradable materials (Pena Serna and Lopes Filho, 2015).

Cheese characteristics such as proteolysis, lipid content, surface color and texture parameters were altered during the storage period as a result of either, cheese ripening or moisture loss (Figs. 4–6).

Ripening is a complex process where cheese flavor and texture are generated by means of the biochemical transformation of curd's triacylglycerides, casein and residual lactose. Thus, a softened cheese texture is obtained as a consequence of the casein hydrolysis

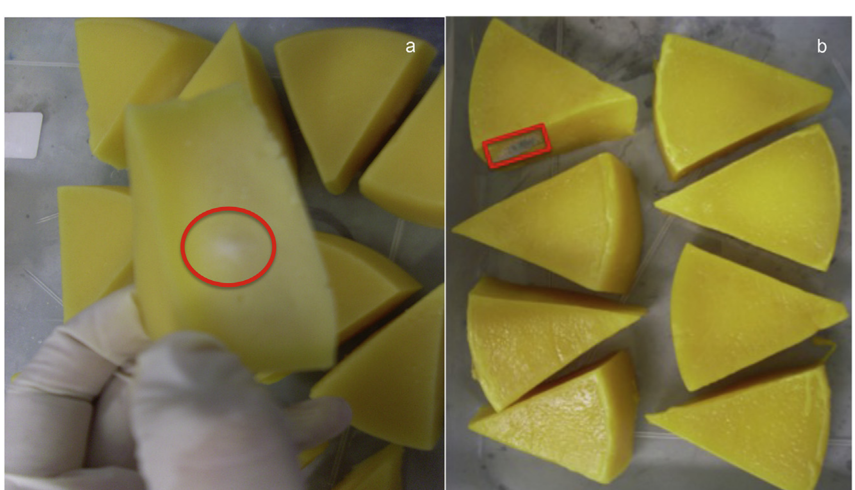

Fig. 1. Microbiological contamination detected on cheese surface during the storage. Unpackaged cheese sample at 21 days **(a)** and Z-OA-XG coated cheese at 53 days **(b)**.

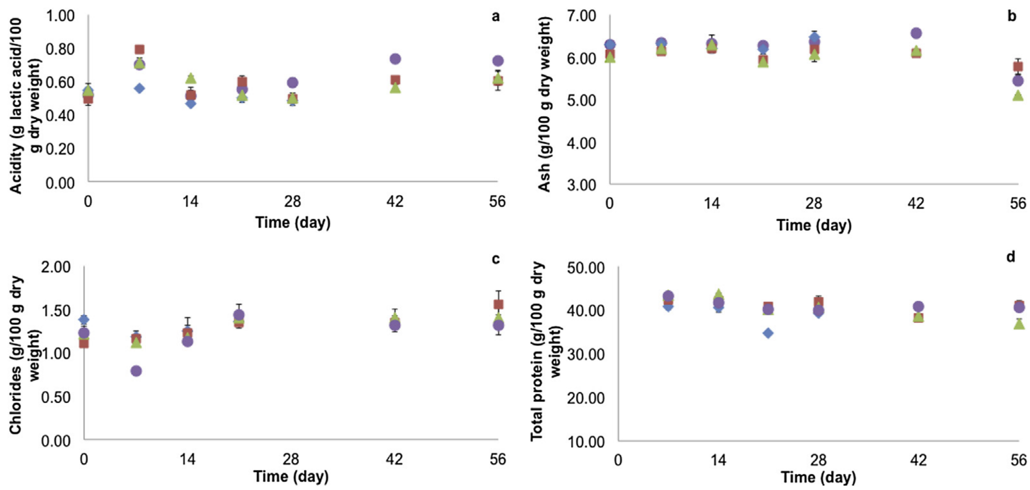

Fig. 2. Effect of the packaging type (◆ unpackaged, ▲ Z-OA-XG, ■ Z-OA, ● plastic) on physicochemical characteristics of the "Minas Padrão" cheese throughout storage. Acidity (**a**), ash (**b**), chlorides (**c**) and total protein (**d**). Data are expressed on dry basis and values are mean ± standard deviation.

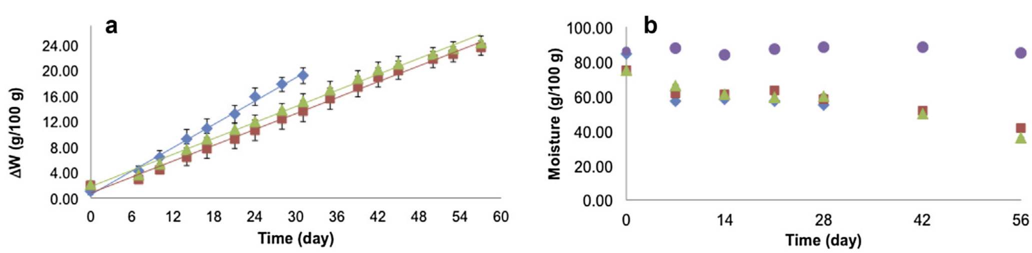

Fig. 3. Effect of the packaging type (◆ unpackaged, ▲ Z-OA-XG, ■ Z-OA, ● plastic) on physicochemical characteristics of the "Minas Padrão" cheese throughout storage. Weight loss (**a**) and moisture content (**b**). Values are mean ± standard deviation.

as well as changes in pH and curd's water-binding ability. Factors such as cheese composition (especially moisture and salt content), temperature, storage time, type of microorganisms and the activity of the added enzymes, strongly influence this process (Farkye and Fox, 1990; McSweeney, 2004).

Due to the several biochemical reactions involved in cheese ripening, there is a difficulty on measuring its evolution. Based on that, cheese proteolysis is used as one of the approaches to quantify

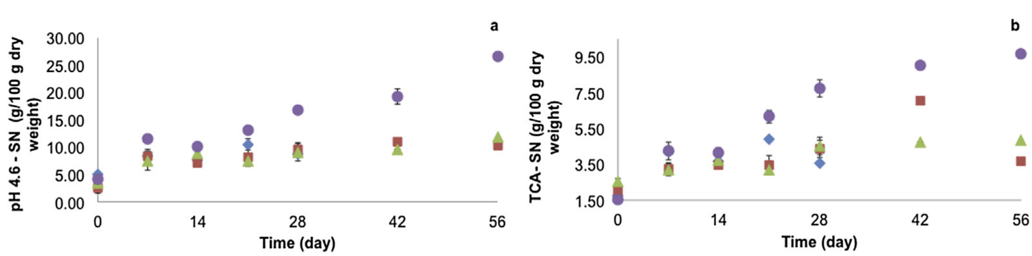

Fig. 4. Effect of the packaging type (◆ unpackaged, ▲ Z-OA-XG, ■ Z-OA, ● plastic) on the ripening of the "Minas Padrão" cheese throughout storage. Proteolysis indices of extension (**a**) and depth (**b**). Data are expressed on dry basis and values are mean ± standard deviation.

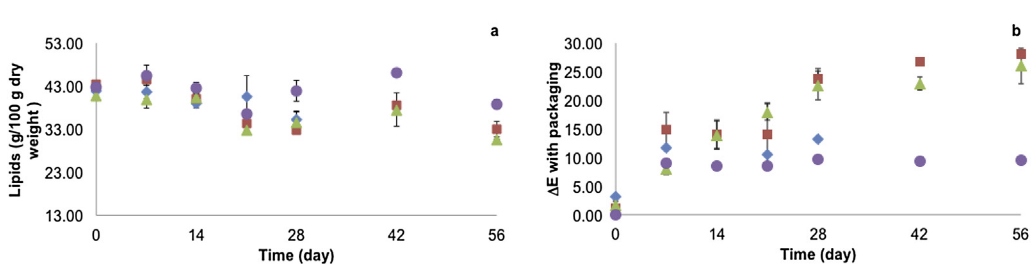

Fig. 5. Effect of the packaging type (◆ unpackaged, ▲ Z-OA-XG, ■ Z-OA, ● plastic) on the "Minas Padrão" cheese lipid content (**a**) and surface color change (**b**) throughout storage. Data are expressed on dry basis and values are mean ± standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

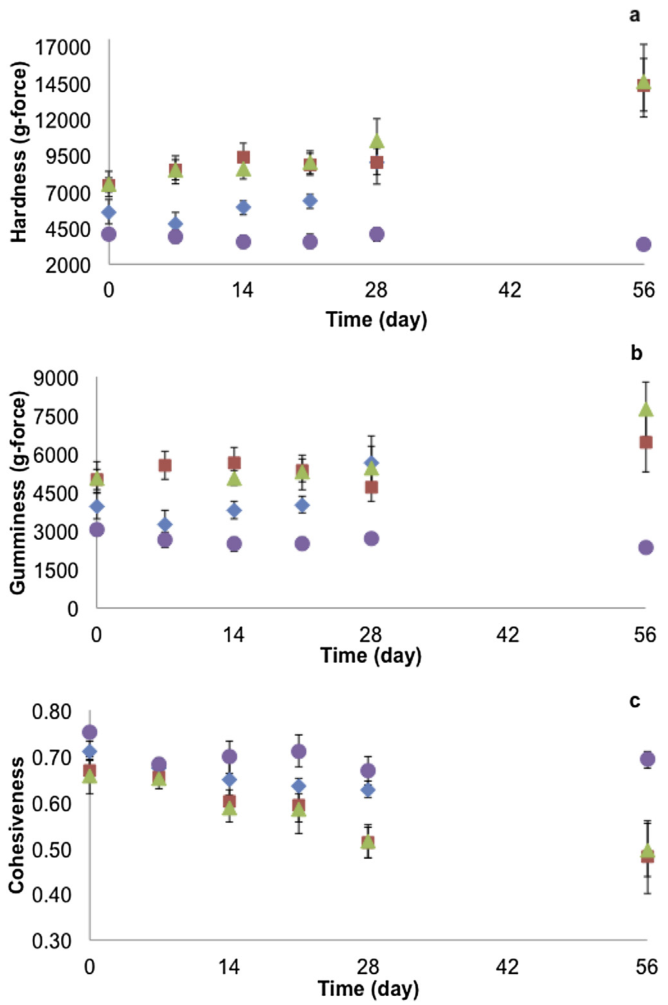

Fig. 6. Effect of the packaging type (◆ unpackaged, ▲ Z-OA-XG, ■ Z-OA, ● plastic) on the texture of the "Minas Padrão" cheese throughout storage. Texture profile: hardness (**a**), gumminess (**b**) and cohesiveness (**c**). Values are mean ± standard deviation.

cheese ripening. Proteolysis measures the degree of hydrolysis of the casein matrix; thus, the pH 4.6-SN, known as the extension index, allows for quantifying mainly the coagulant (rennet) enzymatic activity, i.e. casein hydrolysis into peptides of high molecular weight, whereas TCA-SN or depth index measures small peptides produced by the proteinases of the starter culture (McSweeney, 2004; Panizzolo et al., 2011).

The extension and depth of ripening (Fig. 4a and b) tended to increase throughout the time, displaying the proteolysis evolution along cheese ripening and storage time. Proteolysis extent was influenced by packaging type ($p < 0.05$); thus, plastic-packaged samples exhibited greater proteolytic activity with higher extension and depth indices than biodegradable-coated and unpackaged cheese samples. As an enzymatic process, cheese ripening depends on water activity and moisture content (Cerqueira et al., 2010); consequently, those factors influenced the proteolytic activity of coagulant and starter culture present in the "Minas Padrão" cheese.

The depth index of unpackaged and biodegradable-coated cheese samples showed a reduction on the measurements performed at 28 and 56 days respectively (Fig. 4b). As previously mentioned, unpackaged and coated cheese samples displayed microbiological contamination after 21 and 50 days of storage, respectively. Such reduction was possibly caused by the contaminant microorganism, which likely degraded the small peptides produced during cheese ripening.

Cheese lipid content tended to decrease along time (Fig. 5a) likely caused by the production of volatile flavor compounds during cheese ripening. Furthermore, at the end of the storage period, the lipid content of cheese samples with biodegradable coatings was lower compared to polyethylene-packaged samples ($p < 0.05$). The lipid loss displayed by the biodegradable-coated samples was probably a consequence of the greater affinity between the cheese lipids and the oleic acid-based (hydrophobic) coating (Zhong et al., 2014). Thereby, cheese lipids migrate to cheese surface and may be lost during cheese manipulation and coating removal.

Furthermore, after 21 days of storage, Z-OA and Z-OA-XG coated cheeses exhibited a surface with greasy and wrinkled appearance (data not shown). Mozzarella cheese with SPI coating showed the same surface appearance after 7 days of storage (Zhong et al., 2014).

The "Minas Padrão" cheese underwent color surface variation along the storage time (Fig. 5b) and the packaging type influenced such characteristic ($p < 0.05$). Similarly, Ramos et al. (2012) and Zhong et al. (2014) reported color change throughout the storage on "Saloio" cheese with WPI coating and Mozzarella cheese with SPI coating, respectively.

Initial CIELab color values for the "Minas Padrão" cheese were $L_0 = 84.91$, $a_0 = 7.7$ and $b_0 = 26.26$. Moreover, after one day of coating application, the measured CIELab values were $L = 73.68$, $a = 14.42$ and $b = 73.64$. According to these values, the coated cheese samples experienced color change immediately after coating application as a consequence of the yellow color of the Z-OA and Z-OA-XG coatings produced by the natural color of the zein. Hence, the Z-OA and Z-OA-XG coated cheese samples displayed higher color change compared to plastic-packaged and unpackaged cheese samples; nonetheless, unpackaged samples produced a yellow rind that influenced color surface and texture of the cheese (Figs. 5b and 6).

The textural characteristics of the "Minas padrão" cheese were influenced by the packaging type ($p < 0.05$), as a result of the difference in the cheese moisture content and proteolysis extent (Farkye and Fox, 1990; McSweeney, 2004). Thereby, biodegradable-coated and unpackaged cheese samples were harder, more rubbery and less cohesive than the plastic-packaged cheese (Fig. 6).

According to Fig. 6a, the "Minas Padrão" cheese with Z-OA and Z-OA-XG coatings, exhibited 14% hardness increase after 14 days of storage. During the same period of time, the Mozzarella cheese with SPI coating displayed an increase over 350% (Zhong et al., 2014). Similarly, the "Saloio" cheese coated with WPI (Ramos et al., 2012) and galactomannan (Cerqueira et al., 2010) coatings showed 400% hardness increment. The better water barrier properties exhibited by the Z-OA and Z-OA-XG biodegradable materials compared to other polysaccharide and protein-based films (Pena Serna and Lopes Filho, 2015) allowed for decreasing moisture loss, resulting in a softer and cohesive "Minas Padrão" cheese than Mozzarella and "Saloio" cheeses reported by Zhong et al. (2014), Ramos et al. (2012) and Cerqueira et al. (2010).

Given the harder texture as well as the higher color change exhibited by the biodegradable-coated samples in comparison to cheeses with polyethylene packaging; additional evaluations will be required in order to determine whether these cheese characteristics might cause consumer rejection. Otherwise, the current characteristics of the biodegradable-coated cheese samples may coincide with the physicochemical characteristics of long ripened cheeses with low moisture content. Based on that, Z-OA and Z-OA-XG biodegradable coatings may be applied to the industry of long ripened cheeses and low moisture content, helping to reduce microbiological, chemical or environmental contamination during cheese ripening, storage and commercialization; nevertheless, further studies are required.

4. Conclusions

The impact of Z-OA and Z-OA-XG coatings on the quality of the “Minas Padrão” cheese was studied throughout 56 days of storage. The coated cheese samples exhibited similar preservation of physicochemical characteristics such as chlorides, ash, protein and acidity, compared to unpackaged and plastic-packaged cheese samples. The biodegradable coatings prevented the early microbiological contamination and decreased the cheese moisture loss in comparison to unpackaged samples. Although the Z-OA and Z-OA-XG coatings showed a positive behavior in comparison to unpackaged cheeses, some challenges remain and require further studies. For instance, the texture profile, surface color and proteolysis of the coated cheeses could be improved by decreasing the water vapor permeability of the coating or by using Z-OA and Z-OA-XG biodegradable films instead of the coatings.

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