

# Effect of edible coating based on zedo gum containing *Thymus* essential oil and *Cinnamomum* extract on quality of cheddar cheese

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## ABSTRACT

Effect of edible coatings based on zedo gum (ZG) containing thyme (*Thymus vulgaris*) essential oil (TEO) and cinnamon (*Cinnamomum verum*) extract (CAE) on the quality and proteolysis of cheddar cheese was investigated. Four treatments were prepared using different coating formulas including: C (coated cheese with paraffin and wax mixture as commercial coating), Z treatment (coated cheese with ZG solution), TEO treatment (coated cheese with TEO), and CAE treatment (coated cheese with CAE). The results showed that the highest moisture content and proteolysis indices were related to sample C; the lowest moisture content and number of starter lactic acid bacteria (SLAB) and the highest protein content, FDM content, proteolysis indices and number of non-starter lactic acid bacteria (NSLAB) were related to sample Z; while the TEO and CAE treatments showed similar values. Also, TEO and CAE had an inhibitory effect on growth of moulds and yeasts, without affecting NSLAB and SLAB. Therefore, instead of commercial coating, zedo gum containing *T. vulgaris* essential oil or *C. verum* extract can be used as active coating on cheddar cheese.

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**KEYWORDS**

cheddar cheese, *Cinnamomum* extract, coatings, proteolysis, *Thymus* essential oil, zedo

**1. INTRODUCTION**

Cheddar cheese, a type of hard cheese, is among the most famous varieties of cheese in the world. To prevent spoilage, extend shelf life, and reduce weight loss during ripening of cheddar cheese, mainly petroleum based coatings (such as polyvinyl chloride and paraffin wax) have been used in the past. Nowadays, due to environmental pollution, being allergenic for some consumers, and legal restrictions, the use of edible coatings made from hydrocolloid compounds have been considered (Costa et al., 2018).

Zedo gum or Persian gum is exuded from *Prunus dulcis* trees (Almond). This novel anionic arabino-galactan gum is principally composed of 2.45% proteins, 92.36% polysaccharides, and 0.85% fats (Razmjoo et al., 2021). This gum consists of two fractions. Fraction one is water insoluble (70–75%) partially dissolving in hot water, and fraction two is water soluble (25–30%) dissolving in cold water (Hashemi Gahruei et al., 2022). Its structure is similar to arabic gum. Zedo gum is a nontoxic biodegradable one, which is cheaper than other gums such as arabic, locust bean xanthan, and guar gums (Razmjoo et al., 2021). Several studies have been done on the application of edible films and coatings produced by Zedo gum (Hashemi Gahruei et al., 2022; Razmjoo et al., 2021). Edible coatings also can act as vehicles of antimicrobial and antioxidant compounds. Recently, plant aqueous extracts and essential oils have been considered as suitable alternatives to synthetic food additives due to their safety and significant antioxidant and antimicrobial activity (Valdivieso-Ugarte et al., 2019). Cinnamon and thyme essential oils as natural antioxidants and antimicrobial agents have been increasingly favoured by researchers.

Cinnamon (*Cinnamomum verum*) is widely used as a spice for foods in many countries, especially in the Middle East. Availability of cinnamon throughout the year has made it more popular. Recently, it has been reported that cinnamon has strong antioxidants that are comparable to synthetic antioxidants. Biological activities of cinnamon have been attributed to its active compounds such as *trans*-cinnamaldehyde, 3-methoxy-2-propanediol, methyl eugenol, and cinnamic acid (Parvazi et al., 2016). However, the use of cinnamon as well as its essential oils and oleoresins extracted with organic solvents are not suitable for direct use in food products due to their pungent smell and taste. In addition, cinnamon extracts extracted with organic solvents may have residual solvent in them, which limits their use as a source of strong natural antioxidants and antimicrobials on a large scale in food. It has been reported that water has a lower ability to extract the spicy flavour compounds of cinnamon compared to organic solvents. For this reason, the aqueous extract of cinnamon is more acceptable to the consumer for preparing food. In addition, the aqueous extract is cheaper, non-toxic, and environmentally friendly (Chan et al., 2014).

The whey isolate enriched with thyme and clove essential oils (Kavas et al., 2015) and quince seed mucilage containing thyme oil (Erkaya-Kotan et al., 2023) as coating material have been used in Kashar cheese. Also, Kuorwel et al. (2013) and Mahcene et al. (2021) have used starch containing carvacrol, linalool, and thymol essential oils in Cheddar cheese and sodium alginate enriched with different essential oils in home-made cheese as coating materials.



In the present work, the effect of edible coating based on zedo gum containing *Thymus vulgaris* essential oil and the aqueous extract of *C. verum* were investigated on the physiochemical, proteolysis, and sensory properties and growth of starters and fungi of the cheddar cheese samples during ripening.

## 2. MATERIALS AND METHODS

### 2.1. Raw materials

Zedo gum (ZG) and dried cinnamon bark (*C. verum*) were procured from the traditional market (Urmia, Iran). Thyme essential oil (TEO) was procured from Garreban Co. (Kermanshah, Iran) and one-day old cheddar cheese (moisture 40.06%, salt 1.06%, fat 28.1%, protein 17.37%, and pH 5.26) was procured from Kalleh Dairy Co. (Amol, Mazandaran, Iran).

### 2.2. Preparation of cinnamon aqueous extract (CAE)

Cinnamon bark was first ground and passed through a sieve with 40 mesh. The powder was mixed with hot water at a ratio of 1:10 and stirred for 30 min in a water bath (Memmert, Germany) at 90 °C. The mixture was filtered through a gauze (Parvazi et al., 2016) and was kept in air tight glass container in a cold room until examined.

### 2.3. Preparation of coating solutions

To prepare the coating solution containing cinnamon aqueous extract (CAE), 3 g of ZG was first homogenised in 100 mL of a mixture of cinnamon aqueous extract and distilled water (1:1) at 60 °C using a blender (IKA T25-Digital Ultra Turrax, Staufen, Germany) and then cooled to 50 °C. Next 1.5 g of glycerol (Merck, Darmstadt, Germany) as a plasticiser was added, and the mixture was further blended for 60 s before used.

To prepare the coating solution containing thyme essential oil (TEO), 3 g of ZG in 100 mL hot water (60 °C) was homogenised using a blender (IKA T25-Digital Ultra Turrax, Staufen, Germany), cooled to 50 °C, 1.5 g glycerol as plasticiser was added, and mixed for another 60 s. Then 0.5% thyme essential oil was dissolved in Tween 80 (2:1), added to the gum solution, and mixed with a homogeniser for another 2 min before used.

### 2.4. Cheese coating

Sealed cheese blocks were unwrapped and immediately cut into identical cylindrical pieces (500 g each). The coating was carried out in two layers by gentle brushing. The coating solution was brushed on the samples surface and dried for 60 min. The second layer was applied to the samples, and they were dried at 24 °C at a relative humidity of 50% for 4 h.

Thus, four treatments were prepared using different coating materials, namely C: (control, cheese coated with mixture of paraffin and wax as commercial coating), Z treatment: (coated cheese with ZG solution), TEO treatment: (coated cheese with TEO), and CAE treatment: (coated cheese with CAE). Treated samples were kept at  $8 \pm 2$  °C in a cold room for 90 days and were tested at days 1, 30, 60, and 90 of ripening.



## 2.5. Compositional analysis

Moisture content was measured by drying 5 g of grated cheese in an oven (Memmert, Darmstadt, Germany) at  $102 \pm 2^\circ\text{C}$  until constant weight (AOAC 926.08, 2012), protein content was measured using micro-Kjeldahl method using the conversion factor of 6.38 for nitrogen to protein (AOAC 920.123, 2012), and fat content was measured by the Gerber method using 3 g grated cheese (AOAC 933.06, 2012).

## 2.6. The soluble nitrogen at pH = 4.6 (SNpH = 4.6)

For that, 10 g of crushed samples was homogenised in 20 mL of distilled water, then the pH was set to 4.6 with hydrochloric acid (HCl, 1.0 M). The samples were put for 30 min at room temperature, and then the pH was readjusted to 4.6. The mixture was incubated in water bath for 60 min at  $40^\circ\text{C}$ . Finally, the slurry was centrifuged at  $3,000\text{ g}$  at  $4^\circ\text{C}$  for 30 min. The precipitated casein was then separated using Whatman No. 1 filter paper (Whatman, Camlab, UK). The nitrogen content of the extract was measured with micro-Kjeldahl method.

## 2.7. Trichloroacetic acid soluble nitrogen (TCA-SN)

10 mL of the extracted fraction dissolved at pH = 4.6 was mixed with 10 mL of 24% TCA solution and incubated at room temperature for 60 min. It was then filtered through Whatman No. 1 filter paper (Whatman, Camlab, UK), and the nitrogen content of the extract solution was measured with the micro-Kjeldahl method.

## 2.8. Microbial counting method

2% trisodium citrate was used to prepare the first dilution and 0.1% peptone water was used to prepare the other dilutions.

Non-starter lactic acid bacteria (NSLAB) were cultured in MRS agar (de Man, Rogosa, Sharpe; Merck, Darmstadt, Germany) by pour plate method and incubated under anaerobic conditions using Gas pack (Anaerocult A, Merck, Darmstadt, Germany) at  $30^\circ\text{C}$  for 72 h. Starter lactic acid bacteria (SLAB) were cultured in  $M_{17}$  agar (Merck, Darmstadt, Germany) by pour plate method and incubated at  $30^\circ\text{C}$  for 72 h. Moulds and yeasts were cultured on Sabouraud Dextrose Agar (Merck, Darmstadt, Germany) by surface spreading method and incubated for 5 days at  $25^\circ\text{C}$ . The microbial results were reported as  $\log\text{ CFU g}^{-1}$ .

## 2.9. Statistical analysis

The results were analysed using a factorial statistical design with two factors in three replications. The data were subjected to analysis of variance (ANOVA) using the SAS software. To compare means, Tukey's multiple-comparison test was used at  $P < 0.05$ .

# 3. RESULTS AND DISCUSSION

## 3.1. Cheese composition

As shown in Table 1, for all treatments the moisture content decreased during ripening ( $P < 0.05$ ). This may be due to syneresis and osmosis during the ripening period or to the low relative



Table 1. The effect of coating material on physicochemical properties of cheddar cheese samples during ripening

| Test                       | Treatment | Ripening period, day |                      |                      |                      |
|----------------------------|-----------|----------------------|----------------------|----------------------|----------------------|
|                            |           | 1                    | 30                   | 60                   | 90                   |
| Moisture (%)<br>SEM = 0.35 | C         | 40.06 <sup>aA</sup>  | 39.65 <sup>aA</sup>  | 38.91 <sup>aA</sup>  | 37.82 <sup>bA</sup>  |
|                            | Z         | 40.06 <sup>aA</sup>  | 38.98 <sup>bB</sup>  | 35.68 <sup>cC</sup>  | 33.59 <sup>dC</sup>  |
|                            | TEO       | 40.06 <sup>aA</sup>  | 39.32 <sup>aAB</sup> | 37.91 <sup>bB</sup>  | 36.28 <sup>cB</sup>  |
|                            | CAE       | 40.06 <sup>aA</sup>  | 39.01 <sup>bB</sup>  | 37.53 <sup>cB</sup>  | 36.73 <sup>cB</sup>  |
| FDM (%)<br>SEM = 0.51      | C         | 49.88 <sup>aA</sup>  | 50.21 <sup>aA</sup>  | 50.16 <sup>aB</sup>  | 49.75 <sup>aB</sup>  |
|                            | Z         | 49.88 <sup>bA</sup>  | 50.78 <sup>abA</sup> | 51.65 <sup>aA</sup>  | 51.19 <sup>aA</sup>  |
|                            | TEO       | 49.88 <sup>aA</sup>  | 50.28 <sup>aA</sup>  | 50.41 <sup>aAB</sup> | 49.95 <sup>aAB</sup> |
|                            | CAE       | 49.88 <sup>aA</sup>  | 50.49 <sup>aA</sup>  | 49.95 <sup>aB</sup>  | 49.34 <sup>aB</sup>  |
| Protein (%)<br>SEM = 0.26  | C         | 27.37 <sup>bA</sup>  | 27.74 <sup>abA</sup> | 28.03 <sup>aA</sup>  | 27.77 <sup>aB</sup>  |
|                            | Z         | 27.37 <sup>cA</sup>  | 28.05 <sup>bcA</sup> | 28.38 <sup>bA</sup>  | 28.09 <sup>aA</sup>  |
|                            | TEO       | 27.37 <sup>bA</sup>  | 27.62 <sup>abA</sup> | 27.92 <sup>aA</sup>  | 27.65 <sup>aB</sup>  |
|                            | CAE       | 27.37 <sup>bA</sup>  | 27.61 <sup>abA</sup> | 28.04 <sup>aA</sup>  | 27.48 <sup>aB</sup>  |

Type of coating: C: commercial coating; Z: zedo gum coating; TEO and CAE: ZG coating containing TEO and CAE, respectively. SEM: standard error mean; different lowercase letters within the same row (during storage days) and capital letters within the same column (between cheeses at the same storage day) show significant differences ( $P \leq 0.05$ ).

humidity of the cheese storage room leading to the transfer of moisture from the cheese to the atmosphere depending on the types of coating material. These results were in agreement with the results reported by other researchers (Soleimani-Rambod et al., 2018; Berti et al., 2019).

The highest and the lowest moisture contents were related to the C and Z treatments, respectively. Considering that coatings formulated with polysaccharide gums, such as starch, pectin, cellulose, chitosan, alginate, and as Zedo, are a poor barrier against moisture due to their hydrophilic properties, also not allowing the formation of a crust on the outer surface of the cheese, may lead to moisture loss from cheese (Berti et al., 2019). In the C treatment a mixture of paraffin and wax was used as a coating material that acted as a good barrier against moisture due to its hydrophobicity and non-polar nature. For this reason, the decrease in moisture content in C treatment was significantly lower than in other treatments.

TEO and CAE treatments retained more moisture than Z treatment. It can be due to the effect of essential oils, reducing the permeability of the coating to moisture. Sánchez-González et al. (2011) reported that the films containing essential oils perform better in preventing the passage of water vapor due to the increased hydrophobicity compared to films containing no essential oils. Similar results were obtained by Pires et al. (2013) with the addition of coriander, Indian hyacinth, tarragon, and thyme essential oils to protein based film.

According to Table 1, for all treatments, the protein and FDM contents increased during the ripening period ( $P < 0.05$ ) in parallel with the reduction of moisture, as found in the literature (Soleimani-Rambod et al., 2018).

At the end of the ripening period, lipolysis and proteolysis led to a decrease in fat and protein contents, respectively (Pena-Serna et al., 2016). The samples C, TEO, and CAE showed similar protein and FDM content values.



### 3.2. Evaluation of proteolysis

Proteolysis is an important parameter to determine the degree of ripening of cheese, indicating the development of cheese texture, aroma, and flavour. In this study, primary proteolysis was evaluated by determining soluble nitrogen content at pH = 4.6 (non-casein nitrogen materials) and soluble nitrogen content in TCA (medium and small peptides) as indices of ripening. The ratio of SN<sub>pH = 4.6</sub> on total nitrogen (SN<sub>pH = 4.6</sub>/TN) is mainly the sign of enzymatic activity of the rennet (hydrolysis of casein into high molecular weight peptides), whereas the ratio of TCA-SN on total nitrogen (TCA-SN/TN) is indication of small peptides produced by starter culture proteases (McSweeney et al., 2017).

As shown in Fig. 1, SN<sub>pH = 4.6</sub>/TN and TCA-SN/TN values as proteolysis indices increased during the ripening period ( $P > 0.05$ ) or all treatments. This may be due to continued degradation of caseins into the high and low molecular weight water-soluble peptides by proteolytic activity of starter cultures and residual coagulants (McSweeney et al., 2017).

The increase in the population of lactic acid bacteria (NSLAB) results in the increase in protease enzymes leading to increased proteolysis of cheese and the production of SN<sub>pH = 4.6</sub>/TN and TCA-SN. Similar results have been reported by other researchers (Soleimani-Rambod et al., 2018).

Water-soluble peptides produced by rennet and starter might also be hydrolysed by peptidases and proteases of microorganisms resulting in production of free amino acids. Increasing the free aromatic amino acids value is effective in enhancing the aroma and flavour of cheese (Soleimani-Rambod et al., 2018). In general, in the early stages of the ripening period, the speed of increase of SN<sub>pH = 4.6</sub>/TN and TCA-SN/TN indices is low, as in this stage, the water-soluble peptides are produced only with activity of coagulants and plasmin (McSweeney et al., 2017).

The SN<sub>pH = 4.6</sub>/TN and TCA-SN/TN values were lowest in sample C, but other treatments had similar proteolysis indices at the end of the ripening period (Fig. 1). The results obtained from the proteolysis indices indicated that the coating using zedo gum containing *Thymus* essential oil or *Cinnamomum* extract not only did not have a negative effect on proteolysis, but also increased it, compared to commercial coatings.

### 3.3. NSLAB, SLAB, moulds, and yeasts

According to the role of SLAB and NSLAB in the proteolysis processes in cheddar cheese, their counting is important to determine the negative effect of coatings on the growth of these microbes (Blaya et al., 2018).

According to Fig. 2, on day 1, the number of NSLAB and SLAB in samples were found to be 5.65 and 8.25 log CFU g<sup>-1</sup>, respectively. During the ripening period, the number of NSLAB increased, while the number of SLAB decreased ( $P < 0.05$ ). The increase in the viability of NSLAB can be related to the reduction in the viability of SLAB in cheddar cheese. The NSLAB, by producing lactic acid, make the conditions unfavourable for the growth of SLAB, as a result, the decrease in the viability of starter bacteria leads to an increase in the viability of non-starter lactic acid bacteria in cheese. The SLAB cell lysate is used as nutrient source for NSLAB (Blaya et al., 2018). In this regard, Soleimani-Rambod et al. (2018) also reported the increase of NSLAB and decrease of SLAB in coated cheddar cheese.

At the end of the ripening period, the number of NSLAB was the highest and of SLAB was the lowest for treatment Z, but these numbers were very close to that of the other samples (Fig. 2). By the end of the storage time, the number of NSLAB in samples C, TEO, and CAE increased



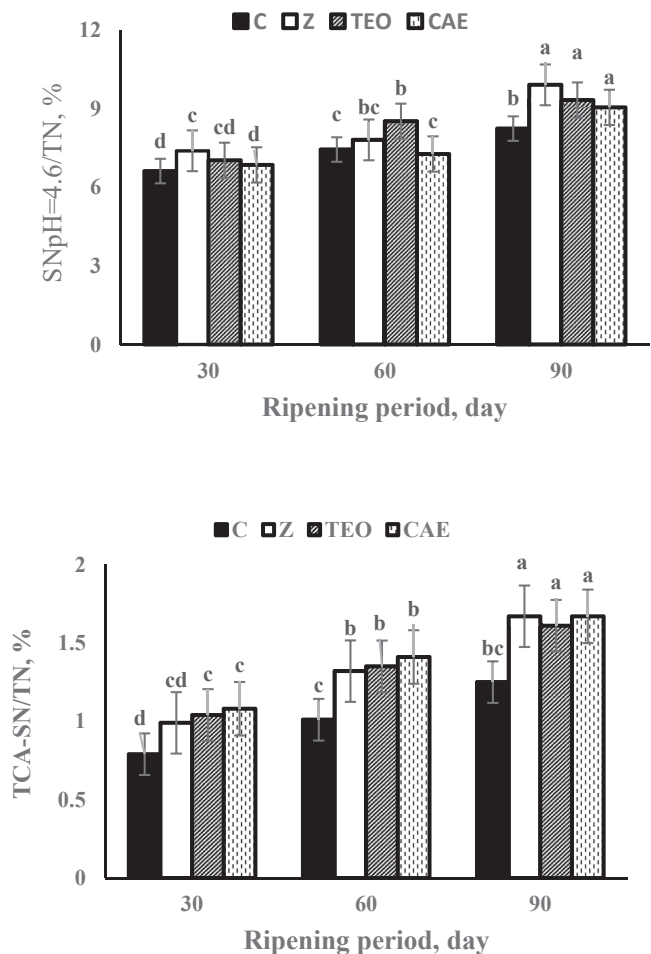


Fig. 1. The effect of coating material on the proteolysis indices in cheddar cheese samples during ripening. Type of coating: C: commercial coating; Z: zedo gum coating; TEO and CAE: ZG coating containing TEO and CAE, respectively. Different letters within the same column indicate significant differences ( $P \leq 0.05$ )

around 2 fold and in the sample Z about 2.5 fold logarithmic, but the number of SLAB decreased around 0.8 and 1.2 fold logarithmic, respectively. The high number of NSLAB in treatment Z can be responsible for the lack of antimicrobial properties of this treatment. These results showed that coating with zedo gum containing thyme essential oil or cinnamon extract had no negative effect on the growth of these microorganisms, which is necessary for cheese maturation.

Yeasts and moulds are important microbial contaminants in the dairy industry, which in many cases make the product unusable. According to the results, no mould and yeast growth was observed in the cheese samples on the first day (Fig. 2). During storage, the number of moulds and yeasts increased significantly ( $P < 0.05$ ) for all samples. This indicates that in the early stages of product storage, microorganisms were in the lag phase, but gradually they entered the log phase and their growth increased. Mahcene et al. (2021) also found that no yeast growth was observed in



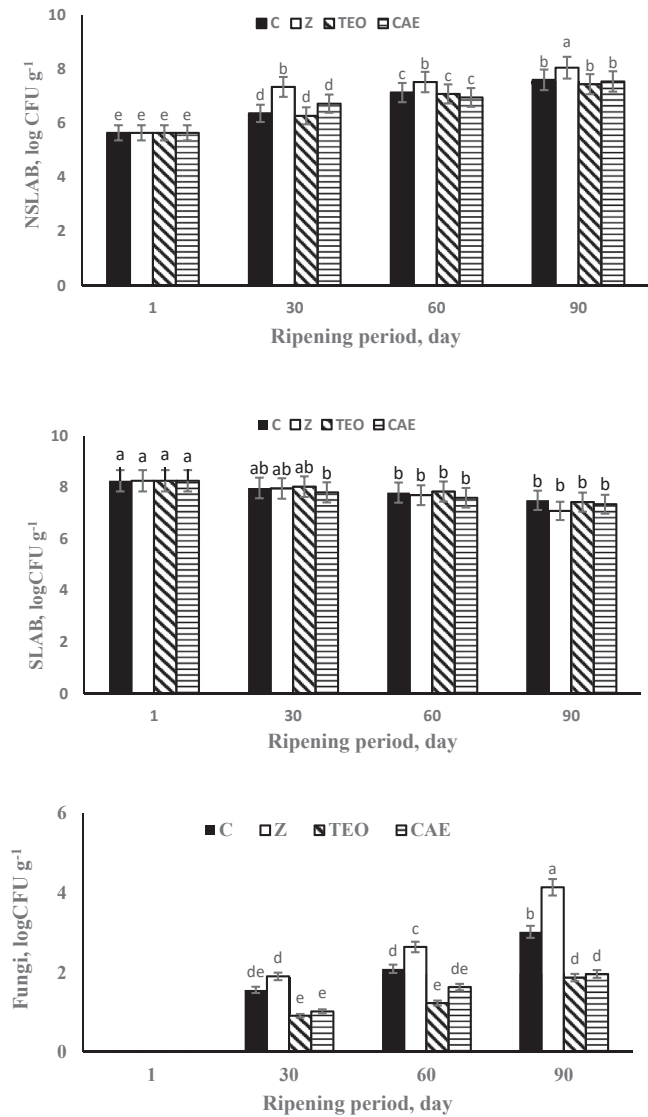


Fig. 2. The effect of coating material on the viability of non-starter lactic acid bacteria (NSLAB), starter lactic acid bacteria (SLAB), and fungi of cheddar cheese samples during ripening. Type of coating: C: commercial coating; Z: zedo gum coating; TEO and CAE: ZG coating containing TEO and CAE, respectively. Different letters within the same column indicate significant differences ( $P \leq 0.05$ )

cheese samples on the first day, but their number increased during storage. They also showed that the addition of different essential oils to the sodium alginate coating reduced the growth of yeasts in cheese, which is in accordance with the results obtained in this study.

According to Fig. 2, during the ripening period, there was a significant difference among treatments in terms of fungi growth. The growth rate of fungi in the Z sample was significantly



higher than for other treatments, which shows that the extract and essential oil have inhibitory effects against them in cheese. The decrease in the microbial population observed in TEO and CAE samples are related to their chemical composition (terpene and phenolic components). The antimicrobial effect of these compounds in dairy products can be affected by several factors, such as their concentration and chemical composition and types of microorganisms to be reduced or eliminated (De Souza et al., 2020).

These results show that the TEO and CAE reduced the growth of moulds and yeasts without affecting NSLAB and SLAB (main and functional dairy microbiota). By the end of the ripening period, the number of moulds and yeasts only in samples C ( $3.02 \log \text{CFU g}^{-1}$ ) and Z ( $4.14 \log \text{CFU g}^{-1}$ ) were higher than the standard range (maximum  $2 \log \text{CFU g}^{-1}$ ).

## 4. CONCLUSIONS

According to the results obtained, using TEO and CAE did not have any adverse effect on the proteolysis indices, textural profiles, and sensory evaluations of cheese. Also, these treatments as coating materials could reduce fungi growth without affecting NSLAB and SLAB (main and functional dairy microbiota) in cheddar cheese. All in all, it can be concluded that the TEO and CAE can be used as a safe and GRAS coating without causing any adverse effect on the quality of cheddar cheese.

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