



Edible active coatings incorporated with *Cinnamomum cassia* and *Myristica fragrans* essential oils to improve shelf-life of minimally processed apples

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ABSTRACT: The effect of the incorporation of cinnamon (*Cinnamomum cassia*) and nutmeg (*Myristica fragrans*) essential oils in alginate-based edible coatings that were applied on minimally processed apples, is reported. The minimum inhibitory concentrations were 1.25mg.mL⁻¹ (cinnamon) and 2.50mg.mL⁻¹ (nutmeg), against both *Escherichia coli* and *Penicillium commune*. Over storage periods there was a significant reduction in the *E. coli* and *P. commune* counts compared to the control. The extent of enzymatic browning was also significantly reduced in the coated samples. In the coated minimally processed apples sensory tests, the flavor had the lowest rating of the properties analyzed, for both treatments, followed by aroma and firmness.

Key words: sodium alginate, cinnamon, nutmeg, *Malus domestica*, food security.

Revestimentos comestíveis ativos contendo óleos essenciais de *Cinnamomum cassia* e *Myristica fragrans* para aumentar a vida útil de maçãs minimamente processadas

RESUMO: O efeito da incorporação dos óleos essenciais de canela (*Cinnamomum cassia*) e noz-moscada (*Myristica fragrans*) em revestimentos comestíveis à base de alginato, aplicados em maçãs minimamente processadas é apresentado. As concentrações inibitórias mínimas foram de 1,25mg.mL⁻¹ (canela) e 2,50mg.mL⁻¹ (noz-moscada), contra *Escherichia coli* e *Penicillium commune*. Durante o armazenamento, houve redução significativa nas contagens de *E. coli* e *P. Commune* em comparação com o controle. A extensão do escurecimento enzimático também foi significativamente reduzida nas amostras revestidas. Nas análises sensoriais, o sabor dos óleos essenciais apresentou a menor classificação das propriedades analisadas, tanto para os óleos essenciais, quanto para aroma e firmeza.

Palavras-chave: alginato de sódio, canela-da-china, noz-moscada, *Malus domestica*, segurança alimentar.

INTRODUCTION

Apple is the most cultivated fruit in Brazil and in 2014 Brazil produced 1,378,617 tons of the fruit (IBGE, 2014). The Global Strategy on Diet, Physical Activity and Health (AGUDO, 2004), recommends a daily intake of 400g of fruit/vegetables. However, this is not usually the case, and is principally due to lack of habit, disagreeable flavors, available time and short shelf-life of fruit/vegetables. To provide consumers with convenient and practical products that maintain characteristics of fresh fruit, Minimally Processed Foods (MPFs) were developed. The MPFs are those submitted to operations such as drying, cutting, sanitizing, centrifuging and conditioning in

appropriate packaging, guaranteeing their sensorial, nutritional and microbiological properties until purchase (ANYADIKE, 2003).

After picking, the biological system of fruits and vegetables remains active, emitting gases and humidity in the packaging, favoring microorganism growth. To avoid darkening and contamination, many MPFs are used in conjunction with active packaging. Active packaging imitates the natural cuticular barrier and is a platform for antimicrobial and antioxidant substances that extend the shelf life of the product (SÁNCHEZ-GONZÁLEZ et al. 2011).

Several studies exist regarding the application of edible active coatings on fresh-fruits such as nectarines (CHIABRANDO &

GIACALONE, 2016), blueberries (VIEIRA et al., 2016), strawberries (PERDONES et al, 2016), pears (SHARMA & RAO, 2015) amongst others. Although, edible coatings incorporating cinnamon essential oil (CEO) have previously been studied, the essential oil (EO) used in this research is from *Cinnamomum cassia* Blume, which is different from *C. zeylanicum*, the concentration used in the present study is half that used and also the formulation proposed in this article is distinct. Moreover, in the present article an alginate based edible coating incorporated with nutmeg (*Myristica fragrans* Houtt) essential oil is proposed, which to the best of our knowledge is new.

MATERIALS AND METHODS

Microorganisms and essential oils

The reference strains, *Escherichia coli* (ATCC 35218) and *Penicillium commune* (INCQS 40062), were activated in Müeller Hinton agar and incubated at 37°C (20h). The fungal strain was reactivated in Malt extract agar and incubated at 25°C (5 days). The methodology adopted was M2-A8 (NCCL, 2003a) for bacteria and M27-A3 (CLSI, 2008) for fungi. Cinnamon essential oil (CEO –[*C. cassia*]) and nutmeg essential oil (NEO –[*M. fragrans*]) were obtained commercially (FERQUIMA®). The main components of CEO are: cinnamic aldehyde (81%), coumarin (3%); and for NEO are: α -pinene (20%), sabinene (14%), β -pinene (14%), myristicin 11% and terpinen-4-ol (6%). The FERQUIMA® emitted the report of the CG-MS.

Minimal inhibitory concentration (MIC)

The MIC was determined using microdilution plates. The subsequent concentrations were obtained by serial dilution that resulted in concentrations of 4% to 0.100% using technique M7-A6 (NCCL, 2003b).

Preparation of edible coating and application on apples: “Gala” apples were obtained from a local supermarket, sanitized using sodium hypochlorite (300ppm/15min), washed in water and dried on paper towels. After sanitization, apples were cut in slices (1cm) and each slice was inoculated, by immersion in the inoculum solutions, containing the microorganisms under study, for 15min. Edible coating was made according to AZARAKHSH et al. (2014), with 1.29% (m/v) of sodium alginate dissolved in distilled water, under heating (70°C) and constant stirring, until the mixture became clear. Following this, glycerol (1.16% (m/v)) and the EOs were added

to the mixture at the previously determined MIC of 0.125% (CEO) and 0.250% (NEO). After inoculation, the residual solution was allowed to drip off and the slices were submerged in the alginate-based edible coating for 2min. Once the excess edible coating was removed, the slices were submerged in a solution of 2% (m/v) calcium chloride, 1% (m/v) ascorbic acid and 1% (m/v) citric acid for 2 minutes to favor cross-link formation. Once dried, samples were packaged separately in atoxic plastic bags, sealed and stored at 3°C for the analysis period (4 days for bacteria and 9 days for the fungus). Four treatments were realized: contaminated apple (MC1); contaminated apple + alginate-based coating without the EOs (MC2); contaminated apple + alginate-based coating + *C. cassia* EO (MC3) and contaminated apple + alginate-based coating + *M. fragrans* EO (MC4).

Microscopic analysis of active films

Microscopic analysis was performed to observe the uniformity of coatings, thickness and adherence to apple surface. Transversal cuts of the covered fruit were performed and analyzed by a digital microscope (MZ8, Leica AG). Thickness of the films was determined at randomly chosen points.

Shelf-life analysis

Total counts of psychrophilic bacteria or molds and yeasts were performed for non-inoculated uncoated and coated apple slices at 15 days (0, 5, 10 and 15 day) to observe the influence of the EO over the native flora. Samples without the inoculation procedure, were prepared as microbiological analyses. A total of 30 samples were prepared and all samples were stored at 3°C until the analysis. Counts of psychrophilic bacteria or molds and yeasts on the apple slices (uncoated and coated) were performed during cold storage. To the samples (10g), was added 90mL of sterile saline peptone water (0.1g peptone/100mL water + 0.85g NaCl/100mL water) and homogenized for 1min. In the sequence, serial dilutions were performed and 0.1mL of this solution was spread over previously prepared plate count agar (PCA) or chloramphenicol glucose agar (CGA) and incubated at 3°C for 10 days and at 25°C for 3 days for psychrophilic bacteria or molds and yeasts determination, respectively. Plate counts were expressed as log₁₀CFU/mL as SALVIA-TRUJILLO et al. (2015).

Browning index: the color change caused by the enzymatic browning was evaluated in triplicate for the samples MC1, MC2, MC3 and MC4 (uncoated and coated samples) using a Chroma Meter calibrated using a standard white

plate CR-A43 and adjusted for the $L^* a^* b^*$ system. The Browning Index (BI) was calculated according to equation (1) (PALOU et al., 1999).

$$BI = \frac{[100(X - 0,31)]}{0,172} \quad X = \frac{(a^* + 1,75L)}{(5,645L + a^* - 3,02b^*)}$$

Sensory analysis

The sensory analyses were performed by 50 volunteers aged 18 – 50 years old, who frequently eat apple. The attributes evaluated were color, aroma, flavor, taste and firmness, using the 7-point hedonic scale, from “extremely unpleasant” (0) to “extremely pleasant” (7). For this sensory analysis, the coated apples (incorporating EOs) were prepared 1 day before the analysis and the control samples (not coated apples) were prepared at the moment of the test. Three samples were offered to each member of the panel: the uncovered control sample (MC1), the sample coated with CEO (MC3) and the sample coated with NEO (MC4). The MC2 was not used in this analysis as the goal was to determine the acceptance of the coating incorporating CEO and NEO and not the acceptance of only the alginate-based coating. The order of presentation was randomized and the panel registered their responses. Results were submitted to variance analysis and the Dunnett test for multiple comparison. This sensory analysis was approved by the Ethics Committee (Process: 1.401.865).

Statistical analysis

The experimental design was randomized in a 4 x 5 factorial scheme, with four treatments (MC1, MC2, MC3 and MC4) and five evaluation periods (0, 1, 2, 3 and 4 days) for *E. coli* and Enzymatic browning (MC1, MC2, MC3 and MC4) and three evaluation periods (5, 7 and 9 days) for *P. commune*. All analyzes were performed in three triplicate and results were submitted to variance analysis (ANOVA) and Tukey test using the Statistica®10 program.

RESULTS AND DISCUSSION

Minimum inhibitory concentration

The Minimum Inhibitory Concentration (MIC), was studied between 4-0.100% for cinnamon and nutmeg EOs, against *E. coli* and *P. commune*. For the CEO, in both microorganisms the MIC was the same, 0.125%. But for the NEO, the MIC was higher in both microorganism, 0.250%. This may be because in the CEO there are more antimicrobial compounds present than the NEO or the antimicrobial activity of the compounds present

in CEO are higher than the ones present in the NEO. Results obtained are lower but in agreement with (HUANG et al., 2014; LIU et al., 2014).

The Gram-negative bacteria are less susceptible to the EOs than Gram-positive ones, as the hydrophilic lipopolysaccharides from the outer membrane of these bacteria, which creates a barrier to the hydrophobic compounds from the EOs (HYLDGAARD et al., 2012). To DORMAN & DEANS (2000), aldehydes, possess significant antimicrobial activity, possibly by the conjugation of the aldehyde group conjugated to a C-C double bond in a highly electronegative arrangement. It suggested that an increase in the electronegativity improve the antimicrobial activity. The authors suggested that electronegative compounds may interfere in biological processes by electron transfer and react with nitrogen components, such as proteins and nucleic acids inhibiting the growth of the microorganisms. According to HYLDGAARD et al. (2012); although, the mode of action of cinnamaldehyde is inconclusive, aldehydes cross-link covalently to DNA and protein amino groups reducing normal function and cinnamaldehyde interacts with the cell membrane. SMYTH et al.(2009) reviews the literature of antimicrobial activity of coumarins and showed that they are selective to Gram-positive microorganisms. Thus, for CEO, both compounds are probably responsible for the antimicrobial activity.

According to DORMAN & DEANS (2000) the stereochemistry of the compounds has an important influence on the antimicrobial activity. The authors observed that α -isomers are inactive or minimally active, which means β -pinene contributes more to the antimicrobial activity in NEO. According to the authors, the alcohols possess bactericidal rather than bacteriostatic activity against vegetative cells. The authors also reported the alcohol terpenoids, such as terpinen-4-ol, exhibited the antimicrobial activity acting as either protein denaturing agents, solvents or dehydrating agents. DORMAN & DEANS (2000) also detailed that an allylic side chain seem to enhance the inhibitory effects of the component, especially against Gram-negative bacteria and also compounds with isopropyl cyclohexane rings or unsaturation on the cyclohexane ring increase the antibacterial activity, such as sabinene. NARASIMHAN & DHAKE (2006) presented several articles reporting the antimicrobial activity of the aromatic alkenyl benzene myristicin. According to the authors the unsaturated side chain on the aromatic ring of myristicin may be responsible for its antibacterial activity, acting on the bacteria cell wall. In *M. fragrans* EO, the myristicin and

the terpinen-4-ol, apparently, are the compounds responsible for the antimicrobial activity of this EO. Normally EOs act synergistically, i.e., the combined EO effects are greater than the sum of the individual effects (TAJKARIMI et al, 2010).

Microscopic analysis of the active films

From microscopic analysis of the cross sections of the samples it was observed that all the studied coatings were homogeneous, covered the entire surface of the fruit, did not present defects and display good adhesion. Thickness of the films was $\sim 120\mu\text{m}$ for the alginate-based coatings MC2, MC3 and MC4. Results obtained were slightly lower than those obtained for ROJAS-GRAÜ et al. (2007), which used alginate and gellan-based coating on apples and observed thicknesses of $132\mu\text{m}$ and $155\mu\text{m}$, respectively.

Microbiological analyses

The counts of *E. coli* and *P. commune* over storage time are presented comparatively in figure 1A and it can be verified that over the storage time the uncoated samples had an increase in the total count from day 2 and continue to increase until day 4 (2 log cycles). The MC2 sample, alginate-based coated with no EO had a discrete decrease in the total count. This is probably due to the presence of ascorbic acid and citric acid in the formulation, as both are known to present antimicrobial activity. But MC3 and MC4 samples presented reductions in the *E. coli* count, with the most significant reduction (3 log cycles). With MC3 and MC4 samples it was observed that, after the 3rd day of storage, it is possible to reduce contamination from 10^5 CFU/g. Comparatively, MC3 was better to reduce the total count than MC4. This result is in agreement with the MIC obtained for the EOs, which the CEO presented greater antimicrobial activity. Results for the edible films with CEO in the present research were comparable to those reported in the literature (RAYBAUDI-MASSILIA et al., 2008 a; RAYBAUDI-MASSILIA et al, 2008 b). However, the authors cited used much higher concentrations. To the best of our knowledge, this present study uses the lowest concentration of CEO. Articles to compare the edible coatings prepared incorporating NEO were not found. Thus, this is the first time NEO is used in an edible coating.

According to Brazilian legislation (BRASIL, 2001) regarding the microbiological standards for direct consumption food, the maximum bacterial count permitted is 5.10^2 CFU/g at 25°C . Both

coatings studied could be an important alternative to avoid bacterial contamination in minimally processed apples. Values obtained for the *P. commune* count over storage time are presented comparatively in figure 1B. According to figure 1B it can be observed that over 9 days of storage, the uncoated sample (MC1) had a total count increase. As observed for *E. coli*, the MC2 sample also presents a reduction in the *P. commune* count. This may be explained due to the presence of ascorbic acid and citric acid in the formulation. But, MC3 and MC4 samples, presented a significant reduction in the *P. commune* count with a decrease of 1log cycle over storage time and 3log cycles compared to the MC1 sample. In this case, the MC3 sample had better reduction on the total count than MC4. This is in agreement with the MIC obtained for the EOs, where the CEO presented a better antimicrobial activity. Brazilian legislation (BRASIL, 2001) does not establish microbiological standards for molds or yeasts in fruit. However, from figure 1B, it can be observed that the count of 10^4 , although low, was sufficient to accelerate the degradation of the product in MC1 case. The literature shows that for films using manioc starch incorporated with CEO, it was observed that the MIC of 0.4% was effective against *P. commune* (PALOU et al, 1999). Although, this concentration is ~ 300 times higher than the used in the present research which means, the present edible coating formulation with 0,125% of CEO shows extremely significant results.

Shelf-life: from figure 1C is possible to see the shelf life variation of the four treatments. From these analyzes was possible to verify a significant extension of the shelf-life of coated samples (15 days), compared to the uncoated slices of apple MC1 (0 days) and alginate-based coated slices of apple but with no EO (3-4 days). According to the results, CEO appears to improve the shelf-life of minimally processed apples, due to its higher effectiveness (0.125%). Although, microbiologically both MC3 and MC4 still presented 1log UFC/g of total count, the samples lost the texture, becoming soft. As for Shelf-Life the sensory and microbiological attributes must be considered. It is possible to say the apples with alginate-based edible coatings with CEO and NEO have a shelf life of 10-13 days because after the 15th day texture was completely lost, despite not presenting a microbial growth. RAYBAUDI-MASSILIA et al (2008a) prepared an alginate-based edible film with cinnamon (*C. zeylanicum*) EO applied on fresh-cut melons. The authors observed that while the *non-coated* melon had a shelf life around 3.6 days, the melons coated with 0.3% of CEO presented a shelf life of approximately 13.1 days for bacterial and >21

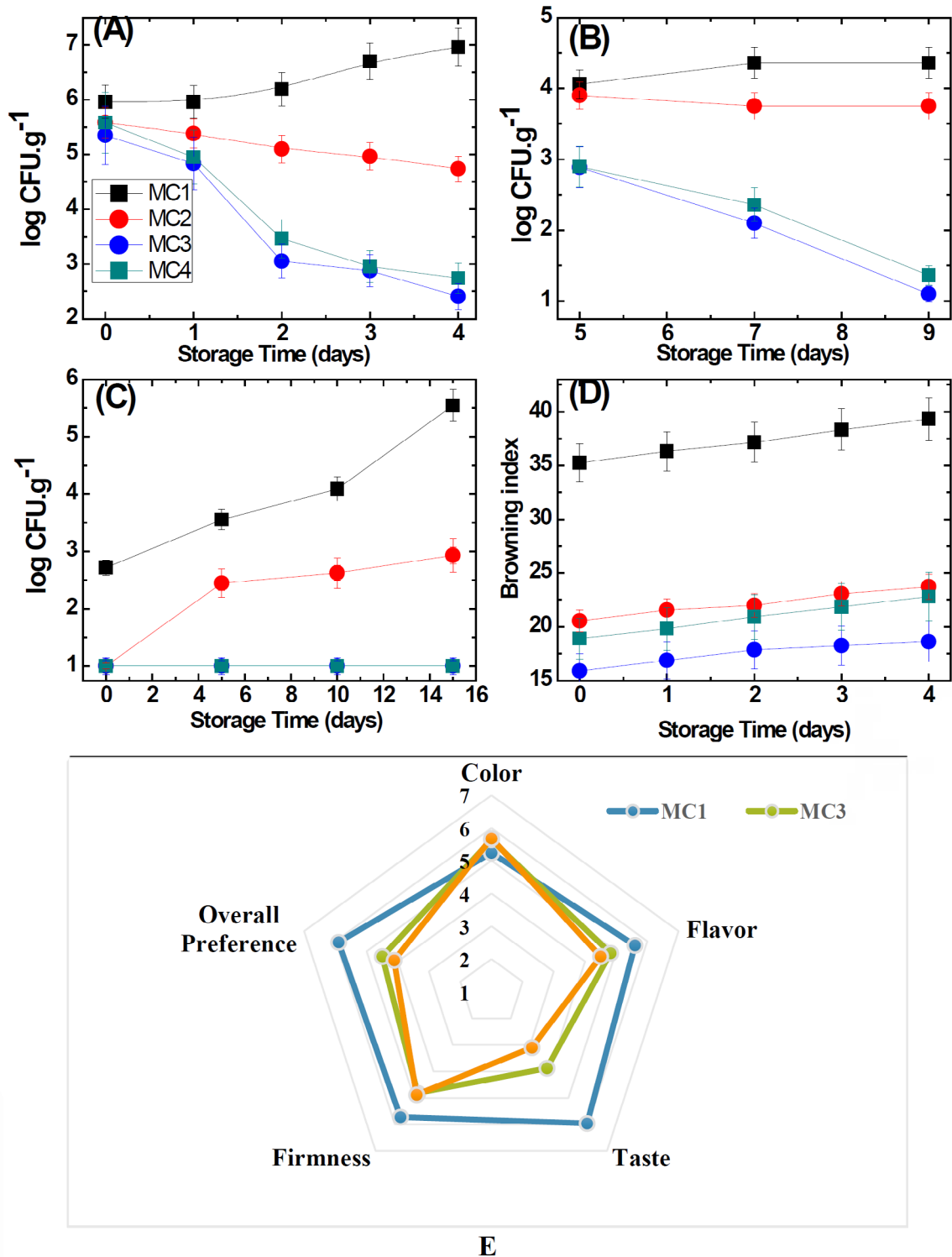


Figure 1 - Comparison of *Escherichia coli* (A) and *Penicillium commune* (B) count over storage time. (C) Shelf life of the coated apples under study. (D) Comparison of the Browning Indexes of the samples during storage. (E) Effect of the incorporation of Cinnamon Essential Oil (CEO) and Nutmeg Essential Oil (NEO) in edible alginate-based coatings on sensorial characteristics of minimally processed apples. (MC1) Control apple, (MC2) apple with alginate film, (MC3) apple with alginate film and CEO and (MC4) apple with alginate film and NEO.

days for yeast and mold. The same edible film reduced the population of *Salmonella enteritidis* approximately $4\log_{10}$ CFU/g. RAYBAUDI-MASSILIA et al. (2008a), prepared edible coatings with alginate, glycerol, malic acid (as an antimicrobial agent), N-acetyl-L-cysteine and glutathione (as anti-browning substances) and calcium lactate pentahydrate as an antisoftening agent. The CEO, was added to the coating in 0.3 and 0.7% concentrations and then the edible coatings were applied to minimally processed Fuji apples. According to the authors, the film inhibited native microbiota for 30 days and *E. coli* for 19 days, reducing by 4log cycles *E. coli* contamination. However, higher EO concentrations were the most microbiologically effective and lower concentrations were the best to maintain the physicochemical properties of the samples. This means that higher EO concentrations adversely affect the physicochemical properties, reducing shelf life. Considering the concentrations of CEO used in these studies, the present formulation has a similar shelf-life of the formulation prepared by RAYBAUDI-MASSILIA et al. (2008b), but with a 2.4 times lower concentration. And, considering RAYBAUDI-MASSILIA et al. (2008 a), the present formulation has a 5.6 times lower concentration of CEO. So, the self-life of the present formulation is proportionally better than that obtained by the authors, indicating that higher EO levels are not necessarily more effective.

Browning index (BI): values obtained for the BI during storage time are given comparatively in Figure 1D. The blank value control sample was measured at a BI = 2.6. As can be observed from Figure 1D, there was a significant difference between in the BI from the control (MC1) and the alginate-based coated apple, without EO (MC2). It may be happened because of the presence of ascorbic and citric acid in the edible coating formulation. However, the reduction in BI was more significant in the alginate-based incorporating the EO-coated apples (MC3 and MC4), showing a gradual decrease over storage time. Additionally, samples MC3 and MC4 present lower BI values, which is due to the presence of antioxidant compounds in the EOs, although, MC3 treatment had a lower enzymatic browning compared to MC4. The CEO and NEO are known to possess antioxidant activity, but PRZYGODZKA et al. (2014) reported that CEO has a higher antioxidant activity than NEO, which agrees with the results obtained here. According to SINGH et al. (2007) cinnamaldehyde and eugenol were the major components of the EOs studied and, the authors concluded that these compounds were responsible for the antioxidant and the antimicrobial activity of the EOs studied. In the present study, eugenol is not present

in the *C. cassia* EO, so the cinnamaldehyde must be the compound responsible for the antioxidant and also the antimicrobial activity of this EO used. TOMAINO et al. (2005), studying the antioxidant activity of several EOs, including *M. fragrans*, reported the major compounds in this EO were, α -pinene, β -pinene and sabinene. The authors also reported the antioxidant activity of these compounds. In the present study, the same compounds are present in the *M. fragrans* EO. Thus, it is possible to conclude these compounds are responsible for the antioxidant activity of the *M. fragrans* EO studied. Generally, it can be concluded that the alginate-based coating was capable, on its own, of reducing the browning process. However, the retardation process was much more efficient in the presence of the EOs, especially with CEO, caused by a more effective antioxidant activity. RAYBAUDI-MASSILIA et al. (2008a), studying alginate-based edible coating with lemongrass, clove and cinnamon EOs, did not find a significant difference between the treatments used on the first day (day 0). Nevertheless, after 14 days of storage at 5°C the authors observed a visible degradation of the lightness. Cinnamon and lemongrass at 0.7% concentration were more rapidly affected. However, generally, the authors reported that the browning index was greatly reduced in coated samples because of the use of N-acetyl-L-cysteine and glutathione in the coating. The authors explained that the anti-browning agents react competitively with polyphenol oxidase and peroxidase to form stable colorless compounds.

Sensory analysis: the sensory analysis data is summarized in figure 1E. The sensory analysis had scores from 1 to 7, in which 7 is the highest grade and 1 the lowest. Taking into account the color attribute, the coated samples (MC3 and MC4) showed the highest score ($P < 0.05$), compared with uncoated control samples (MC1). This is due to enzymatic browning that started immediately after slicing the control samples. Conversely, flavor obtained the lowest score, followed by aroma and firmness. Despite the low concentrations of the CEO (0.125%) and NEO (0.250%) incorporated in the alginate-based edible-coating formulation, the panels detected their presence, which contributed to decrease the scores of the aroma and taste when compared to the control sample. Regarding the overall acceptance of the samples, the incorporation of the EOs negatively affected the samples (MC3 and MC4) since the scores were also significantly lower than those of the control sample (MC1). Similar results were observed in minimally processed apples covered with alginate-based films incorporating 1 and 1.5% lemongrass and

oregano EOs studied by ROJAS-GRAÜ et al. (2007). The authors reported that the use of the EOs had a significant effect on all sensory attributes, reducing the overall preference of these samples. AZARAKHSH et al. (2014), reported that the incorporation of 0.5% lemongrass EO in the alginate-based coating applied on minimally processed pineapples also had a negative effect on the taste scores on the texture, thus affecting the overall acceptance of the samples. A study of alginate-based films incorporated with different EOs in minimally processed melons, performed by RAYBAUDI-MASSILIA et al. (2008 a), showed that the incorporation of 0.3 and 0.7% of CEO in the films significantly affected the odor and flavor of the samples, since these presented less acceptance by the panels.

CONCLUSION

Alginate-based edible coatings incorporating CEO and NEO display significant improvements in the sample properties. The microscopic analysis demonstrated thinner coatings (~120µm) compared to the literature but still reduced 3 log cycles the *E. coli* and 1 log cycle the *P. commune* counts over storage time. Treatments improved the shelf-life of coated samples by proximately in 15 days and the treatment using CEO presented the best results. Reduction in browning index was more significant in the coated samples (14 days), with better results for the CEO. In sensory tests for coated minimally processed apples, the flavor had the lowest rating of the properties analyzed, for both treatments, followed by aroma and firmness.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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