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The effects of polyethylene film coated with menthol and carvacrol on the physicochemical, microbiological and sensory properties of low-moisture Mozzarella cheese

Elahe, Jahdkaran[1], Seyed Ebrahim Hosseini[1,*], Abdorreza, Mohammadi Nafchi[2,3], Leila Nouri[2]

1- Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2- Food Science and Technology Department, Damghan Branch, Islamic Azad University, Damghan, Iran
3- Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia.

ABSTRACT

The aim of this research was to evaluate the effect of polyethylene (PE) films coated with menthol and carvacrol on the physicochemical, microbiological, and sensory properties of low-moisture Mozzarella cheese. At first, PE films were coated with MC-HPMC solution containing different concentrations of menthol and carvacrol (0, 1, 1.5 and 2%), Mozzarella cheeses were then packed with these active films, and physicochemical, antimicrobial, and sensory properties of cheese treatments for 30 days storage in refrigerator were studied. The results showed that over time, the pH and moisture amount of all treatments significantly were decreased, whereas, the acidity and hardness amounts of cheeses were increased, but their exchange rate in control significantly was higher than other samples ($p<0.05$). Packaging of cheeses with active PE films leads to decrease the growth rate of mesophyll bacteria and molds and yeasts in cheeses. At the end of storage period, the lowest number of molds and yeasts and microbial load were for samples packed in films containing 2% menthol and carvacrol. From the point of *E. coli* and *S. aureus* contamination, the samples packed in active films were safe until the last day of storage (30th days), while control sample was incredible at 17th of storage. Packaging of Mozzarella cheese in PE films containing different levels of menthol and carvacrol also leads to improved sensory acceptance of cheeses during refrigerated storage. Finally, the results demonstrated that coated PE films with solutions containing menthol and carvacrol active components had a positive effect on the extension of the Mozzarella cheese shelf life, especially, at 2% concentration.

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*Corresponding Author E-Mail:

1- Introduction

Today, with the increase in the world's population, the demand for food products and food packaging has increased. Packaging protects the food contained in it against various environmental factors such as oxygen, light, heat, pressure and humidity [1]. Food packaging also increases the shelf life of food products and improves the safety of products by reducing destructive reactions such as chemical reactions and microbial growth [2]. In recent years, in order to preserve the quality of food, active packaging has been developed [3]. In these packaging systems, active agents such as antioxidants and antimicrobial agents are used, which are gradually released into the packaging over time [4]. However, in order to produce active packages based on synthetic polymers, it is not possible to combine active materials during the film production process, because high temperatures are used during the production process of these films, which can cause the destruction of the active compounds used. . Coating synthetic films with active agents is a good solution to overcome this problem [5].

Today, a lot of attention has been directed towards the extraction of bioactive compounds with antioxidant and antimicrobial activities from different natural plant sources [6-8]. Carvacrol (2-methyl-5-(1-methylethyl)-phenyl) is an active monoterpene phenolic compound found in aromatic plants and some plant essential oils such as oregano, thyme, and ginseng [9]. The significant antimicrobial activity of carvacrol against a wide range of microorganisms has been confirmed by previous researchers [10]. Researchers found that the antimicrobial activity of this bioactive compound is due to its effect on the structure and function of the microbial cytoplasmic membrane [11]. Menthol is a cyclic terpene alcohol that can be extracted from various plant sources and has various medical uses. This compound is abundantly present in peppermint. Previous researches indicated the high antimicrobial and antifungal activity of menthol [12]. The antimicrobial activity of menthol has also been attributed to its effect on the permeability of the microbial cell membrane and, as a result, the leakage of intracellular contents to the outside [13].

Mozzarella cheese is one of the most important types of cheese and based on its moisture content, it is classified into two categories, including low moisture types (45-54% moisture) and high moisture types (56-65% moisture). Mozzarella cheese with low moisture content is known as pizza cheese [14]. This type of cheese is one of the perishable products, because it can only be stored for 30 days at refrigerator temperature [15]. In recent years, various studies have been conducted in the field of increasing the shelf life of these cheeses using active packaging films. For example, Cardosa et al. (2022) increased the microbial shelf life of mozzarella cheese by using an active film based on polybutylene adipate-tetraphthalate (PBAT) combined with oregano essential oil [16]. Cheng et al. (2021) also reduced the microbial load of mozzarella cheese during storage by using polyethylene-based active films containing linalool and thymol [15]. In the research conducted by Da Silvadanenberg et al. (2017), the antibacterial efficiency of active films based on cellulose acetate combined with pepper essence in reducing the microbial load of cheese was reported [17]. Therefore, this research was conducted to determine the effect of polyethylene films coated with carvacrol and menthol as antimicrobial active agents on the qualitative and microbiological characteristics of low-moisture mozzarella cheese.

2- Materials and methods

2-1- Materials

Polyethylene film with a thickness of 0.02 mm was purchased from Sultan Printing Company (Karaj, Iran). Carvacrol, menthol, microbial culture media and chemicals used for experiments were purchased from Merck (Germany).

2-2- Preparation of polyethylene films coated with carvacrol and menthol

The coating solution was prepared from methylcellulose and hydroxypropyl methylcellulose (HPMC). 7 grams of methyl

cellulose and 3 grams of HPMC were slowly added to 200 cc of 95% ethanol and heated on a hot plate with a magnetic stirrer. Heating was stopped when the temperature reached 65 °C. Simultaneously with stirring, a mixture of polyethylene glycol and 100 cc of distilled water was added to the cooled MC-HPMC mixture as a plasticizer. Then, carvacrol and menthol were added to the coating solution at different levels, until the coating materials containing antimicrobial agents were obtained at the final levels of 0, 1, 1.5 and 2% w/w. The coating medium was placed on the polyethylene film using a roller, and then the films were dried under normal conditions. Addition of antimicrobial agents and coating of films with prepared coatings was done at low temperature to minimize the loss of antimicrobial agents due to their volatility [18].

2-3- Preparation of packaged mozzarella cheese samples

Fresh low-moisture mozzarella cheese was purchased from Pegah factory and divided into different batches of 150 grams, each of these pieces of cheese was packed with polyethylene films coated with methyl cellulose-hydroxypropyl methyl cellulose containing different levels of active compounds carvacrol and menthol and in They were kept at 4°C (refrigerator temperature) for 30 days. On days 0, 7, 17 and 30 of storage, relevant tests were performed on cheese samples.

2-4- Analysis of the chemical composition of cheeses

pH and acidity of the treatments were measured according to the method stated by the National Standard Organization No. 2852 [19]. The sample was poured into a 50 ml beaker and the electrode of the pH meter was completely inserted into the sample. The temperature of the sample was around 20 degrees Celsius and the minimum contact time of the sample with the electrode was 45 seconds. Then the pH was read. In order to measure the acidity, first the cheese sample was completely softened and mixed in a suitable mixer. Then 20 grams of it was weighed in a bowl and dissolved in some water. After putting all its contents into a 250 ml volumetric flask, it was brought up to volume and filtered using a strainer, and 25 ml of the filtered solution was transferred to a suitable beaker and 0.5 ml of phenolphthalein was added to it. It was titrated with 0.1 normal sodium hydroxide. This process continued until the appearance of a pale pink color and its stability for 5 seconds. The amount of acidity was calculated through the following equation, in which: N is the value of 0.1 ml of soda normal; M1 sample weight equal to 20 grams; V sample volume is equal to 25 ml.

$$Acidity = \frac{N \times 0.009 \times 100}{M1} \times \frac{Use\ case\ of\ volume\ balloon}{IN}$$

The moisture percentage of cheese samples was measured according to the method provided by Iran's National Standard Organization No. 1753. In short, 3 grams of the sample was weighed in a plate and dried in an oven at a temperature of 105 degrees Celsius until reaching a constant weight, and finally, the moisture content of cheeses was determined using the following equation [20].

$$100 \times \frac{initial\ weight\ final\ weight}{Sample\ weight} = \text{humidity (\%)}$$

5-2- Microbial analysis of cheeses

2-5-1- Total microbial count

The total microbial count in mozzarella cheese samples was done using the mixed culture method stated by the Iranian National Standard No. 5272-1. At first, pity[3-]10 cheese samples were prepared. Then, using a sterile pipette, 1 ml of the desired dilution was transferred to each of the two sterile petri dishes. About 12-15 ml of PCA culture medium It was poured into each plate at a temperature of 44-47 degrees Celsius. The plates were carefully mixed with the culture medium by rotating movement and placed on a horizontal and cool surface until they became completely solid. Then the prepared plates were placed upside down in a greenhouse at 30 degrees Celsius for 72 hours. After the incubation period, the number of microbial colonies was counted and the number of aerobic microorganisms per gram of samples (log CFU/g) was reported as a logarithm [21].

2-5-2- Attendance check*Escherichia coli*

Check*Escherichia coli* In the cheese samples, it was done according to the method stated by the Iranian National Standard Organization No. 5234. First, the desired dilution was prepared from the sample. Then, 3 large test tubes containing 10 ml of enrichment culture medium with double concentration were selected and 10 ml of the initial suspension (initial dilution) was added to each of them using a sterile pipette. 3 other small tubes containing 10 ml of enrichment culture medium with normal concentration were selected and 1 ml of the initial suspension was added to each of them by pipette. With the help of a suitable mixer, the cultivated material was well mixed with the environment. The test tubes were placed in a 37 degree Celsius incubator for 24 hours. After this time, if no gas was observed, the tubes were kept in a greenhouse for another 24 hours. To investigate in the second selective medium, the greenhouse tubes containing gas were cultured by means of a culture ring in the second selective medium which was previously heated in a water bath to 44 degrees Celsius. Then the cultivated material was mixed. The cultured tubes were placed in a greenhouse for 24 hours at a temperature of 44 degrees Celsius in a water bath. If no gas was observed at this stage, the greenhouse was continued for 48 hours. In order to investigate in peptone water, from the greenhouse tubes that formed gas, it was cultured and mixed with the culture ring in tryptone water, which was previously placed in a water bath with a temperature of 44 degrees Celsius. The cultured tubes were placed in a water bath at a temperature of 44 degrees Celsius for 48 hours. Finally, to test the production of indole, 0.5 ml of indole reagent was added to the tubes containing tryptone water placed in the greenhouse, mixed well and checked after one minute. The appearance of red color in the alcoholic phase indicates the presence of indole (positive tubes) [22].

2-5-3- Attendance check*Staphylococcus aureus* Coagulase positive

Bacteria examination*Staphylococcus aureus* In the samples, it was done using the method stated by the National Standard Organization of Iran No. 3-6806. In this way, a certain amount of the initial suspension was inoculated in the selected culture medium. The inoculated medium was kept at 37 degrees Celsius for 24 and 48 hours in anaerobic greenhouse conditions. Existence*Staphylococcus aureus* By reducing potassium tellurite[1] It was found. From the possible positive tubes after 24 hours and from all the remaining tubes after 48 hours on the surface of Brad-Parker agar selective culture medium.[2] Cultivated. The plates were kept in a greenhouse at 37 degrees Celsius for 24 and 48 hours. By performing the coagulase test, specific or indeterminate colonies were confirmed [23].

2-5-4- Counting mold and yeast

Counting the number of mold and yeast in mozzarella cheese samples was done using the mixed culture method stated by the Iranian National Standard Organization No. 10154. Using a sterile pipette, 1 ml of the desired dilution was transferred to each of two sterile petri dishes. About 12-15 ml of YGC culture medium with a temperature of 44-47 degrees

[1]- Potassium tellurite (K2TeO3)

[2]- Baird – Parker agar

Celsius was poured into each plate. The plates were carefully mixed with the culture medium by rotating movement and placed on a horizontal and cool surface until they became completely solid. Then the prepared plates were placed upside down in a greenhouse at a temperature of 25°C for 5 days. After the greenhouse period, the number of mold and yeast colonies were counted [24].

6-2- Evaluation of sensory acceptance of cheeses

Control low-moisture mozzarella samples packed in polyethylene films coated with methylcellulose-hydroxypropylmethylcellulose combination containing different levels of active compounds carvacrol and menthol were analyzed by 7 trained evaluators. The evaluators were asked to evaluate the overall acceptance by considering the taste, color, smell and texture of the low-moisture mozzarella cheese samples. The cheese samples were provided to the evaluators and they were asked to rate the overall acceptability of the samples based on a 5-point hedonic test, such that 5: very good, 4: good, 3: average, 2: bad and 1: It is very bad.

9-2- Method of statistical data analysis

The mean of each parameter was analyzed by analysis of variance in a completely randomized design with factorial arrangement and using SPSS 22.0 software. The differences between the treatments were expressed in Duncan's test and at the 95% confidence level, and the corresponding graphs were drawn with Excel 2013.

3. Results and Discussion

3-1- Mozzarella cheese pH values

The changes in pH values of low-moisture mozzarella cheese samples during the storage period are shown in Figure 1. At the beginning of the storage period, the pH of mozzarella cheese was 5.78. In all cheese samples, during the storage period, the pH Significantly decreased, but the pH changes in the control sample were more than the samples packed in antimicrobial active films. The decrease in pH during the storage period can be related to various reasons, including the consumption of lactose, hydrolysis of fat and protein, which cause the production of lactic acid, fatty acids, and amino acids, so that the production of all these acids causes a decrease in pH in cheese [25]. . The effect of antimicrobial films coated with different levels of carvacrol and menthol on the reduction of pH changes during the storage period is related to the reduction of the proliferation rate of bacteria in cheese samples, especially lactic acid bacteria, because these bacteria produce lactic acid by consuming the lactose sugar found in cheeses. and reduce the pH, the active compounds of carvacrol and menthol can reduce the production of acid in the environment by reducing the rate of reproduction of these bacteria and preventing their growth, and less changes in the pH level occur [26].

On the last day of storage at refrigerator temperature, the highest pH value was obtained in samples containing 2% menthol and carvacrol (similarly: 5.64) and there was a statistical difference between these two treatments and cheeses containing 1.5% menthol and carvacrol. There was no significance (<0.05).*p*). According to the Iranian National Standard Organization No. 4658, the pH of low-moisture mozzarella cheese should be in the range of 5.60-5.80 [27]. By comparing the results obtained in this research with the national standard of Iran, it can be seen that the pH level of all the treatments examined in this research was within the range determined by the standard in all days of storage, except for the control sample, which reached less than this limit on the last day.

Researchers stated that plant essential oils reduce lactose consumption and acid production by inactivating lactic acid bacteria [28]. Kavas et al. (2015) also showed in agreement with the results of this research that during the storage period, the pH of cheese samples decreased, but the use of active coating containing ginger essential oil was able to reduce the speed of pH changes compared to the control [29]. . Haras (2010) also reported that packaging cheddar cheese with LDPE film containing different levels of the antimicrobial agent thymol, through the inhibitory effect on the growth of lactic acid bacteria, caused a decrease in pH changes during 36 days of storage [30].

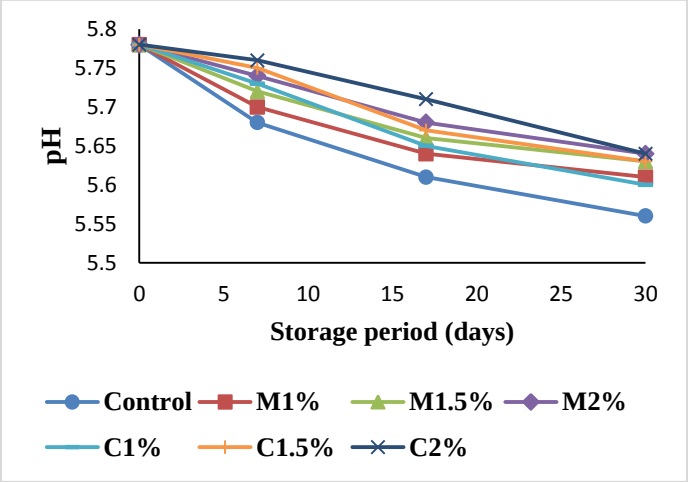

Figure 1. Changes in pH values of Mozzarella cheese samples during the 30-days storage period

2-3- Mozzarella cheese acidity

Cheese is a fermented dairy product that during the production and ripening stages of this product, it is necessary to control the production of lactic acid and other acids from lactose. Acidity prevents the growth of spoilage microorganisms and affects coagulant activity during the production and ripening stages. Acidity also dissolves colloidal calcium phosphate and increases synergistic properties and affects the activity of enzymes during cheese ripening. Also, acidity is effective on the texture quality and taste of cheese [31]. Changes in the average acidity values of different cheese samples during the 30-day storage period are shown in Figure 2. As can be seen in the graph, at the beginning of the storage period, the acidity level of different cheese samples was 0.58%, and with the passage of time, from day 0 to day 30 of storage in the refrigerator, the acidity level of all cheese samples increased significantly (0.5 0>*p*), but the speed of its increase in the control sample was significantly higher than that of the cheeses packaged in polyethylene films coated with the active compounds menthol and carvacrol, so that in the samples containing the highest levels of menthol and carvacrol (concentration 2%), the acidity From 0.58% on the first day, it increased to 0.74% and 0.76% until the 30th day, respectively, while in the control sample, the acidity reached 0.97% on the last day. However, on the last day of storage, there was no statistically significant difference between the acidity values of cheeses containing 1.5 and 2% menthol and carvacrol and 1% menthol (<0.05).*p*). The increase in the acidity of different cheese treatments during the storage period is due to the production of lactic acid by the cheese microbial flora [25]. Lactose decomposition is the introduction of all transformations, acid production and proper pH adjustment in cheese, which during natural ripening of cheese leads to proteolysis in the right direction and prevents its spoilage. The pH and acid of the rind environment have a significant effect on the percentage of water release during the production stage, the activity of enzymes and microbial flora during the production stages and the ripening period, as well as the taste and consistency of the cheese. In general, one of the important reactions in the production of cheeses is the decomposition of

carbohydrates [32]. The National Standard Organization of Iran No. 4658 has determined the maximum acidity of low-moisture mozzarella cheese as 0.80 (percentage in terms of lactic acid) [27], which according to the results obtained in the present study, the acidity of the control treatment on day 17 and 30 and the amount of acidity of cheeses packaged in films containing 1% menthol and carvacrol on the 30th day of refrigerator storage was higher than recommended by the standard. Other treatments were within the specified range until the last day of storage. In the study conducted by Kavas and Kavas (2014), it was observed that the acidity level of the control cheese was significantly higher than the samples coated with whey protein-based films containing different concentrations of mint essential oil. Increasing the storage period led to an increase in the acidity of all cheese treatments, which was consistent with the results of the present study [33]. In Khazaei et al.'s (2022) research, during the 60-day storage period, the acidity of cheese samples increased significantly, but the use of active films containing garlic, ginger, and cinnamon essential oils could increase the rate of acid production in cheese samples compared to the control. significantly reduce [34].

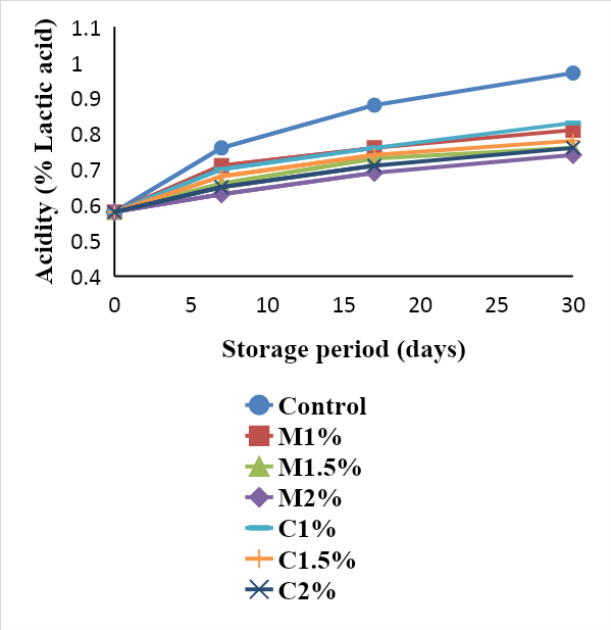

Figure 2. Changes in acidity values of Mozzarella cheese samples during the 30-days storage period

3-3- Mozzarella cheese moisture

Changes in the average moisture values of different samples of low-moisture mozzarella cheese during the storage period are shown in Figure 3.

given. At the beginning of the storage period, the moisture content of the cheeses was 59.76%, and with the passage of time from the first day to the thirtieth day of storage in the refrigerator, the moisture content of the cheeses decreased significantly (<0.05).*p*), but as expected, the rate of moisture loss in the control sample was significantly higher than the samples packed in the active film containing different levels of menthol and carvacrol. The decrease in the moisture content of cheese samples during the storage period in the refrigerator is related to the exit of moisture from the cheese to its surrounding environment. The reduction of moisture loss during the storage period due to the packaging of mozzarella cheeses with active films containing different levels of carvacrol and menthol is due to the barrier properties of these films against water vapor. Adding active agents to polyethylene film reduces the permeability to water vapor of produced films [18], As a result, the evaporation of moisture from the surface of the cheeses decreases.

Overall, in the control sample the moisture content decreased to 45.93% on the last day, while in the samples packaged with films containing the highest levels of menthol and carvacrol (2% level), the moisture content on the last day decreased to 10%, respectively. It reached 54.54 and 53.97 percent. Iran's National Standard Organization No. 4658 has determined the moisture content of low-moisture mozzarella cheese at a maximum of 61% by weight [27]. The comparison of the results obtained in this research with the amount recommended by the national standard of Iran showed that the moisture content of all the treatments examined in this research was acceptable until the last day of storage in the refrigerator. Kanwambuena et al. (2016) also reported that coating fresh goat milk cheese with active coatings containing rosemary and oregano essential oils significantly reduced the moisture loss of cheeses during the storage period due to the good barrier properties of these films against water vapor [35]. Ramos et al. (2012) also observed a decrease in the moisture content of cheeses during the 60-day storage period. These researchers stated that the use of edible coating based on whey protein could reduce the rate of moisture loss in cheeses [36].

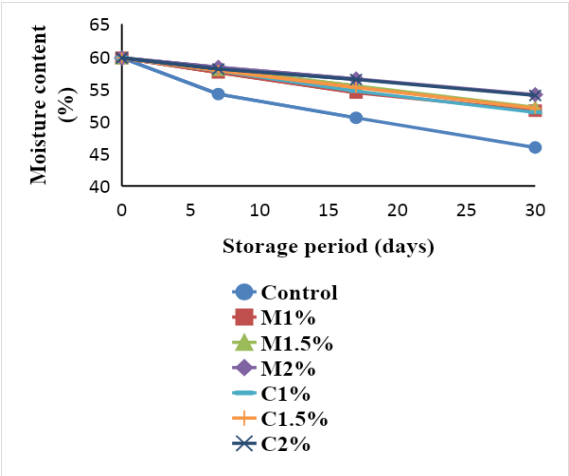

Figure 3. Changes in moisture content of Mozzarella cheese samples during the 30-days storage period

3-4- Mozzarella cheese microbial test results

3-4-1- General microbial count

The changes of the average logarithm of the total microbial count in different samples of low-moisture mozzarella cheese during the 30-day storage period at refrigerator temperature are shown in Figure 4. At the beginning of the storage period, there was no difference between the total microbial counts in different cheese samples. With the increase of the storage period in the refrigerator temperature, due to the growth and proliferation of microorganisms in the cheeses, the total microbial count increased significantly (<0.05).*p*) and as expected, the highest rate of growth and proliferation of microorganisms was related to the control sample, which increased from 3.11 to 6.41 log CFU/g from the first day to the thirtieth day. The rate of microbial load increase in cheeses packaged in polyethylene film coated with the highest level of menthol and carvacrol (concentration 2%) during the storage period was significantly lower than other treatments (<0.05).*p*), so that on the last day of storage, the total microbial load in these two treatments was 4.31 and 4.42 log CFU/g, respectively, and no statistically significant difference was observed between these two treatments (0.05 <*p*). The antimicrobial activity of polyethylene films coated with the active agents carvacrol and menthol was concentration-dependent, so that with the increase in the concentration of these two antimicrobial agents, the functional activity of the active packaging films increased significantly and the overall bacterial count decreased. In general, it has been confirmed that at higher concentrations of antimicrobial agents in films, the migration of more compounds occurs, and as a result, more of these compounds are found in the medium [37]. The antimicrobial activity of carvacrol and menthol is basically related to their inherent hydrophobic properties [38]. An increase in the antibacterial activity of the active coating containing cardamom essence in reducing the microbial load of white cheese was also observed in the research of Hamzaei et al. (2022) [39]. Sopakol (2004) also found that coating Cheddar cheese with films containing linalool[3] and methylcavicol[4] It reduced the microbial load of all cheeses during the storage period of 9-15 days [40]. In the research conducted by Cardosa et al. (2022), the reduction of microbial load in mozzarella cheese samples due to the use of active film based on polybutylene adipate-tetraphthalate (PBAT) combined with oregano essential oil was reported [16].

[3]- Linalool

[4]- Methylchavicol

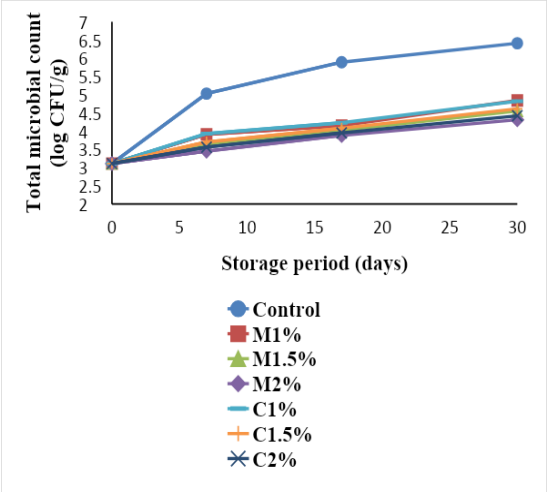

Figure 4. Changes in total microbial count of Mozzarella cheese samples during the 30-days storage period

3-4-2- Attendance check *Escherichia coli* in cheeses

bacteria*Escherichia coli* is a pathogenic and gram-negative bacterium that can contaminate cheeses [41]. The results of examining the presence of gram-negative bacteria*Escherichia coli* In low moisture mozzarella cheese samples, it is shown in Table 1. These results show that in the control sample until the seventh day*Escherichia coli* It was not found, but on the 17th day, this bacteria was observed in the control sample and its number increased significantly until the 30th day. However, in the samples packaged in active films containing different levels of linalool and thymol compounds by the last day*Escherichia coli* not found. Menthol and carvacrol are active antimicrobial compounds that cause the disintegration of the outer membrane in gram-negative bacteria and release lipopolysaccharides and increase the production of colloidal compounds [42]. National Standard Organization of Iran No. 2406 stated that*Escherichia coli* In mozzarella cheese, it should be negative [43], therefore, the control sample is microbially inedible from the 17th day onwards. Kavas and Kavas (2014) investigated the effect of active coating containing different concentrations of peppermint essential oil on the microbial properties of bay cheese and showed, in agreement with the results of the present study, that increasing the concentration of peppermint essential oil in edible coatings, the number*Escherichia coli* and significantly reduced molds and yeasts in cheeses, so that at the highest concentration of this essential oil (4% level),*Escherichia coli* Not found [33]. The effect of active bionanocomposite film combined with cinnamon essential oil in reducing the no*Escherichia coli* It was also observed in cheese in the research of Sharma et al. (2022) [44].

Table 1. Changes in *Escherichia coli* number of Mozzarella cheese samples during the 30-days storage period

Samples	Day 0	Day 7	Day 17	Day 30
Control	ND	ND	14.69 ± 0.08 [b]	29.44 ± 0.16 [a]
M1%	ND	ND	ND	ND
M1.5%	ND	ND	ND	ND
M2%	ND	ND	ND	ND
C1%	ND	ND	ND	ND
C1.5%	ND	ND	ND	ND
C2%	ND	ND	ND	ND

*Values represent mean ± SD. Different letters in each row represent statistical significant difference at 5% level.

3-4-3- Attendance check *Staphylococcus aureus* in cheese samples

The results of examining the presence of gram-positive bacteria*Staphylococcus aureus* In low moisture mozzarella cheese samples, it is shown in Table 2. in the control sample until the seventh day*Staphylococcus aureus* It was not found, but on the 17th day, this bacterium was observed in the control sample, and until the 30th day, the number of this microorganism increased significantly. However, in films containing high levels of carvacrol and menthol (1.5 and 2%), up to the last day of refrigeration,*Staphylococcus aureus* Not found, these results are related to the gradual transfer of antimicrobial agents from the film layer to the food in antimicrobial coating systems. With the gradual transfer of antimicrobial agents, the concentration of these active compounds at the food level is optimal until the last day of storage and protects the food product against microorganisms [45]. According to the National Standard Organization of Iran No. 2406,*Staphylococcus aureus* In mozzarella cheese it should be negative [43], hence, the control sample is microbiologically inedible from the 17th day onwards. Kavas et al. (2016) observed in agreement with the results of the present study that the use of

active films containing ginger essential oil could increase the growth of bacteria.*Escherichia coli* And*Staphylococcus aureus* in Kashar cheese significantly reduced during the storage period [46]. Siddim et al. (2020) also reported that in Kasar cheeses inoculated with*Staphylococcus aureus*After 24 hours, the use of active films containing oregano essential oil could reduce this bacteria by 2.15 logarithms compared to the control [47].

Table 2. Changes in *Staphylococcus aureus* number of Mozzarella cheese samples during the 30-days storage period

Samples	Day 0	Day 7	Day 17	Day 30
Control	ND	ND	26.35 ± 0.11 [b]	37.77 ± 0.24 [a]
M1%	ND	ND	ND	ND
M1.5%	ND	ND	ND	ND
M2%	ND	ND	ND	ND
C1%	ND	ND	ND	ND
C1.5%	ND	ND	ND	ND
C2%	ND	ND	ND	ND

*Values represent mean ± SD. Different letters in each row represent statistical significant difference at 5% level.

3-4-4- Counting mold and yeast in cheeses

The changes of the average logarithm of the number of molds and yeasts in different samples of mozzarella cheese during the 30-day storage period at refrigerator temperature are shown in Figure 5. At the beginning of the storage period, there was no difference between the number of molds and yeasts in different cheese samples. With the passage of time from the first day to the thirtieth day, due to the proliferation of molds and yeasts in the samples, the number of these microorganisms increased significantly (<0.05).*p*) and as expected, the highest number of molds and yeasts was related to the control sample, which increased from 0.85 to 2.23 log CFU/g from the first day to the thirtieth day. The rate of increase in the number of molds and yeasts in cheeses packaged in polyethylene film coated with the highest level of menthol and carvacrol (2% concentration) during the storage period was significantly lower than other treatments (<0.05).*p*), so that on the last day of storage, the number of mold and yeast in these two treatments was 1.25 and 1.29 log CFU/g, respectively, and there was no statistically significant difference between these two treatments (0.5 <0*p*). Carvacrol and menthol have hydroxyl group in different places of their phenolic ring. These compounds destroy the external membrane of microorganisms and cause the release of liposaccharides and increase the permeability of the cell membrane to ATP. The release of ATP leads to the exhaustion of the cell's energy reserve and its death [48]. According to the National Standard Organization of Iran No. 2406, the maximum number of molds and yeasts in mozzarella cheese should be Log cfu/g 2 [43]. The results of this research showed that the number of molds and yeasts in the control sample was within the acceptable limit determined by the Iranian standard until the 17th day and in the other samples until the last day. Aguirre et al. (2013) stated that carvacrol is an antimicrobial compound with activity against a wide range of spoilage or pathogenic fungi, yeasts and bacteria [49]. Hamzaei et al. (2022) also observed that the active coating containing cardamom essential oil showed significant antimicrobial activity and was able to inhibit the growth of molds and yeasts in white cheese [39].

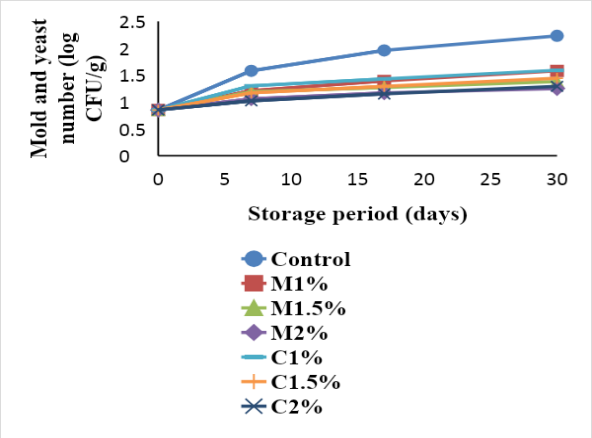

Figure 5. Changes in mold and yeast number of Mozzarella cheese samples during the 30-days storage period

3-5- Sensory evaluation of mozzarella cheese

The changes in the average overall acceptance scores of the control sample and cheeses packaged in polyethylene films coated with solutions containing different levels of the active compounds menthol and carvacrol during the 17-day storage period at refrigerator temperature are shown in Figure 6 and show that on the first day, all treatments The cheese examined in this research got a full score of overall acceptance. Over time from the first day to the last day of refrigeration, the overall acceptance score. The control sample was significantly reduced ($p<0.05$), while the samples packed in different active films had a full overall acceptance score on the seventh day, but on the seventeenth day the overall acceptance score of all treatments decreased significantly ($p<0.05$). The reduction of the overall acceptance score on the last day of storage in the refrigerator is related to the reduction of the sensory characteristics of taste, texture, smell and color of the treatments. In general, since menthol and carvacrol

compounds have antimicrobial and antioxidant activity, they maintain different sensory characteristics in products. Therefore, all cheeses packaged with active films containing different levels of carvacrol and menthol were acceptable in terms of all sensory characteristics until the 17th day. Kanwambuena et al. (2016) showed that coating fresh goat's milk cheese with an active coating containing rosemary and oregano essential oils had no adverse effect on the sensory characteristics of cheese samples. In the study conducted by Mahsen et al. (2021), cheeses coated with active coatings containing basil essential oil had high sensory acceptance [50]. Khazaei et al. (2022) also reported that the use of active films containing cinnamon and ginger essential oils not only did not adversely affect the overall acceptance of jarred cheese, but also improved the sensory characteristics of packaged cheeses [34].

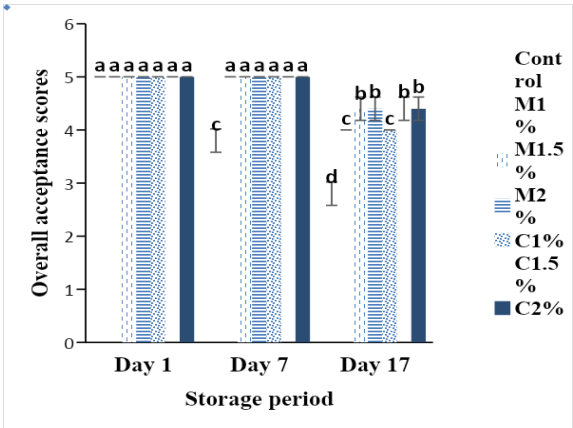

Figure 6. Changes in overall acceptance scores of Mozzarella cheese samples during the 30-days storage period

4 - Conclusion

The results of investigating the effect of active films coated with different levels of menthol and carvacrol on the physicochemical, microbial and sensory characteristics of mozzarella cheese during a 30-day storage period at refrigerator temperature showed that the packaging of cheese samples with polyethylene films coated with different levels of menthol and carvacrol, By reducing the speed of growth and proliferation of spoilage and disease-causing microorganisms, it increased the shelf life of cheeses and reduced the speed of pH and acidity changes in them during the period of storage in the refrigerator. Packing the cheese in the mentioned active films also kept the moisture of the cheeses during the storage time. By packaging mozzarella cheese in active packages containing different levels of menthol and carvacrol, the sensory acceptance of the cheese improved during the storage period and these samples were acceptable until the last day. In general, according to the results reported in this research, it was determined that the use of active films coated with different levels of menthol and carvacrol could increase the shelf life of low-moisture mozzarella cheese by at least 23 days, and the difference between the effects caused by menthol and carvacrol No significant was observed.

5- Resources

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مقاله علمی_پژوهشی

اثرات بسته بندی با فیلم پلی‌اتیلنی پوشش‌دهی شده با منتول و کارواکرول بر خصوصیات فیزیکوشیمیایی، میکروبیولوژیکی و حسی پنیر موزارلای کم‌رطوبت

الهه جهدکاران[۱]، سید ابراهیم حسینی[۲*]، عبدالرضامحمدی نافچی[۳]، لیلا نوری[۴]

۱-دانشجوی دکتری علوم و صنایع غذایی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

۲- دانشیار علوم و صنایع غذایی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

۳- استاد دانشگاه علوم مالزیUSM ، پنانگ، مالزی.

۴- استادیار علوم و صنایع غذایی، واحد دامغان، دانشگاه آزاد اسلامی، دامغان، ایران

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فساد میکروبی،

انبارمانی

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* مسئول مکاتبات:

چکیده

هدف از این تحقیق، بررسی تأثیر فیلم‌های پلی‌اتیلنی پوشش‌دهی شده با منتول و کارواکرول بر خصوصیات فیزیکوشیمیایی، میکروبیولوژیکی و حسی پنیر موزارلای کم‌رطوبت بود. در ابتدا، فیلم‌های پلی‌اتیلنی با محلول‌های متیل‌سلولز-هیدروکسی پروپیل‌متیل‌سلولز حاوی غلظت‌های مختلف منتول و کارواکرول (۰، ۱، ۱/۵ و ۲ درصد) پوشش‌دهی شدند و سپس، پنیر موزارلا با این فیلم‌های فعال بسته‌بندی شده و خصوصیات فیزیکوشیمیایی، ضدمیکروبی و حسی تیمارهای پنیر طی دوره نگهداری ۳۰ روزه در یخچال بررسی گردید. نتایج نشان داد که با گذشت زمان، مقادیر pH و رطوبت کلیه تیمارها به طور معنی‌داری کاهش یافت، در حالی که مقادیر اسیدیته و سفتی بافت پنیرها افزایش پیدا کرد، ولی سرعت تغییرات آن‌ها در نمونه شاهد به طور قابل توجهی بیشتر از سایر نمونه‌ها بود (p<۰/۰۵). بسته‌بندی پنیرها با فیلم‌های پلی‌اتیلنی فعال سبب کاهش سرعت رشد باکتری‌های مزوفیل و کپک‌ها و مخمرها در پنیرها گردید. در پایان دوره انبارمانی، کمترین تعداد کپک‌ها و مخمرها و بار میکروبی در نمونه‌های بسته‌بندی شده در فیلم‌های حاوی ۲٪ منتول و کارواکرول مشاهده شد. از لحاظ آلودگی *اشرشیاکلی* و *استافیلوکوکوس اورئوس*، نمونه‌های بسته‌بندی شده در فیلم‌های فعال تا روز آخر انبارمانی (روز سی‌ام) ایمن بودند، ولی نمونه شاهد در روز هفدهم غیرقابل قبول بود. بسته‌بندی پنیر موزارلا در فیلم‌های پلی‌اتیلنی حاوی سطوح مختلف منتول و کارواکرول همچنین موجب بهبود مشخصات پذیرش حسی پنیرها طی دوره انبارمانی در یخچال گردید. در نهایت نتایج حاصله نشان داد که فیلم‌های پلی‌اتیلنی پوشش‌دهی شده با محلول‌های حاوی ترکیبات فعال منتول و کارواکرول، مخصوصاً در غلظت ۲٪، تأثیر مثبتی بر توسعه دوره ماندگاری پنیر موزارلا داشت.