Evaluation of Microbial and Sensory Properties of Jug Cheese Packed in the Biodegradable Film of Polyvinyl Alcohol and Pinto Bean Starch-Containing Garlic, Ginger, and Cinnamon Essential Oils

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ABSTRACT: *Biodegradability and antimicrobial activity of food packaging materials are the most important parameters of modern food packaging industries. Therefore, the present study aimed to use a biodegradable film of polyvinyl alcohol and pinto bean starch-containing cinnamon, garlic, and ginger essential oils to increase the shelf life and reduce the microbial load of the jug cheese and compared with conventional packaging. For this purpose, jug cheese in the biodegradability film of polyvinyl alcohol/pinto bean starch (20/80%) respectively and dissolved in distilled water for 60 minutes at 90 °C was heated at 400 rpm stir. T10% glycerol was added to the filtered solutions on a magnetic stirrer (400 rpm) for 30 minutes at 37 °C to obtain a completely homogeneous solution. To make an antimicrobial biocomposite, three concentrations of each of the essential oils containing different concentrations (3.125, 6.25, and 12.5%) of cinnamon, garlic, and ginger The prepared films were poured into Teflon containers with a diameter of 15 cm and exposed to ambient temperature for 24 hours. The dried film layers were removed from the Teflon surface and stored in a zip kept at 25 °C until their microbial and sensory properties were evaluated during 60 days of storage at 4 ° C and compared with the control sample. The results showed that the use of biodegradable film containing essential oil significantly reduced the microbial load in jug cheese samples. Total count of microorganisms, amount of Staphylococcus aureus, coliform, mold, and yeast in samples of jug cheese packed in a biodegradable film containing 6.25 and 12.5% cinnamon essential oil and 12.5% ginger essential oil after 60 days of storage was within the acceptable national standard. The highest general acceptance score among the acceptable samples of national standard belonged to the sample of jug cheese packed in a biodegradable film containing 6.25% cinnamon essential oil. The use of biodegradable films based on pinto bean starch and polyvinyl alcohol along with cinnamon and ginger essential oils are solutions that can reduce the microbial load increase the safety of food products during storage and prevent environmental damage.*

KEYWORDS: *Biodegradable film; Jug cheese; Essential oil; Garlic; Ginger; Cinnamon.*

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INTRODUCTION

Due to the problems of plastic packaging for the environment, edible and biodegradable films with antimicrobial properties have expanded and can be effective in controlling chemical and microbial properties in food. Antimicrobials added to biodegradable films containing antimicrobial compounds slowly migrate from the film layer into the food and begin their activity against contaminating microorganisms [1].

Plant essential oils are an important group of natural antimicrobial compounds that have great potential for use in a variety of foods to combat pathogenic and spoilage microorganisms. These compounds are volatile and aromatic extracts obtained from various parts of plants, including flowers, seeds, buds, leaves, and roots. Among plant essential oils, cinnamon, garlic, and ginger essential oils have been used successfully for food preservation many times due to their known antimicrobial and antioxidant properties [2].

Garlic with the scientific name *Allium Sativum* is a plant belonging to the *Liliaceae* family and is native to Central Asia. However, this plant is found all over the world today. Garlic has antibacterial, antiviral, antifungal, antioxidant, and anti-inflammatory properties and has been identified as a potent antibacterial agent. Its main antimicrobial agent is an organosulfur compound called thio-2-propene 1-sulfonic acid S-allyl ester, which is called allicin [3]. Diallyl tetrasulfide is the main sulfur compound composed of allicin and the most important antibacterial compound of garlic essential oil [4].

Cinnamon, scientifically known as *Cinnamomum zeylanicum*, was a *Lauraceae* shrub of the *Laurales* order, [5]. Its compounds include cinnamic acid, phenolic compounds such as eugenol, Flandre, and safrole, tertiary compounds such as limonene and linalool, trans-cinnamaldehyde, tannin, coumarin, resin, phenyl propionic compounds such as hydroxy-cinnamaldehyde and mannitol, which have a sweet taste due to mannitol [6]. Important ingredients of cinnamon include cinnamic aldehyde 65-80% and eugenol 5-10%, which has the most antibacterial effect related to cinnamic aldehyde, and the oil in it has antibacterial properties [7]. The Cinnamon essential oil has been recognized by many researchers as a suitable source of antifungal and bacterial compounds [8]. Inhibitory effects of cinnamon peel essential oil on pathogenic and spoilage bacteria of meat (*Pseudomonas fluorescens, Escherichia coli, Lactobacillus sakei, Lactobacillus Plantarum, Listeria monocytogen*) under special and controlled conditions were studied by the Ojagh and colleagues in 2012 and the results showed that cinnamon essential oil and its main antibacterial compounds have a high potential for use as a natural food preservative [9]. Also, in 2014, *Han et al*., after examining the oral film of polypropylene-polyvinyl alcohol with different concentrations of rhubarb ethanolic extract and cinnamon essential oil for preserving fresh beef, stated that this oral film prevents the growth of bacteria [10].

Ginger is obtained from a yellow plant with purple veins called *zingiber Officinale*. Sesquiterpenoids and monoterpenoids as the main compounds in ginger essential oil are essentially phenolic compounds that have antimicrobial activity [11].

Traditional Iranian cheeses, despite their high microbial load, are one of the most widely used dairy-fermented products produced in Iran. These cheeses are traditionally made from the raw milk of sheep, goats, or cows without the use of cultivation cheese. Most traditional cheesemakers believe that the use of raw milk creates a pleasant aroma and taste in the cheese, which is due to the activity of proteolytic and lipolytic enzymes in milk produced by the microbial flora of raw milk. Jug cheese is one of the most popular and widely consumed cheeses in the northwestern provinces of the country, It is traditionally produced from raw milk and its high microbial load can transmit pathogenic bacteria to consumers [12].

The constant efforts and information of environmentalists about the bio-pollution of polymers and the need for their safety introduce new consumption patterns, which include the packaging of products with biodegradable materials. Packaging food with biodegradable films containing natural antimicrobial compounds can also be a new approach to reducing the microbial load of food during storage.

Abdolsattari et al., (2016) examining the active packaging of Lighvan cheese with polyethylene nanocomposite films containing silver nanoparticles, stated that after 60 days of storage the growth rate of coliform and *Staphylococcus aureus* in fresh cheese, using a composite film containing equal percentages of silver, copper oxide, and zinc oxide is significantly less ($P \leq 0.05$) than other films [13].

Other researchers as a result of their research pointed to the antibacterial and antifungal properties of nanoparticles Ag NPs [14] and Fe_2O_3, NiO, and CoO NPs [15] which can be used in the formulation of biodegradable films.

The overall purpose of this study was to produce a biodegradable film of polyvinyl alcohol and pinto bean starch-containing cinnamon, garlic, and ginger essential oils to increase shelf life and reduce the microbial load of the jug cheese compared with conventional packaging.

EXPERIMENTAL SECTION

Materials

To prepare the film, pinto beans from a local market in Tehran (Tehran, Iran), garlic essential oils, cinnamon, and ginger from Adonis (Tehran, Iran), PVA pellets (98% purity) from GC (Guangdong, China), and dimethyl sulfide, Tryptone Soya Agar (TSA) and Müller-Hinton (MHB) broth were provided by Sigma-Aldrich (London, UK). Other chemicals and reagents were purchased from Merck Chemical (Darmstadt, Germany).

Jug cheese was prepared from the local market in Qazvin. The culture media used are Kant agar plate, Bird-Parker Agar (containing egg yolk and potassium tellurite), VRBA, EC Broth, YGC Agar, which were purchased from Ibersco (Iran).

Method of preparing research samples

Khazaei et al., (2020) showed that biodegradable films based on polyvinyl alcohol and pinto bean starch mixed in proportions of 80% to 20% had the desired physical and mechanical properties as follows: Thickness 66.4 µm, water vapor permeability 0.29 (g.mm/(k.Pa.h.m^2), solubility 58%, water absorption capacity 297%, turbidity 24.7 (AUnm), tensile strength 43.2 (MPa) and increase in length to the point of tension 198.9% [16].

These films were prepared using the casting method (wet method) proposed by *Jayakumar et al*., (2019) with some minor modifications as follows:

To prepare the composite film, the ratio of polyvinyl alcohol to pinto bean starch was mixed in 80 to 20% (PV-PB (80:20)) respectively and dissolved in distilled water for 60 minutes at 90 °C was heated at 400 rpm stir. To remove impurities and bubbles, the solutions were aerated under a vacuum pump and then passed through the Whatman 3 filter paper. 10% glycerol was added to the filtered solutions on a magnetic stirrer (400 rpm) for 30 min at 37 °C to obtain a completely homogeneous solution. To make an antimicrobial biocomposite, three concentrations of each of the essential oils of garlic, ginger, and cinnamon (3.125, 6.25, and 12.5%) were added to the mixed film. Tween 80 was used as an emulsifier (surfactant) to mix the essential oil well in the composite film. The film solution was homogenized using an Ultra Turrax homogenizer for 5 minutes at a stirring rate of 15,000 rpm. The prepared films were poured into Teflon containers with a diameter of 15 cm and exposed to ambient temperature for 24 hours. The dried film layers were removed from the Teflon surface and stored in a zip kept at 25 °C until the films were evaluated [17].

The concentrations of essential oils were determined based on the amount of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) results published in another study [18] after pre-treatment. This film was used to pack jug cheese. The jug of cheese prepared from the market was packaged by the mentioned film by observing the sterile conditions and under the hood by a hand-made heat press (heating element). To evaluate the antimicrobial efficacy of biodegradable films containing essential oils of garlic, ginger, and cinnamon, a sample of jug cheese was packed in ordinary polyethylene nylon and considered as a control. Microbial properties of packaged cheese samples were performed on the first, 30, and 60 days of storage, and sensory evaluation was performed on cheese samples at 30 and 60 days at 4 °C with three replications.

Microbial tests

The total count of microorganisms following Iranian National Standard No. 5484 was performed on Kant agar plate culture medium at 30 ℃ for 72 hours [19].

Staphylococcus aureus coagulase-positive count according to Iranian National Standard No. 1-6806 was performed on Bird-Parker Agar culture medium plus egg yolk and potassium tellurite at 37 ° C for 48 hours [20].

The counting of coliforms was performed according to the national standard of Iran, number 11166, on VRBA environment and by a two-layer Pure pallet method at 35° C for 18 to 24 hours [21].

Escherichia coli was counted according to Iranian National Standard No. 5234 on EC Broth culture medium at 35 ° C for 23 hours [22].

Mold and yeasts were counted according to the Iranian National Standard No. 10154 on the culture medium, YGC Agar for 5 days at 25 ° C [23].

Sensory evaluation tests

Sensory properties (taste, texture, smell, color, and total acceptance) of 5-point hedonic packed cheese samples were performed by 10 semi-trained evaluators. Panelists rated 5 for very excellent samples, 4 for excellent samples, 3 for good samples, 2 for acceptable samples, and 1 for unacceptable samples [24]. It should be noted that to observe the safety of the evaluators, before performing the sensory evaluation test, the cheese samples were completely sterilized by 5 kGy gamma irradiation.

Data analysis method

Microbial tests were performed on the treatments with 3 replications. The test results were analyzed by Duncan's one-way analysis of variance with 95% confidence by Minitab16 software.

RESULTS AND DISCUSSION

Examining the results of total counting of microorganisms

Evaluation of changes in the total count of microorganisms in jug cheese samples packed in the optimal biodegradable film based on 20% pinto bean starch and 80% polyvinyl alcohol-containing different concentrations of cinnamon, garlic, and ginger essential oils and comparing it with the control sample in Table 1 was given. The results showed that the total number of microbes in all treatments decreased significantly with increasing storage time and increasing the concentration of essential oils in biodegradation films ($P\leq 0.05$). The lowest total count (1.328 Log cfu/g) after 60 days of storage belonged to the jug cheese sample packed in a biodegradable film of cheese containing 12.5% cinnamon essential oil and the highest total count (5.222 Log cfu /g) belonged to the control treatment which was packed in polyethylene nylon.

The results showed that biodegradable films containing cinnamon essential oil were more effective in reducing the microbial count of microorganisms in jug cheese during the storage period than biodegradable films containing garlic and ginger essential oil.

According to National Standard No. 2406 [25], the highest total count of microorganisms in pasteurized milk cheeses should have a maximum of 10^3 cfu/g, so the total amount of microorganisms in samples containing 6.25% and 12.5% Cinnamon essential oil and 12.5% ginger essential oil were within the standard allowable range after sixty days of storage.

The reason for the decrease in the total count of microorganisms in cheese samples may be related to the essential oils used in the biodegradable film along with the production of acids and the reduction of pH during the processing period. The reason for the difference in the total count among the samples of jug cheese can be related to the difference in the number of antimicrobial compounds of different essential oils such as eugenol and especially cinnamic aldehyde in cinnamon.

The most common compounds involved in inhibiting essential oils are their phenolic compounds. Aromatic phenolic compounds such as thymol and carvacrol have been identified in marjoram and thyme, eugenol in cloves and cinnamon, and cinnamic aldehyde in cinnamon. These compounds sensitize the cell's phospholipid bilayer membrane, increasing the permeability and leakage of essential intracellular components (such as iron, ATP, nucleic acids, and amino acids), or damaging the bacterial enzyme system [26]. In confirmation of the results of this study, *Hasas et al*., (2019) produced low-fat jug cheese containing beta-glucan powder and ethanolic extract of oregano and reported that the total count of jug cheese samples decreased with increasing concentration of ethanolic extract of oregano. The reason for this decrease was attributed to the presence of antimicrobial compounds such as Polygon 1 and 8 Synthol in the ethanolic extract of peppermint [12].

Evaluation of Staphylococcus aureus count results

Evaluation of *Staphylococcus aureus* count changes in jug cheese samples packed in an optimal biodegradable film based on 20% pinto bean starch and 80% polyvinyl alcohol-containing different concentrations of cinnamon, garlic, and ginger essential oil and comparison with control sample was given in Table 1. The results showed that the count of *Staphylococcus aureus* in all treatments decreased significantly with increasing storage time and increasing the concentration of essential oils in biodegradable films ($P\leq 0.05$). The lowest rate of *Staphylococcus aureus* (0 Log cfu/g) after 60 days of storage belonged to the samples of jug cheese packed in a biodegradable film that contained 12.5% essential oil

Table 1: Evaluation of changes in the total count of microorganisms (Log cfu /g) and Staphylococcus aureus (Log cfu/g) jug cheese packed in the potential biodegradable film based on 20% pinto bean starch and 80% polyvinyl alcohol-containing different concentrations of cinnamon, garlic, and ginger essential oils and its comparison with a control sample

Sample	Total count of microorganisms (Log cfu/g)			*Staphylococcus aureus* (Log cfu/g)		
	Day one	Day 30	Day 60	Day one	Day 30	Day 60
Polyethylen^{e} (control)^{1}	5.222± 0.007^{aA}	3.670 ± 0.210^{dA}	4.814± 0.065^{aB}	3.279± 0.020^{aA}	1.977± 0.019^{aB}	0.950± 0.050^{aC}
Pva 80/20 starch (film)^{2}	5.219± 0.014^{aA}	3.910 ± 0.180^{dA}	4.522 ± 0.052^{bB}	3.279± 0.025^{aA}	1.859± 0.030^{aB}	0.933 ± 0.028 ^{bC}
Film + Garlic 3.125	5.218± 0.018^{aA}	2.840 ± 0.270^{eA}	4.111 ±0.016^{Cc}	3.278± 0.006^{aA}	1.545± 0.011^{bB}	0.660±0.50^{bC}
Film + Garlic 6. 25	5.203 ± 0.026^{aA}	2.110 ± 0.24^{fB}	3.927±0.026^{DC}	3.272± 0.027^{aA}	1.223± 0.006^{cB}	0.356±0.098^{CC}
Film + Garlic 12. 5	5.213± 0.010^{aA}	1.680 ± 0.19^{fB}	3.490± 0.042^{Ec}	3.259± 0.019^{aA}	0.916± 0.028^{eB}	0.000± 0.000^{dc}
Film + Ginger 3.125	5.212± 0.019^{aA}	4.550 ± 0.17^{abcA}	3.865±0.016^{dC}	3.283± 0.009^{aA}	1.260±0.09^{CB}	0.413±0.098^{cC}
Film + Ginger 6.25	5.206± 0.015^{aA}	4.510 ± 0.160^{abcA}	3.259 ±0.028^{fC}	3.266± 0.021^{aA}	0.856± 0.075^{DB}	0.000 ±0.000^{dC}
Film + Ginger 12. 5	5.197±0.011^{aA}	4.020 ± 0.110^{bcdA}	2.581±0.070^{gC}	3.263±0.015 ^{aA}	0.256± 0.238^{eB}	0.000 ±0.000^{db}
Film + Cinnamon 3.125	5.218± 0.007^{aA}	4.650± 0.150^{abcA}	3.138±0.050^{fC}	3.277± 0.013^{aA}	0.933±0.028^{dB}	0.356±0.098^{cC}
Film + Cinnamon 6. 25	5.186± 0.018^{aA}	4.510±0.150^{abcA}	2.250±0.023^{hC}	3.267± 0.006^{aA}	0.413±0.098^{eB}	0.000±0.000^{dC}
Film + Cinnamon 12. 5	5.191± 0.001^{aA}	3.980±0.120 ^{cdA}	1.328±0.030^{iC}	3.258± 0.014^{aA}	0.000± 0.000^{fB}	0.000±0.0000^{dB}

1) Control: Jug cheese packed in polyethylene nylon.

2) Films: a biodegradable film based on 80% polyvinyl alcohol and 20% pinto bean starch

Results are shown as mean standard deviation.

The lowercase letters indicate a significant difference in each column.

Different uppercase letters indicate a significant difference in each row.

(cinnamon, ginger, and garlic) and 6.25% essential oil (ginger and cinnamon) and the highest count of *Staphylococcus aureus* (0.950 Log cfu/g) belonged to the control treatment, which was packed in polyethylene nylon.

The reason for the decrease in the number of *Staphylococcus aureus* in all samples of jug cheeses during storage can be the presence of salt, decrease in pH, increase in acidity, and growth of lactic acid-producing bacteria in all treatments, which has limited the growth of *Staphylococcus aureus* coagulase positive.

The results showed that biodegradable films containing cinnamon essential oil were more effective than biodegradable films containing garlic and ginger essential oil in reducing the count of *Staphylococcus aureus* in jug cheese during the storage period. This can be due to the presence of antimicrobial compounds such as cinnamic aldehyde in a cinnamon essential oil [7] compared to antimicrobial compounds in garlic essential oil such as diallyl tetrasulfide [4] and ginger essential oil such as sesquiterpenoids and monoterpenoids [11] have had a greater effect on reducing the growth of *Staphylococcus aureus* coagulase positive.

According to National Standard No. 2406, the maximum permissible level of *Staphylococcus aureus* coagulase positive in grams of pasteurized milk cheeses must be negative [25]. Therefore, the amounts of *Staphylococcus aureus* coagulase positive except in samples of jug cheese packed in biodegradable films containing 12.5% cinnamon, garlic, and ginger essential oil and also samples containing 6.25% ginger and cinnamon essential oil after 60 days of storage within the permitted range was not standard.

In a study of *Staphylococcus aureus* coagulase-positive bacteria in a variety of dairy products in the United Kingdom, the results emphasized that by creating the right hygienic conditions, the growth of this bacterium can be prevented, and cheeses made from raw milk has a higher content of *Staphylococcus aureus* than cheeses made from pasteurized milk [27].

Mahmoudi et al., (2010) stated that oregano essential oil in the brain and heart infusion culture medium (BHI) at 0.015 and 0.03 inhibits the growth of *Staphylococcus aureus* in Iranian white cheese [28]. *Akhundzadeh Basti et al.*, (2007) stated that Shirazi multiflora essential oil in

Table 2: Evaluation of changes in coliforms (Log cfu/g) and mold and yeast (Log cfu/g) jug cheese packed in the optimal biodegradable film based on 20% pinto bean starch and 80% polyvinyl alcohol-containing different concentrations of cinnamon, garlic, and ginger essential oils and comparing it with the control sample.

Sample	coliforms (Log cfu/g)			Mold and yeast (Log cfu/g)		
	Day one	Day 30	Day 60	Day one	Day 30	Day 60
Polyethylen (control)[1]	3.113± 0.011[aA]	2.246± 0.011[bB]	1.288±0.011[aC]	2.920± 0.021[abA]	2.583± 0.012[aB]	2.374±0.008[aC]
Pva 80/20 starch (film)[2]	3.103± 0.013[aA]	2.797± 0.582[aA]	1.150± 0.012 [bB]	2.935± 0.027[aA]	2.550± 0.034[aB]	2.326± 0.033[aC]
Film + Garlic 3.125	3.108± 0.008[aA]	1.786± 0.036[bB]	0.971±0.19[cC]	2.911± 0.029[abA]	2.185± 0.014[bB]	1.726±0.25[bC]
Film + Garlic 6. 25	3.100±0.005[aA]	1.060± 0.013[cB]	0.500±0.027[CC]	2.893±0.0135[abcA]	2.065± 0.030[cB]	1.541±0.041[CC]
Film + Garlic 12. 5	3.096± 0.012[aA]	0.686± 0.085[cdeB]	0.000± 0.000[ec]	2.859± 0.018[abcA]	1.680± 0.023[fB]	1.335± 0.027[dec]
Film + Ginger 3.125	3.106± 0.008[aA]	1.085±0.007[CB]	0.586±0.110[dC]	2.911± 0.022[acA]	1.904±0.052[dB]	1.446±0.053[cdC]
Film + Ginger 6.25	3.103± 0.005[aA]	0.716± 0.046[cdeB]	0.000 ±0.000[eC]	2.864± 0.038[abcA]	1.664 ±0.036[fB]	1.276 ±0.049[eC]
Film + Ginger 12. 5	3.093±0.015[aA]	0.413± 0.098[defB]	0.000 ±0.000[eb]	2.853±0.049[bcA]	1.246± 0.030[hB]	0.966 ±0.028[fC]
Film + Cinnamon 3.125	3.113± 0.098[aA]	0.916±0.028[cdB]	0.00±.0.000[eC]	2.886± 0.016[abcA]	1.782±0.011[eB]	1.259±.0.016[eC]
Film + Cinnamon 6. 25	3.100± 0.003[aA]	0.356±0.098[efB]	0.000±0.000[eC]	2.867± 0.009[abcA]	1.366±0.040[gB]	1.040±0.069[FC]
Film + Cinnamon 12. 5	3.094± 0.010[aA]	0.000± 0.000[fB]	0.000±0.0000[eB]	2.828± 0.016[bcA]	0.983± 0.028[iB]	1.356±0.098[Gc]

1) Control: Jug cheese packed in polyethylene nylon.
2) Films: a biodegradable film based on 80% polyvinyl alcohol and 20% pinto bean starch
Results are shown as mean ± standard deviation.
The lowercase letters indicate a significant difference in each column.
Different uppercase letters indicate a significant difference in each row.

concentrations above 0.005% inhibit the growth of *Staphylococcus aureus* in feta cheese [29].

Evaluation of the results of coliform changes

Evaluation of changes in coliforms in jug cheese samples packed in an optimal biodegradable film based on 20% pinto bean starch and 80% polyvinyl alcohol-containing different concentrations of cinnamon, garlic, and ginger essential oil and comparing it with the control sample has been shown in Table 2. The results showed that the number of coliforms in all treatments decreased significantly with increasing storage time and increasing the concentration of essential oils in biodegradable films ($P \leq 0.05$). The highest number of coliforms was observed after 60 days of storage in the control treatment (1.288 Log cfu/g). The lowest number of coliforms (0 Log cfu /g) was observed after 60 days of storage in jugs of cheese packed with a biodegradable film containing 12.5% cinnamon, garlic, and ginger essential oil, and 6.25% of ginger and cinnamon essential oil, and 3.125% of cinnamon essential oil.

Since according to the national standard No. 2406 [25], the maximum allowable number of coliforms in cheeses made from pasteurized milk is 10 cfu/g, so the number of coliforms in all samples of jug cheese after sixty days of storage was within the standard range.

This can be due to the antimicrobial effect of cinnamon, ginger, and garlic essential oils, lowering the pH, the presence of salt, and the growth of acid-producing bacteria in all treatments, which has limited the growth of coliforms. Among the mentioned essential oils (cinnamon, ginger, and garlic), cinnamon essential oil is more effective in preventing the activity of coliforms. The amount of coliform in the cheese sample packed in a biodegradable film containing 12.5% cinnamon essential oil after 30 days of storage at 4 ° C was zero.

The antibacterial activity of essential oils has been attributed to volatile compounds [26]. Among the components of cinnamon essential oil, cinnamaldehyde has the strongest antibacterial activity, which is mainly due to the presence of a benzyl ring and a double bond attached to it. For this reason, between different cinnamon aldehyde derivatives, the effect of cinnamaldehyde is> cinnamic acid> cinnamyl alcohol> cinnamyl acetate. The aldehyde group seems to have the highest antibacterial properties [30].

Numerous reports have shown that gram-positive bacteria are more sensitive to antibacterial compounds than gram-negative bacteria, and this high susceptibility to gram-positive bacteria is due to the lack of a lipopolysaccharide cell wall that this wall in gram-negative bacteria may prevent active compounds from entering the cytoplasmic membrane [31].

Resistance of gram-negative bacteria to antibacterial agents with a hydrophilic surface of the outer membrane of bacteria, which is rich in lipopolysaccharide molecules and provides a barrier against the penetration of various antibiotic molecules and also with peripheral space enzymes (Which can break down imported molecules) is also related. Gram-positive bacteria do not have such an outer membrane in the cell wall structure [26].

Vaziri and *Norouzi* (2011) stated that the contamination of fresh cheese samples with coliforms is high. Naturally, the activity of coliforms stops at pH< 1.5, so the activity of this bacterium was possible in the initial stages and before the acidification of the environment. Decreasing the pH and increasing the percentage of salt are effective factors in reducing the number of these bacteria. However, *Escherichia coli*, which is one of the most resistant species of coliforms, can survive under these conditions [32].

Hasas et al., (2019) stated that the use of the ethanolic extract of peppermint and increasing its concentration had a significant effect on increasing the antimicrobial properties of the tested treatments. According to the results, after sixty days of storage, the highest number of coliforms in the control treatment and the lowest number of coliforms (0 log cfu/g) in samples containing 0.2 and 0.3% ethanolic extract of peppermint, which is consistent with the results of this study [12].

Hayaloglu et al., (2008) also mentioned the antimicrobial effect of using plant powders in Otlu cheese and their results showed that reducing foodborne pathogens, especially coliforms, using aromatic plant powders (thyme, garlic, mint, cumin, black pepper) which are consistent with the present results [33].

Evaluation of the results of mold and yeast changes

Evaluation of mold and yeast changes in jug cheese samples packed in the optimal biodegradable film based on 20% pinto bean starch and 80% polyvinyl alcohol-containing different concentrations of cinnamon, garlic, and ginger essential oils and comparison with the control sample in Table 2 has been shown. The results showed that with increasing storage time and increasing the concentration of essential oils in biodegradable films, any amount of mold and yeast (Log cfu/g) in all treatments of jug cheese had a significant decrease ($p \leq 0.05$). The highest amount of mold and yeast (2.374 Log cfu /g) after 60 days of storage belongs to the control cheese sample packed in polyethylene and the lowest amount of mold and yeast (0.356 Log cfu /g) belongs to the jug cheese sample packed in the optimal biodegradable film contained 12.5% cinnamon essential oil.

The main ingredient in cinnamon oil is cinnamaldehyde, which is a compound containing an aldehyde group and a conjugated double bond located outside the ring. This compound has very strong antifungal activity and this may be a potential compound for the production of antifungal drugs by controlling the β- (1-3) synthesis of glucan and chitin in yeasts and fungi [34].

According to the national standard of Iran No. 2406 [25], the maximum permissible limit for mold and yeast in all types of cheese is 10^2 cfu/g. The amount of mold and yeast in all samples of jug cheese packed in biodegradable films containing essential oil (garlic, ginger, and cinnamon) was within the standard allowable range after 60 days of storage at 4 °C. The amount of mold and yeast in the samples of jug cheese packed in control polyethylene film and biodegradable film that did not contain essential oil after 60 days of storage was higher than the national standard.

Oily essential oils increase permeability and disrupt the membrane integrity of microorganisms and destroy them [35]. *Hasas et al*., (2019) used the ethanolic extract of oregano in the formulation of jug cheese and reported that the amount of mold and yeast in the mentioned samples compared to the control sample which did not have the extract was significantly reduced [12].

Sensory evaluation (texture, color, smell, taste, and general acceptance) of jug cheese

Evaluation of texture changes in jug cheese samples packed in the biodegradable optimal film based on 20% pinto bean starch and 80% polyvinyl alcohol-containing different concentrations of cinnamon, garlic, and ginger essential oils and comparing it with the control sample have been shown in Table 3. The texture score in all

Table 3: Evaluation of score changes in texture and taste of jug cheese packed in the optimal biodegradable film based on 20% pinto bean starch and 80% polyvinyl alcohol-containing different concentrations of cinnamon, garlic, and ginger essential oils and comparing it with the control sample during the period of storage.

sample	Texture score changes		Taste score changes	
	Day 30	Day 60	Day 30	Day 60
Polyethylen (control) [1]	4.52 ± 0.16^{aA}	4.51 ± 0.17^{aA}	3.750 ± 0.220^{abcA}	3.820 ± 0.260^{abA}
Pva 80/20 starch (film) [2]	4.53 ± 0.19^{abA}	4.50 ± 0.12^{aA}	3.81 ± 0.260^{abcA}	3.920 ± 0.160^{abA}
Film + Garlic 3.125	4.17 ± 0.22^{abA}	4.01 ± 0.15^{bcdA}	3.510 ± 0.520^{bcdA}	3.110 ± 0.430^{bcA}
Film + Garlic 6. 25	4.05 ± 0.15^{abA}	3.85 ± 0.16^{cdA}	3.160 ± 0.420^{cdA}	2.540 ± 0.380^{cdA}
Film + Garlic 12. 5	3.85 ± 0.15^{abA}	3.55 ± 0.21^{dA}	2.800 ± 0.280^{dA}	1.940 ± 0.340^{dB}
Film + Ginger 3.125	4.58 ± 0.19^{bA}	4.51 ± 0.19^{aA}	4.150 ± 0.350^{abA}	4.380 ± 0.190^{aA}
Film + Ginger 6.25	4.61 ± 0.17^{aA}	4.54 ± 0.14^{aA}	4.580 ± 0.190^{aA}	4.510 ± 0.280^{aA}
Film + Ginger 12. 5	4.48 ± 0.19^{aA}	4.20 ± 0.13^{abcA}	4.320 ± 0.16^{abA}	3.920 ± 0.350^{abA}
Film + Cinnamon 3.125	4.56 ± 0.27^{aA}	4.48 ± 0.16^{abA}	4.450 ± 0.25^{aA}	4.620 ± 0.190^{aA}
Film + Cinnamon 6. 25	4.61 ± 0.230^{aA}	4.54 ± 0.20^{aA}	4.350 ± 0.280^{abA}	4.410 ± 0.230^{aA}
Film + Cinnamon 12. 5	4.28 ± 0.35^{abA}	4.11 ± 0.18^{abcA}	3.920 ± 0.280^{abcA}	3.950 ± 0.270^{abA}

1) Control: Jug cheese packed in polyethylene nylon.
2) Films: a biodegradable film based on 80% polyvinyl alcohol and 20% pinto bean starch
Results are shown as mean ± standard deviation.
The lowercase letters indicate a significant difference in each column.
Different uppercase letters indicate a significant difference in each row

samples of jug cheese decreased slightly during 60 days of storage, but this decrease was not statistically significant ($P\leq 0.05$), so the highest texture score was observed in the control cheese sample packed in polyethylene (4.50) and the lowest texture score was observed in the sample of jug cheese packed in the optimal biodegradable film containing 12.5% of garlic essential oil (3.550).

The results showed that increasing storage time, the concentration of essential oils, and the type of packaging (polyethylene and biodegradable film) had no significant effect on color score changes o treatments ($P\leq 0.05$), and the color score was 5.

Evaluation of changes in taste scores of jug cheese packed in an optimal biodegradable film based on 20% pinto bean starch and 80% polyvinyl alcohol-containing different concentrations of cinnamon, garlic, and ginger essential oil and comparing it with the control sample during storage have been shown in Table 3.

Evaluation of changes in smell scores of jug cheese packed in an optimal biodegradable film based on 20% pinto bean starch and 80% polyvinyl alcohol-containing different concentrations of cinnamon, garlic, and ginger essential oil and comparing it with the control sample during storage have been shown in Table 4.

With increasing storage time, the amount of smell and taste scores in the samples of jug cheese packed in polyethylene, a film without essential oil, and a biodegradable film containing cinnamon essential oil (3.125, 6.25, and 12.5%) increased. It was not statistically significant. The reason for this increase in taste and smell during storage can be related to the processes of proteolysis and lipolysis of jug cheese during storage. The development of flavor and taste in ripe cheeses is a complex process involving various microbial and biochemical activities that results in the production of a heterogeneous mixture of volatile and non-volatile compounds. In most cheeses, the process of ripping is accompanied by hydrolysis of triglycerides to free fatty acids with a chain length of more than 4 carbon atoms. Free fatty acids are considered as the precursors to the formation of many flavoring compounds such as alcohols, esters, aldehydes, ketones, and electrons. One of the biochemical reactions that play an important role in the formation of flavor and smell compounds in ripe cheeses

Table 4. Evaluation of changes in smell and total acceptance (score) of jug cheese packed in the optimal biodegradable film based on 20% pinto bean starch and 80% polyvinyl alcohol-containing different concentrations of cinnamon, garlic, and ginger essential oils and comparing it with the control sample during the period of storage.

Sample	Smell score changes		Total acceptance (score)	
	Day 30	Day 60	Day 30	Day 60
Polyethylen[1](control)	3.580 ± 0.240^{bcA}	3.670 ± 0.210^{dA}	3.850±0.170^{bA}	4.010± 0110^{cA}
Pva 80/20 starch[2] (film)	3.650± 0.210^{bcA}	3.910 ± 0.180^{dA}	4.020± 0.160^{abA}	4.220± 0.150^{bcA}
Film + Garlic 3.125	3.220 ± 0.170^{cdA}	2.840 ± 0.270^{eA}	3.150± 0.140^{cA}	2.950 ± 0.190^{dA}
Film + Garlic 6. 25	2.850 ± 0.22^{dA}	2.110 ± 0.24^{fB}	2.720± 0.350^{cA}	2.160 ± 0.240^{eA}
Film + Garlic 12. 5	2.150 ± 0.19^{eA}	1.680 ± 0.19^{fB}	2.110 ±0.180^{dA}	1.580± 0.320^{fA}
Film + Ginger 3.125	4.450 ± 0.37^{aA}	4.550 ± 0.17^{abcA}	4.420±0.270^{abA}	4.660± 0.180^{abA}
Film + Ginger 6.25	4.380 ± 0.26^{aA}	4.510 ± 0.160^{abcA}	4.300±0.120^{abA}	4.540± 0.140^{abcA}
Film + Ginger 12. 5	4.080 ± 0.23^{aA}	4.020 ± 0.110^{bcdA}	4.060±0.240^{abA}	4.110±0.200^{bcA}
Film + Cinnamon 3.125	4.420 ± 0.25^{aA}	4.650± 0.150^{abcA}	4.550±0.21^{aA}	4.720±0.130^{aA}
Film + Cinnamon 6. 25	4.240±0.172^{abA}	4.510±0.150^{abcA}	4.350±0.130^{abA}	4.510±2.121^{abcA}
Film + Cinnamon 12. 5	3.950±0.200^{abA}	3.980±0.120 ^{cdA}	4.060±0.170^{abA}	4.100± 0.110^{cA}

1) Control: Jug cheese packed in polyethylene nylon.
2) Films: a biodegradable film based on 80% polyvinyl alcohol and 20% pinto bean starch
Results are shown as mean ±standard deviation.
The lowercase letters indicate a significant difference in each column.
Different uppercase letters indicate a significant difference in each row

is proteolysis. Free amino acids and peptides resulting from the hydrolysis of milk proteins, in addition to being a direct cause of flavor, by participating in reactions such as deamination, decarboxylation, desulfurization, etc. also indirectly play a role in the formation of cheese flavor compounds [36].

The smell and taste score in jug cheese samples packed with a biodegradable film containing garlic essential oil (3.125, 6.25, and 12.5%) and ginger essential oil (12.5%) decreased during storage. This decrease in taste can be related to the intense aroma-producing compounds of garlic essential oil such as organic sulfur compounds such as thiosulfates, especially allicin (*Molaei et al*., 2016), and ginger essential oil such as sesquiterpenes and hydrocarbons (Zingiberrn, [T]-ar-curcumin, B-secquiphellanderene, B-bisabolene, monoterpenes, aldehydes, and alcohols) (*Sadeghi Mahani et al*., 2015) in high concentrations.

The highest score of smell (4.650) and taste (4.620) was observed in the sample of jug cheese packed in a biodegradable optimal film containing 3.125% cinnamon essential oil and the lowest score of odor (1.680) and taste (1.940) was observed in the sample of jug cheese packed in the optimal biodegradable film containing 12.5% of garlic essential oil.

Evaluation of changes in the total acceptance score of jug cheese packed in the optimal biodegradable film based on 20% pinto bean starch and 80% polyvinyl alcohol-containing different concentrations of cinnamon, garlic, and ginger essential oil and comparing it with the control sample during storage in have been shown in Table 4.

With increasing storage time, the overall acceptance score of jug cheese samples packed in polyethylene increased without essential oil and a biodegradable film containing cinnamon essential oil and ginger essential oil (3.125, 6.25, and 12.5%). The increase was not statistically significant.

The total acceptance score in jug cheese samples packed with a biodegradable film containing garlic essential oil (3.125, 6.25, and 12.5%) decreased during storage.

After 60 days of storage, the highest total acceptance score (4.720) was observed in the sample of jug cheese packed in the optimal biodegradable film containing 3.125% cinnamon essential oil, and the lowest total acceptance score (1.580) was observed in the sample of jug cheese packaged in the optimal biodegradable film containing 12.5% garlic essential oil. In general, the samples of cheese packed in films containing cinnamon essential oil and then ginger essential oil had a higher total acceptance score than films containing garlic essential oil, especially in higher concentrations.

Embuena et al., (2016) reported that no significant difference was observed between the taste of cream cheese coated with oregano essential oil and the control sample [37]. In another study, *Giani Pieretti et al*. (2019) coated fresh cheese with rosemary and peppermint essential oil and reported that samples of cheese coated with peppermint essential oil showed better organoleptic properties than rosemary essential oil. They attributed the result to people being more accustomed to the taste of peppermint [38].

Ksouda et al., (2019) reported that samples of Pimpinella saxifrage-coated cheese showed a higher flavor rating than uncoated cheeses [39].

CONCLUSIONS

In this study, jug cheese was packed with the biodegradable film of polyvinyl alcohol/pinto bean starch-containing cinnamon, garlic, and ginger essential oils and its microbial load and sensory properties were evaluated during 60 days of storage at 4 ° C and were compared with the sample of control jug cheese packed in polyethylene.

The use of essential oils and increasing their concentration in biodegradable film significantly reduced the microbial load of jug cheese samples during storage. Cinnamon essential oil and then ginger essential oil had the greatest effect on reducing the microbial load of jug cheese samples during the storage period.

The total count of microorganisms, *Staphylococcus aureus*, coliform mold, and yeast of jug cheese samples in biodegradable polyvinyl alcohol/bean starch containing 6.25 and 12.5% cinnamon essential oil and 12.5% ginger essential oil was within the acceptable national standard after 60 days of storage. The highest total acceptance score among the acceptable samples of national standard belonged to the sample of jug cheese packed in a biodegradable film containing 6.25% cinnamon essential oil. By using a biodegradable film containing 6.25% cinnamon essential oil, the microbial load of jug cheese can be significantly reduced during storage without damaging its sensory properties. Therefore, it is believed that the resulting environmentally friendly biodegradable film can be used as an excellent antimicrobial agent against microorganisms or as a pack of antimicrobial products.

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