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Original Research Article

Effect of *Zizyphus spina-christi* and *Cinnamomum zeylanicum* Essential Oils as Antimicrobial Agents on the Microbiological Characteristics of White Cheese

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Abstract: The purpose of this study is to assess the microbiological characteristics of white cheese supplemented with *Ziziphus spina-christi* and *Cinnamomum zeylanicum* essential oils during the storage period. The cheese was made from warmed (45°C) raw cow milk with *Ziziphus spina-christi* and *Cinnamomum zeylanicum* oils added, and after manufacture, the cheese was kept in sterile plastic bags at 4°C for 21 days, and microbiological tests were performed at 1, 7, 14 and 21-day intervals. Results showed that total viable bacteria (TVB), *Staphylococcus aureus*, and yeasts and moulds counts were significantly lower in cheese supplemented with both oils, according to the findings. The concentration of oil had a significant effect on the counts, with TVB, *S. aureus*, yeasts and moulds, and coliform bacteria counts being significantly higher in the control cheese and significantly lower in the cheese supplemented with 0.5% *Ziziphus spina-christi* oil. The storage period of cheese made with *Ziziphus spina-christi* and *Cinnamomum zeylanicom* oils had a significant effect on TVB, coliform bacteria, and *S. aureus* counts. The microbial counts were significantly affected by the concentrations of 0.3% and 0.5% *Ziziphus spina-christi* oil during the storage period, except for yeasts and moulds, which were unaffected by the 0.3% *Ziziphus spina-christi* oil. The counts of microbes in cheese supplemented with 0.3% and 0.5% *Cinnamomum zeylanicom* oils decreased significantly as the storage period progressed. The study concluded that essential oils, as natural antimicrobials, could be useful in preserving cheese made traditionally from raw milk without the addition of starter culture.

Keywords: *Ziziphus spina-christi*, *Cinnamomum zeylanicum*, essential oil, white cheese, microbiological characteristics, storage period.

INTRODUCTION

The dairy industry is improving the processing techniques to extend the shelf life and ensure the safety of milk while meeting consumers' needs and demands for more appealing and natural products [1]. Nonthermal preservation methods and natural antimicrobials have received a lot of attention due to the negative effects of thermal processing on the sensory and nutritional properties of food, as well as the potentially harmful effects of chemical preservatives [2]. Food safety is a major global health concern today, with approximately one-tenth of the world's population becoming ill as a result of contaminated foods, and nearly 420,000 people dying each year as a result of foodborne diseases [3]. Foods must be pathogen-free and protected against microbial spoilage during their shelf life [4]. As consumer demand for safe and natural products free of chemical preservatives has grown, research into alternative techniques for preserving microbiological quality while maintaining nutritional and sensory properties developed [4].

All steps of food production in the food industry are typically sterilized, but at the final step, where the food is packaged, it is frequently exposed to post-process surface contamination resulting in a reduction in shelf life [5]. The scientific evaluation of natural products' bioactive properties could be a solution not only to the spread of antimicrobial resistance but also as natural preservatives with added value in the food industry as a possible preservative against food spoilage as well as an antioxidant activity that could help in the retardation of the harmful effects that could be caused by oxidative stress [6]. Plants produce a variety of secondary metabolites, the majority of which have been reported as defensive products against pathogens, and some of these products have

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been found in specific plant organs during specific phenological periods of the plant [7]. Because of the misuse and mishandling of antibiotics, as well as increased consumer awareness of the potential negative impact of synthetic preservatives on health, the use of natural antimicrobial compounds in food has received much attention from consumers and the food industry [8]. Essential oils are used as an ingredient to improve the functionality of a variety of products, including foods, drinks, perfumes, pharmaceuticals, cosmetics, and green pesticides, due to their biological properties [9].

Ziziphus is a well-known genus for its medicinal properties as a hypoglycemic, hypotensive, anti-inflammatory, antimicrobial, antioxidant, antitumor, and liver-protective agent, as well as an immune system stimulant [10]. Many antibacterial compounds are found in some *Cinnamomum* species, which are commonly used as spices [11]. Cinnamaldehyde is an important component of cinnamon oils formed by aromatic plants of cinnamon, and this compound has been shown in vitro to have antimicrobial properties in laboratory media, as well as in animal feeds and human foods contaminated with pathogenic bacteria [12]. White cheese is a pickled cheese made traditionally in rural areas from cow milk without pasteurizing milk or adding starter cultures which can cause spoilage while being stored [13].

However, to our knowledge, there have been few studies on the antibacterial properties and potential mechanisms of *Ziziphus spina-christi* and *Cinnamomum zeylanicum* essential oils in white cheese. The purpose of this study is to determine the effect of essential oils of *Ziziphus spina-christi* and *Cinnamomum zeylanicum* as antimicrobial agents on the microbiological characteristics of white cheese during storage.

MATERIALS AND METHODS

Extraction of essential oil from *Ziziphus spina-christi*

Powdered, dried seeds of *Z. spina-christi* (300 gm) were macerated with n-hexane at room temperature for 48 hr. The solvent was removed under reduced pressure to obtain the oil, which was sterilized by filtration through 0.45 µm Millipore filters, and kept in the freezer (-18°C) until used [14].

Extraction of essential oil from *Cinnamomum zeylanicum*

Essential oil of *C. zeylanicum* was extracted from cinnamon barks using Clevenger apparatus. 100 gm of barks were mixed with 500 ml of distilled water and transferred into oil distillation apparatus at 90°C for 1-2 hr. The essential oil was collected, sterilized by filtration through 0.45 µm Millipore filters, and kept in the freezer (-18°C) until used [15].

Preparation of *Solanum dubium* extract

The whole seeds were coarsely powdered using an electric grinder, and 20 gm of the powder were soaked in 100 ml distilled water for 3 hr, followed by filtering, and 40 ml of the liquid were used for the coagulation of milk.

Cheese manufacture

Fresh raw milk (25 L) was warmed (45°C), followed by the addition of *Solanum dubium* enzyme extract (2 ml/L). The milk was stirred and left to develop a curd, and after 5 minutes the cured was tested by knife for coagulation. The curd was then cut for whey separation, and the essential oils were added aseptically to the curd as follows: control, to which no oil was added; 0.3% (v/w) of *Ziziphus spina-christi* oil; 0.5% (v/w) of *Ziziphus spina-christi* oil; 0.3% (v/w) of *Cinnamomum zeylanicom* oil; 0.5% (v/w) of *Cinnamomum zeylanicom* oil. The curd was mixed thoroughly to distribute the oil evenly and the curd was poured into a wooden mould lined with a clean sterile cloth and pressed overnight (2.5 kg weight). After pressing, the curd was removed from the mould, cut into small cubes (2x2x2 cm size), and immersed into salted (2% w/w), heat-treated (62°C/30 min) and cooled brine solution for 48 hr. The cheese was then transferred to plastic bags and stored without whey in the refrigerator at 4°C for 21 days. The microbiological examination was carried out at 1, 7, 14, and 21-day intervals. The analysis was carried out in triplicate.

Microbiological examination

Preparation of serial dilutions

For the preparation of cheese samples, 11 gm of cheese were weighed aseptically in a sterile mixer, and 99 ml of sterile peptone water were added and mixed for two minutes to make the first dilution (10^{-1}), followed by preparation of serial dilutions for up to 10^{-8} [16].

Total viable bacteria count

The plate count agar (Himedia, M091) was used for the enumeration of TVB. The plates were incubated in an inverted position at 32±1ºC for 48±3 hr, and the colonies were counted using a manual colony counter (scan 100) and recorded as cfu/gm [17].

***Staphylococcus aureus* count**

Mannitol salt agar (Micro master, DM160) was used for the enumeration for coagulase-positive staphylococci. The plates were incubated in an inverted position at 37ºC for 48 hr, and the typical colonies were counted with a manual colony counter (Scan 100) and regarded as cfu/gm [17].

Coliform bacteria count

MacConkey agar was used to determine the coliform count. The plates were incubated in an inverted position at 37ºC for 48 hours, and the typical

colonies were counted by a manual colony counter (Scan 100) and recorded as cfu/gm [18].

Yeast and moulds count

Yeast extract agar was used for the enumeration of yeasts and moulds. The plates were incubated in an inverted position at 25ºC for 5 days, and the colonies were counted by a manual colony counter (Scan 100) and recorded as cfu/gm [19].

STATISTICAL ANALYSIS

The statistical analysis was carried out using Statistical Analysis Systems (SAS, ver.9). The general linear model (GLM) procedure was used to determine the effect of type, concentration of oil, and the storage period on the microbiological characteristics of cheese. Duncan's multiple range test was conducted for mean separation between treatments (P≤0.05).

RESULTS AND DISCUSSION

Microbiological characteristics of cheese manufactured with *Ziziphus spina-christi* and *Cinnamomum zeylanicum* essential oils compared to the control cheese

According to the results in Table 1, all microorganisms under study had significantly higher counts in the control cheese, while cheese supplemented with *Ziziphus spina-christi* and *Cinnamomum zeylanicum* essential oils had the lowest count. When compared to control samples, the essential oils of *Ziziphus spina-christi* and *Cinnamomum zeylanicum* had antimicrobial activities against the microorganisms under study. These results agree with Nkafamiy et al. [20] who reported that *Zizphus spina-christi* extracts had inhibitory activity against various germination stages of

Table-1: Effect of type of oil on the microbiological characteristics (log_{10} cfu/gm) of white cheese (mean±SD)

Microorganisms	Type of oil			SL
	Control	*Ziziphus spina-christi*	*Cinnamomum zeylanicum*	
Total viable bacteria	8.73±0.93[a]	7.88±0.76[b]	7.52±0.61[b]	**
Coliform bacteria	6.98±1.06[a]	6.88±0.59[a]	6.85±0.73[a]	NS
Staphylococcus aureus	7.25±0.79[a]	6.62±0.83[b]	6.88 ±0.58[b]	*
Yeasts and moulds	8.31±0.68[a]	7.47±0.65[b]	7.45±0.51[b]	**

Means in each row bearing similar superscripts are not significantly different (p>0.05)
**=P<0.01; *=P<0.05; NS = Not significant
SL = Significance level
SD = Standard deviation

Staphylococcus aureus. Motamedi et al. [21] discovered significant antibacterial activity against *S. aureus*. According to Nasir et al. [22], *Cinnamomum zeylanicum* oil has antimicrobial properties against *Candidia albicans, Aspergillus niger*, and streptococci. Cinnamon oil exhibited high antibacterial activity against several bacteria in vitro, and its use in milk demonstrated its antibacterial properties [23]. The essential oil of *Ziziphus spina-christi* demonstrated more potent antimicrobial activity against all microorganisms tested, which was attributed to the absence of sugars present in its extracts such as lactose, glucose, galactose, arabinose, xylose, and rhamnose [14]. Sugars' mode of action was attributed to disruption of the cytoplasmic membrane, which disrupted the proton motive force, electrolyte flow, active transport, and coagulation of bacterial cell contents [24]. Because the essential oil components may have ATPase inhibiting activity, the bacterial cytoplasmic membrane is disrupted by increasing its non-specific permeability at bactericidal concentrations [25].

Effect of concentration of essential oil on the microbiological characteristics of cheese

TVB and *S. aureus* counts were significantly higher in the control cheese, and lower in cheese supplemented with 0.3% *Ziziphus spina-christi* essential oil, while coliform bacteria count was higher in cheese supplemented with 0.5% *Ziziphus spina-christi* essential oil and lower in cheese supplemented with 0.3% *Ziziphus spina-christi* essential oil and 0.5% *Cinnamomum zeylanicum* essential oil, and yeasts and moulds count was significantly (P<0.05) higher in the control cheese and lower in cheese supplemented with 0.5% *Cinnamomum zeylanicum* essential oil (Table 2). According to Nasir et al. [22], all test strains were sensitive to the action of cinnamon oil, and a concentration of 625.0 mg/ml was able to inhibit all microorganisms. Many researchers reported similar findings. At 1000 gm/ml concentrations, *Z. spina-christi* fruit oil showed promising activity against *Pectobacterium carotovorum* and *Dickeya solani* [26]. Cinnamon oil at a subinhibitory concentration (0.78%) inhibited the adhesion of *S. aureus*, *E. coli*, and *Salmonella enterica* to polystyrene surfaces, highlighting the potential application of essential oils as an alternative natural compound that can help to prevent bacterial adhesion on surfaces, extending shelf life and improving segregation [27]. The essential oil of cinnamon bark was found to have antibacterial activity against *E. coli* O157:H7, *Yersinia enterocolitica* O9, *Proteus* spp., and *Klebsiella pneumonia* [28].

Table-2: Effect of concentration of oil on the microbiological characteristics (log_{10} cfu/mg) of white cheese (mean±SD)

Type of oil	Concentration of oil (%)	Microorganisms			
		TVBC	Coliform bacteria	*S. aureus*	Yeasts and moulds
Control	0	8.73±0.93[a]	6.98±1.06[a]	7.25±0.79[a]	8.31±0.68[a]
Ziziphus spina-christi	0.3	7.01±2.75[d]	6.70±0.55[b]	6.57±0.88[b]	7.43±0.74[b]
	0.5	7.84±0.79[b]	7.06±0.58[a]	6.68±0.78[b]	7.52±0.54[b]
Cinnamomum zeylanicum	0.3	7.42±0.65[c]	7.00±0.81[a]	6.90±0.49[ab]	7.59±0.62[b]
	0.5	7.61±0.29[c]	6.70±0.60[b]	6.89±0.65[ab]	7.30±0.29[b]
SL		***	**	**	*

Means in each column bearing similar superscripts are not significantly different (p>0.05)
*** = P<0.001; ** = P<0.01; * = P<0.05
SL= Significance level
SD = Standard deviation

The essential oil of *Ocimum gratissimum* had significant fungistatic activity against all of the tested species at concentrations of 200, 400, 600, 800, and 1000 mg/L, and increasing the essential oil level reduced mycelial growth [29]. Coliform bacteria were not found in cheese fortified with 0.1% *Ocimum compactum* and *Thymus vulgari* essential oils on the first day of storage [30]. *Mentha spicata* essential oil at a concentration of 2% or 2.5% (v/w) significantly reduced the growth of *L. monocytogenes*, but there was no significant difference between the two concentrations used, and the number of *L. monocytogenes* was reduced as the essential oil level, salt water percentage, ripening temperature, and storage period were increased [31]. The essential oil of *Cuminum cyminum* L. and the probiotic bacteria had synergistic effects on decreasing the growth of *S. aureus* in Iranian white-brined cheese, with samples containing 30 µl//100 ml essential oil plus 0.5% probiotic bacteria showing the greatest inhibitory activity after 75 days of storage [32]. However, 0.04% cinnamon essential oil, alone or in combination with EDTA and/or polyethylene glycol, failed to demonstrate antimicrobial activity against aerobic mesophilic bacteria and yeasts and moulds in yogurt [4]. At lower concentrations, the essential oil of *Thymus vulgaris* did not affect the counts of *S. aureus* and *L. monocytogenes*, but at 2.5 µl/ml, the counts of *S. aureus* and *L. monocytogenes* decreased by 0.3 to 1 log_{10} cfu/gm after 72 hours of exposure [33].

Effect of the storage period on the microbiological characteristics of cheese

During the storage period of control cheese, TVB, coliform bacteria and yeasts and moulds counts decreased as the storage period progressed, while *S. aureus* count increased (Table 3). TVB and coliform bacteria counts of cheese supplemented with *Ziziphus spina-christi* and *Cinnamomum zeylanicum* essential oils decreased during the storage period, while *S. aureus* count increased, and the count of yeasts and moulds was not significantly affected by the storage period (Table 4). TVB and coliform bacteria counts of cheese supplemented with 0.3% and 0.5% *Ziziphus spina-christi* essential oil, *S. aureus* count in cheese supplemented with 03% *Ziziphus spina-christi* essential oil, and yeasts and moulds count in cheese supplemented with 0.5% *Ziziphus spina-christi* essential oil significantly decreased during the storage period, while *S. aureus* count of cheese supplemented with 0.5% *Ziziphus spina-christi* essential oil significantly increased, and yeasts and moulds count of cheese supplemented with 0.3% *Ziziphus spina-christi* essential oil was not significantly affected by the storage period (Table 5).

Table-3: Effect of storage period on the microbiological characteristics (log_{10} cfu/gm) of control white cheese (mean±SD)

Microorganisms	Storage period (days)				SL
	1	7	14	21	
Total viable bacteria	8.89±0.45[a]	8.59±0.84[b]	8.50±1.34[c]	7.70±1.79[d]	***
Coliform bacteria	6.67±0.22[a]	6.64±0.21[a]	6.42±0.03[a]	6.20±0.45[a]	NS
Staphylococcus aureus	6.64±0.76[b]	6.49±0.13[c]	7.29±0.45[a]	7.35±1.34[a]	**
Yeasts and moulds	8.04±0.35[b]	8.31±0.99[b]	8.89±0.05[a]	7.88±0.46[b]	***

Means in each row bearing similar superscripts are not significantly different (p>0.05)
*** = P<0.001; ** = P<0.01; NS = Not significant
SL= Significance level
SD = Standard deviation

Table-4: Effect of type of oil on the microbiological characteristics (log_{10} cfu/gm) of white cheese during the storage period (mean±SD)

Type of oil	Storage period (days)	Total viable bacteria	Coliform bacteria	*S. aureus*	Yeasts and moulds
Ziziphus spina-christi	1	7.91±0.65^{a}	6.80±0.45^{b}	6.73±0.51^{b}	7.67±0.92^{a}
	7	8.24±0.85^{a}	6.89±0.62^{b}	5.94±0.59^{c}	7.53±0.34^{a}
	14	7.37±0.75^{b}	7.45±0.12^{a}	6.81±0.42^{ab}	7.29±0.79^{a}
	21	7.94±0.55^{a}	6.38±0.50^{c}	7.02±1.18^{a}	7.40±0.28^{a}
	SL	**	**	***	NS
Cinnamomum zeylanicum	1	7.82±0.78^{a}	6.99±0.57^{a}	6.24±0.41^{c}	7.37±0.75^{a}
	7	7.44±0.37^{b}	6.92±0.53^{a}	7.24±0.45^{a}	7.74±0.43^{a}
	14	7.69±0.38^{ab}	6.88±1.00^{a}	6.90±0.48^{b}	7.47±0.37^{a}
	21	7.13±0.61^{c}	6.61±0.73^{b}	7.15±0.37^{ab}	7.22±0.19^{a}
	SL	***	*	**	NS

Means in each column bearing similar superscripts are not significantly different (p>0.05)
*=P<0.05; **=P<0.01; ***=P<0.001; NS = Not significant
SL = Significance level
SD = Standard deviation

Table-5: Effect of concentration of oil on the microbiological characteristics (log_{10} cfu/gm) of white cheese supplemented with *Ziziphus spina-christi* during the storage period (mean±SD)

Concentration of oil (%)	Storage period (days)	Total viable bacteria	Coliform bacteria	*S. aureus*	Yeasts and moulds
0.3%	1	8.27±0.58^{a}	6.59±0.07^{b}	7.05±0.54^{a}	7.27±1.10^{a}
	7	8.44±0.54^{a}	6.58±0.19^{b}	6.15±0.29^{c}	7.45±0.46^{a}
	14	7.02±0.33^{c}	7.39±0.09^{a}	6.50±0.06^{b}	7.43±0.89^{a}
	21	7.91±0.43^{b}	6.27±0.69^{c}	6.59±1.55^{b}	7.57±0.14^{a}
	SL	*	*	**	NS
0.5%	1	7.64±0.54^{b}	7.02±0.55^{b}	6.41±0.11^{c}	8.07±0.39^{a}
	7	8.03±1.02^{a}	7.21±0.73^{ab}	5.74±0.73^{d}	7.63±0.05^{b}
	14	7.73±0.86^{b}	7.51±0.10^{a}	7.11±0.38^{b}	7.15±0.65^{c}
	21	7.98±0.65^{a}	6.50±0.04^{c}	7.45±0.04^{a}	7.24±0.29^{c}
	SL	***	**	**	*

Means in each column bearing similar superscripts are not significantly different (p>0.05)
*=P<0.05; **=P<0.01; ***=P<0.001; NS = Not significant
SL = Significance level
SD = Standard deviation

All microorganisms under study decreased during the storage period of cheese supplemented with 0.3% and 0.5% *Cinnamomum zeylanicum* essential oil (Table 6). When compared to the other treatments, the results showed that *Cinnamomum zeylanicum* essential oil had a significant effect on the microbes under study. Despite the appropriate efficacy of essential oils in limiting the growth and survival of microorganisms in cheese, some limitations in their application have been identified due to the interaction of essential oil constituents with food components such as fat, carbohydrate, and proteins, which can reduce the antimicrobial effects of the mentioned essential oils [34]. Many studies have shown that essential oils from various plants have antimicrobial properties. Caraway and dill essential oils had the most inhibitory activity against *L. monocytogenes* and *S. typhimurium* after 7 and 14 days in cheese yogurt samples, and caraway essential oil reduced *S. aureus* and *E. coli* counts during the 14-day storage period, while dill essential oil reduced *S. aureus* and *B. cereus* counts during the 14-day storage period [35]. Cinnamon essential oil reduced *Salmonella typhimurium* populations by 2 and 2.2 log_{10} cycles, and *L. monocytogenes* populations by 2.5 and 3 log_{10} cycles, respectively, at the end of storage [2]. The number of *E. coli* in Kashar cheese samples coated with edible films containing thyme and clove essential oils decreased from log_{10} 5.86 cfu/gm and log_{10} 6.12 cfu/gm on day 0 to log_{10} 2.25–4.49 and log_{10} 1.57–4.91 cfu/gm on day 60, respectively, whereas the count of *L. monocytogenes* decreased from log_{10} 5.66 cfu/gm and log_{10} 5.93 cfu/gm on day 0 to log_{10} 4.40 cfu/gm and log_{10} 4.81 cfu/gm on day 60, the count of *S. aureus* decreased from log_{10} 5.49 cfu/gm and log_{10} 5.64 cfu/gm on day 0 to log_{10} 4.16 cfu/gm [36]. The effect of *Mentha pulegium* essential oil at 7.5, 15 or 30 µl/ml on the growth of *L. monocytogenes* inoculated at 10^3 cfu/ml in Iranian white-brined cheese over 60 days was studied, and it was found that the maximum growth lasted for 14 days after a log reduction in the count on the other days [37]. The number of *E. coli* O157:H7 and *L. monocytogenes* colonies decreased in Iranian white

cheese samples containing essential oil over a 45-day storage period, with a higher level of essential oil being more effective and *L. monocytogenes* being more sensitive than *E. coli* O157:H7 [38]. The edible coating of ginger essential oil applied in the coating of Kashar cheese during 30 days of storage at 4°C resulted in a reduction of *S. aureus* count to $\log_{10}$ 2.48 cfu/gm on the 7th day, while the effect of oil on *E. coli* was observed from the first day with the maximum level being observed on the 30th day [39].

Table-6: Effect of concentration of oil on the microbiological characteristics of white cheese supplemented with *Cinnamomum zeylanicum* during the storage period (mean±SD)

Concentration of oil (%)	Storage period (days)	Total viable bacteria	Coliform bacteria	*S. aureus*	Yeasts and moulds
0.3%	1	8.27±0.54^{a}	6.59±0.07^{a}	6.55±0.10^{a}	6.52±0.75^{a}
	7	7.69±0.16^{b}	6.58±0.19^{a}	5.65±0.80^{b}	5.70±0.04^{b}
	14	6.66±0.62^{c}	6.38±1.04^{b}	5.50±1.03^{b}	4.93±0.40^{c}
	21	6.16±0.48^{c}	5.27±0.35^{c}	4.59±0.52^{c}	4.07±0.38^{d}
	SL	*	*	**	*
0.5%	1	8.39±0.25^{a}	6.52±1.06^{a}	5.91±0.51^{a}	6.82±0.91^{a}
	7	7.53±0.33^{b}	6.21±0.31^{b}	5.24±1.24^{b}	5.62±1.04^{b}
	14	6.23±0.06^{d}	5.51±0.10^{c}	5.11±1.56^{b}	4.65±0.98^{a}
	21	6.98±1.68^{c}	4.50±0.04^{d}	4.20±1.33^{c}	4.49±1.11^{c}
	SL	***	**	**	*

Means in each column bearing similar superscripts are not significantly different (p>0.05)
*=P<0.05; **=P<0.01; ***=P<0.001
SL = Significance level
SD = Standard deviation

When compared to green fruits, the essential oil from ripe and mature *Schinus terebinthifolius* Raddi fruits was more effective against *L. monocytogenes*, and bacterial growth was reduced by 1.3 $\log_{10}$ cfu/gm in 30 days [40]. The addition of 2.0% cinnamon oil to tahini reduced *E. coli* O157:H7 numbers by 1.38, 1.79, or 2.20 $\log_{10}$ cfu/ml at 10, 25, or 37°C, respectively by 28 days, while the addition of 1.0% resulted in the absence of visible *E. coli* 0157:H7 cells at 10°C after 21 days, and addition of 0.5% resulted in the absence of visible *E. coli* 0157:H7 cells at 25°C and 37°C for 14 days [41]. Some studies, however, produced contradictory results. After 24 hours of storage, 0.6 µl/ml *Origanum vulgare* L. essential oil had no inhibitory effect on *S. aureus* and *L. monocytogenes* in cheese broth [42]. *Thymus vulgaris* L. and *Origanum vulgare* L. essential oils did not affect *S. aureus* count in cheese samples after 7 days of storage, which was attributed to the interaction of essential oil active components with cheese matrix [43]. *L. monocytogenes* and *E. coli* O157:H7 could survive up to 18 and 22 days of storage, respectively in feta cheese stored under modified atmosphere packaging (50% CO_2 and 50% N_2) at 4°C at a concentration of 0.1 ml/100 gm of oregano essential oil [44].

CONCLUSION

When compared to the control sample, the essential oils of *Ziziphus spina-christi* and *Cinnamomum zeylanicum* had antimicrobial activity against the microorganisms under study, and the concentrations of the oils (0.3% and 0.5%) showed lower microbial counts. Except for yeasts and moulds, the storage period had a significant impact on the counts of all microorganisms tested, and except for *S. aureus* and yeasts and moulds, all microorganisms significantly decreased during the storage period of cheese supplemented with 0.3% and 0.5% *Ziziphus spina-christi* essential oil. All microbes in the study decreased significantly during the storage period of cheese supplemented with 0.3% and 0.5% *Cinnamomum zeylanicum* essential oil. The study concluded that these essential oils could be used as antimicrobial agents in cheese manufacturing if the sensory analysis is performed to determine consumer acceptance.

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