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Effect of *Ziziphus spina-christi* and *Cinnamomum zeylanicum* essential oils on the microbiological quality of braided cheese during the storage period

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Abstract

The purpose of this study was to understand how the addition of Ziziphus spina–christi and Cinnamomum zeylanicum essential oils as antimicrobial agents affect the microbiological properties of braided cheese. Cheese was made from warmed (45°C) raw cow milk to which essential oils of Z. spina–christi and C. zeylanicum (0.3% and 0.5%) were added. Microbiological characteristics were determined at 1, 7, 14 and 21-day intervals. The essential oils of Z. spina–christi and C. zeylanicum significantly reduced the counts of total viable bacteria (TVB), coliform bacteria, S. aureus, yeasts and moulds in cheese supplemented with the oils compared to the control cheese. As the concentration of oil increased, the number of microbes decreased. The storage period had a significant effect on the microbial counts in both the control cheese and the cheese supplemented with essential oils of Z. spina–christi and C. zeylanicum. The addition of the oils as preservatives to the braided cheese was effective against microbial populations and could be used in cheese preparations to extend the shelf life of cheese, particularly for cheese made from raw milk without the addition of starter culture.

Keywords: Braided cheese; Microbiological; *Ziziphus spina-christi*; *Cinnamomum zeylanicum*; Essential oil

1. Introduction

Food technology research continues to face significant challenges in finding effective ways to add value to produce foodstuffs while also maintaining their quality and safety [1]. However, the growing consumer demand for safe and natural products free of chemical preservatives has prompted food authorities and researchers to conduct extensive research into the feasibility of mild preservation techniques and to improve the microbial quality and safety of products while retaining their good nutritional and organoleptic properties [2]. As a result, new methods of reducing or eliminating foodborne pathogens are still required, possibly in conjunction with existing method and furthermore, the World Health Organization has recently called for a global reduction in salt consumption in order to reduce the prevalence of cardio-vascular disease [3].

Herbs and spices have been used since ancient times because of their antimicrobial properties, which increase food safety and shelf life by acting against foodborne pathogens and spoilage bacteria, and the plants have long been used in traditional medicine as a source of natural antimicrobial substances for the treatment of infectious diseases [4, 5]. Several food preservation methods have been developed to ensure microbial food safety, as well as nutritional and sensory properties, however, consumer concern about health issues is driving up demand for natural antimicrobial agents [6]. In today's nutrition food system, dairy products are a unique carrier that has been successfully used to deliver phytochemicals and other nutrients for health benefits [7].

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Essential oils have long been used in the medical, cosmetic, and food industries. In recent years, extensive research has been conducted to investigate and determine the antimicrobial activity of essential oils [8]. Essential oils derived from medicinal herbs have numerous applications in ethnomedicine, cosmetics, food, beverages, preservation, fragrance, and pharmaceutical industries [9]. These oils have antioxidant, insecticidal, antiviral, antibacterial, antifungal, and antibiofilm properties [10].

The genus *Zizphus* is well-known for its antimicrobial, antioxidant, anti-inflammatory, and immune-stimulant properties [11]. The cinnamon oil contains antibacterial and antifungal compounds that can be used to prevent food spoilage due to microbial contamination [12]. To the best of our knowledge, no research reports on the antibacterial properties of *C. zeylanicum* and *Z. spina-christi* oils in cheese, as well as their possible mechanisms. This study was carried out to determine the effect of adding essential oil on the microbiological quality of braided cheese during the storage period.

2. Material and methods

2.1. Extraction of essential oil from *Z. 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 [13].

2.2. Extraction of essential oil from *C. zeylanicum*

Essential oil of C. zeylanicum was extracted from cinnamon barks using Clevenger apparatus. 100 gm of barks with 500 ml of distilled water was transferred into oil distillation at 90°C for 1-2 hr. The essential oil was collected and determined by calibrated tube, sterilized by filtration through 0.45 μm Millipore filters, and kept in the freezer until used [12].

2.3. Preparation of *Solanum dubium* extract

The whole seeds were coarsely powdered using 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.

2.4. Manufacture of braided cheese

Aseptically, milk (25 L) was warmed to 45°C, then S. dubium extract (2 ml/50 ml) was added. After 5 minutes the curd was tested by knife and then the curd was cooked and the whey was drained. The curd was divided evenly into 5 equal batches: Control, to which no essential oil is added, 0.3% (v/w) of Z. spina-christi essential oil, 0.5% (v/w) of Z. spina-christi essential oil, 0.3% (v/w) of C. zeylanicom essential oil and 0.5% (v/w) of C. zeylanicom essential oil. The curd was mixed thoroughly and preserved at room temperature for 12 min, followed by elasticity test. The curd was cooked and braided. Salt (6% w/w) was added to the whey, heat treated (63°C/30 min), and the cheese was preserved in the whey for 24 hr, after which cheese was preserved in the whey at 4°C. Microbiological analyses were carried out at 1, 7, 14, and 21-day intervals.

2.5. Microbiological examination

2.5.1. Preparation of serial dilutions

For the preparation of serial dilutions, 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} using sterile peptone water [14].

2.5.2. 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±1°C for 48±3 hr, and the colonies were counted using a manual colony counter (scan 100) and recorded as cfu/gm [15].

2.5.3. 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 37°C for 48 hr, and the typical colonies were counted with a manual colony counter (Scan 100) and recorded as cfu/gm [15].

2.5.4. Coliform bacteria count

MacConkey agar was used to determine the coliform count. The plates were incubated in an inverted position at 37°C for 48 hours, and the typical colonies were counted by a manual colony counter (Scan 100) and recorded as cfu/gm [16].

2.5.5. 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 25°C for 5 days, and the colonies were counted by a manual colony counter (Scan 100) and recorded as cfu/gm [17].

2.6. Statistical analysis

The statistical analysis was carried out using Statistical Analysis Systems (SAS, ver.9). General linear model (GLM) procedure was used to determine the effect of type and 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 ($P \leq 0.05$).

3. Results and discussion

3.1. Microbiological characteristics of cheese supplemented with *Z. spina-christi* and *C. zeylanicum* essential oils compared to control cheese

Table 1 shows that TVB, coliform bacteria and *S. aureus* counts were significantly higher in the control cheese ($\log_{10}$ 8.11±0.97 cfu/gm, $\log_{10}$ 7.09±1.17 cfu/gm and $\log_{10}$ 5.18±1.63 cfu/gm, respectively) and lower in cheese supplemented with *C. zeylanicum* essential oil ($\log_{10}$ 7.16±2.01 cfu/gm, $\log_{10}$ 6.07±1.36 cfu/gm and $\log_{10}$ 4.36±1.52 cfu/gm, respectively).

Table 1 Effect of type of oil on the microbiological characteristics ($\log_{10}$ cfu/gm) of braided cheese (mean±SD)

Microorganisms	**Type of oil**			**SL**
	Control	*Z. spina-christi*	*C. zeylanicum*	
Total viable bacteria	$8.11±0.97^a$	$8.03±0.86^b$	$7.16±2.01^b$	**
Coliform bacteria	$7.09±1.17^a$	$6.50±0.66^b$	$6.07±1.36^c$	***
S. aureus	$5.18±1.63^a$	$4.58±1.51^{ab}$	$4.36 ±1.52^b$	*
Yeasts and moulds	$6.15±1.41^a$	$6.15±0.51^a$	$6.03±0.54^a$	NS

Means in each row bearing similar superscript letters 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

Although yeasts and moulds count was not significantly different between treatments, control cheese and cheese supplemented with *Z. spina-christi* essential oil showed higher count ($\log_{10}$ 6.15±1.41 cfu/gm and $\log_{10}$ 6.15±0.51 cfu/gm, respectively). These findings are consistent with Gupta et al. [18] who reported that *C. zeylanicum* oil was very effective against *Bacillus sp*., *Listeria monocytogenes*, *E. coli*, *Klebsiella sp*. and *Rhizomucor sp*., and Behbahani et al. [9] who reported an antibacterial activity of *C. zeylanicum* bark essential oil against Gram-positive bacteria (*Listeria innocua*, *S. aureus*, and *B. cereus*), compared to Gram-negative ones (*E. coli, P. aeruginosa,* and *S. typhi*) through disrupting cell envelope and facilitating the leakage of intracellular compounds. The oil extract of *Z. spina-christi* seed showed good antimicrobial activity against *E. coli* and *Candida albicans*, partially active against *S. aureus, P. aeruginosa*, and *Aspergillus niger*, and inactive against *B. subtilis* [19]. Abdel Karim et al. [13] reported that *Z. spina-christi* oil has a remarkable lethal effect on *E. coli*, *S. typhimurium*, and *Aspergillus niger* by inhibiting their survival. The essential oil of *Z. spina-hristi* was most sensitive to *Aspergillus niger*, *Penicillium digitatum*, and *Klebsiella pneumonia*, while *P. aeruginosa*, *S. aureus*, and *B. cereus* showed no sensitivity [20]. The oil of *Z. spina-christi* L. was found to be highly active against *S. auras* and *Candida sp*., and inactive against *P. aeruginosa*, *B. subtilis* and *E. coli* [21].

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

Table 2 shows that control cheese and cheese supplemented with 0.5% *Z. spina-christi* essential oil had the highest TVB count ($\log_{10}$ 8.11±0.97 cfu/gm and $\log_{10}$ 8.12±0.69 cfu/gm, respectively), while the lowest count ($\log_{10}$ 6.80±2.71

cfu/gm) was in cheese supplemented with 0.5% *C. zeylanicum* essential oil. Coliform bacteria and *S. aureus* counts were significantly higher in control cheese ($\log_{10}$ 6.84±0.85 cfu/gm and $\log_{10}$ 6.90±1.30 cfu/gm, respectively) and lower in cheese supplemented with 0.5% *C. zeylanicum* essential oil ($\log_{10}$ 5.71±2.36 cfu/gm and $\log_{10}$ 5.71±2.28 cfu/gm, respectively). Yeasts and moulds count was significantly (P<0.01) higher in control cheese ($\log_{10}$ 8.15±1.04 cfu/gm), whereas no significant difference was observed in the other treatments. Research studies showed that *Z. spina-christi* var. *aucheri* essential oil inhibited the growth of *Aspergillus niger, Penicillium digitatum,* and *Klebsiella pneumonia* in concentrations of 32 gm/ml, 128 gm/ml, and 512 gm/ml, respectively [20]. Cinnamaldehyde, the main component of *C. zeylanicum* and *C. cassia* essential oils, was found to kill 99% of *Streptococcus pyogenes, Pseudomonas aeruginosa* and *E. coli* at a concentration of 2 mg/ml [10], and 2.9 mg/ml *C. verum* essential oil inhibited the growth of *S. typhi* and *P. fluorescens* [22]. *C. burmannii* bark essential oil has been shown to have antibacterial activity against *S. aureus* [23].

Table 2 Effect of concentration of oil on the microbiological characteristics ($\log_{10}$ cfu/gm) of braided cheese (mean±SD).

Type of oil	Concentration of oil (%)	TVB	Coliform bacteria	*S. aureus*	Yeasts and moulds
Control	0	8.11±0.97^{a}	6.84±0.85^{a}	6.90±1.30^{a}	8.15±1.04^{a}
Z. spina-christi	0.3	7.94±0.60^{a}	6.43±0.76^{c}	5.49±2.25^{c}	6.86±0.65^{b}
	0.5	8.12±0.69^{a}	6.56±0.69^{b}	6.42±0.41^{b}	6.76±0.71^{b}
C. zeylanicum	0.3	7.52±2.19^{a}	6.42±0.59^{c}	6.27±1.05^{b}	6.75±0.66^{b}
	0.5	6.80±2.71^{b}	5.71±2.36^{d}	5.71±2.28^{c}	6.74±0.68^{b}
SL		***	***	**	**

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

3.3. Effect of storage period on the microbiological characteristics of cheese

Table 3 shows the microbiological characteristics of control cheese during the storage period. TVB count decreased significantly (P<0.01) during the storage period, while coliform bacteria and yeasts and moulds counts increased until days 14 and then decreased towards the end, and *S. aureus* count gradually increased throughout the storage period.

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

Microorganisms	Storage period (days)				SL
	1	7	14	21	
Total viable bacteria	8.48±0.11^{a}	8.38±0.92^{a}	7.98±1.54^{b}	7.59±0.46^{c}	**
Coliform bacteria	5.95±0.31^{c}	6.65±0.75^{b}	7.57±0.12^{a}	7.20±0.96^{a}	*
S. aureus	5.09±0.99^{d}	6.83±0.37^{c}	7.40±0.34^{b}	8.27±0.30^{a}	***
Yeasts and moulds	6.92±1.02^{c}	8.00±0.41^{b}	8.94±0.32^{a}	8.74±0.39^{a}	*

Means in each row 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.

The results of TVB contradict those of Cetinkaya and Soyutemiz [24], who reported an increasing trend of TVB count in Kaskar cheese during ripening. Cetinkaya and Soyutemiz [24] discovered an increasing pattern of yeasts and moulds content during ripening, which is consistent with the findings in this research.

TVB count of cheese supplemented with *Z. spina-christi* essential oil significantly (P<0.01) decreased during the storage period, while coliform bacteria count significantly (P<0.01) increased, and *S. aureus* and yeasts and moulds counts fluctuated during the storage period showing peaks on days 7 and 14, respectively (Table 4).

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

Type of oil	Storage period (days)	Total viable bacteria	Coliform bacteria	*S. aureus*	Yeasts and moulds
Z. spina-christi	1	8.69±1.06^{a}	5.96±0.31^{a}	5.09±0.14^{a}	6.92±0.25^{a}
	7	8.38±0.47^{b}	5.65±0.66^{b}	6.82±0.81^{b}	7.00±0.54^{a}
	14	7.98±1.01^{b}	5.56±0.92^{b}	6.02±0.74^{c}	7.94±0.32^{a}
	21	6.01±0.52^{c}	6.00±0.64^{a}	5.71±2.47^{d}	6.78±0.56^{b}
	SL	**	**	***	*
C. zeylanicum	1	8.69±1.06^{a}	5.95±2.14^{a}	5.09±0.14^{c}	6.92±0.25^{a}
	7	7.74±0.58^{b}	5.53±0.84^{b}	5.29±0.85^{b}	5.81±0.54^{b}
	14	6.15±1.26^{d}	5.78±0.92^{b}	5.22±0.67^{b}	5.76±0.59^{b}
	21	6.74±3.03^{c}	5.55±0.75^{b}	5.51±2.19^{a}	5.71±0.52^{b}
	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

All microorganisms significantly decreased during the storage period of cheese supplemented with 0.3% *and* 0.5% *Z. spina-christi* essential oil indicating an inhibitory effect of the oil against microbial population (Table 5). These findings are consistent with those of Nkafamiya et al. [25], who discovered that *S. aureus* was sensitive to *Z. spina-christi* oil. The findings, on the other hand, contradict the findings of Coopoosamy et al. [26], who reported that water extracts of leaves, roots, and stem bark of other species of *Z. mucronata* showed no activity against all Gram-negative and Gram-positive bacteria tested.

Table 5 Effect of concentration of oil on the microbiological characteristics (log10 cfu/gm) of braided cheese supplemented with *Z. spina-christi* during the storage period (mean±SD).

Concentration (%)	Storage period (days)	Total viable bacteria	Coliform bacteria	*S. aureus*	Yeasts and moulds
0.3%	1	7.91±0.62^{a}	5.88±0.45^{c}	5.31±1.25^{b}	6.50±0.30^{c}
	7	7.81±0.41^{a}	6.32±0.22^{b}	6.86±0.32^{a}	7.34±0.55^{a}
	14	8.07±1.14^{a}	7.09±0.56^{a}	6.54±0.74^{a}	6.82±0.58^{b}
	21	7.97±0.40^{a}	6.42±0.60^{b}	3.25±3.47^{c}	6.78±0.61^{b}
	SL	NS	*	**	*
0.5%	1	8.47±0.62^{a}	5.72±0.45^{c}	6.22±0.87^{c}	6.33±0.71^{c}
	7	7.87±0.41^{c}	6.80±0.67^{a}	6.93±0.60^{a}	7.03±0.54^{a}
	14	8.07±1.15^{b}	6.94±0.52^{a}	6.37±0.97^{b}	6.91±0.66^{b}
	21	8.05±2.75^{b}	6.79±0.78^{b}	6.17±0.49^{d}	6.79±0.46^{b}
	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.

Al-Mutairi et al. [27] reported that *Z. spina-christi* leaves extract had an inhibitory effect against all tested bacterial species. The ethanolic and butanolic extracts were bactericidal and bacteriostatic against *E. coli, S. aureus,* and *C. albicans* [28]. *Z. spina-christi* oil was found to have antibacterial activity against *Staphylococcus aureus* and *E. coli* [29].

Cheese supplemented with *C. zeylanicum* essential oil inhibited the growth of TVB, coliform bacteria, yeasts, and moulds, while the growth of *S. aureus* increased during storage (Table 4). Coliform bacteria, *S. aureus*, yeasts, and moulds counts significantly increased during the storage period of cheese supplemented with 0.3% *C. zeylanicum* essential oil, while TVB count decreased. However, the concentration of 0.5% *C. zeylanicum* essential oil had antimicrobial activity against microorganisms under study because the count decreased as the storage period progressed (Table 6). Previous research found no coliform bacteria, but yeasts, moulds, and *S. aureus* were found in trace amounts in some treated labneh containing 0.3% cinnamon oil [30]. The essential oil of *C. zeylanicum* significantly influenced *S. aureus, Enterococcus, Enterobacter,* and harmful fungi [31]. *C. zeylanicum* oil was found to have adequate antimicrobial activity against coliform bacteria [1]. The essential oil concentrations of 10, 20, and 30 µl/ml caused a rapid and steady decrease in the number of viable cells of Gram-positive and Gram-negative bacteria by 2 to 5 log cycles over 24 hours [12]. *S. aureus* counts in milk were reduced by 0.35-2.77 log cfu/ml during storage, with significantly greater decreases observed when cinnamaldehyde was added, regardless of concentration, when compared to the control [32].

Table 6 Effect of concentration of oil on the microbiological characteristics (log_{10} cfu/gm) of braided cheese supplemented with *C. zeylanicum* during the storage period (mean±SD).

Concentration (%)	Storage period (days)	Total viable bacteria	Coliform bacteria	*S. aureus*	Yeasts and moulds
0.3%	1	$8.91±0.62^a$	$5.97±0.45^b$	$5.16±1.25^c$	$5.84±0.30^c$
	7	$7.17±0.41^c$	$6.03±0.22^b$	$6.50±0.32^b$	$6.89±0.55^b$
	14	$8.06±1.14^b$	$6.95±0.56^a$	$7.01±0.74^a$	$7.23±0.58^a$
	21	$5.93±0.40^d$	$6.74±0.60^a$	$6.42±3.47^b$	$7.04±0.61^a$
	SL	*	*	**	*
0.5%	1	$7.59±0.62^b$	$5.54±2.71^c$	$5.78±0.48^c$	$6.07±0.71^b$
	7	$7.32±0.41^b$	$6.53±0.67^b$	$7.07±0.60^a$	$6.98±0.54^a$
	14	$8.24±1.15^a$	$7.42±0.52^a$	$6.38±0.97^b$	$7.04±0.66^a$
	21	$4.05±4.34^c$	$6.36±0.78^b$	$3.61±3.85^d$	$6.88±0.46^a$
	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

4. Conclusion

When compared to the control cheese, the essential oils of *Z. spina-christi* and *C. zeylanicum* inhibited the growth of microorganisms in cheese during storage. Microbial counts decreased as oil concentration increased. Traditionally, cheese is made from raw milk with no starter culture added; therefore, preservation methods such as the use of essential oils should be tried, provided that the question of cheese acceptability to consumers is answered through sensory analysis of cheese.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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