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Rosemary Essential Oil as a Natural Alternative to Potassium Sorbate: Impact on Shelf-Life and Quality Attributes of Low-Salt White Soft Cheese

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Abstract

This study investigated the potential of incorporating rosemary (Rosmarinus officinalis) essential oil at various concentrations (0.1%, 0.2%, and 0.3% v/v) into milk as a natural alternative to potassium sorbate for the production and bio-preservation of low-salt soft white cheese. The volatile profile of the rosemary essential oil was characterized using Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS analysis demonstrated a rich profile of bioactive volatile compounds in rosemary essential oil. The fortified soft cheese samples were evaluated for their total antioxidant capacity, antimicrobial efficacy against spoilage and pathogenic microorganisms, and overall microbiological profile during storage. The findings showed that functional characteristics improved as the essential oil content increased in cheese. Notably, the 0.3% concentration emerged as the most effective treatment, exhibiting superior antioxidant capacity, microbial inhibition, and optimal preservation of the cheese's quality. Furthermore, the preservative efficacy of the 0.3% rosemary essential oil treatment was closely comparable to that of the conventional synthetic preservative, potassium sorbate. Therefore, RMEO at 0.3% offers a viable and effective way to reduce synthetic preservatives and promote natural bio-preservation in low-salt white soft cheese.

Keywords: Soft white cheese; Rosemary essential oil; Potassium sorbate; GC-MS analysis; Antioxidant activity; Antimicrobial efficacy; Bio-preservation.

1. Introduction

Cheese is a highly nutrient-dense dairy product that is widely consumed worldwide. The different types of milk and production methods used are responsible for the differences in cheese products' sensory attributes **(Boukria et al., 2020).**

Spoilage is primarily associated with microbial proliferation, including yeasts, moulds, and psychrotrophic bacteria, which lead to textural degradation, off-flavours, and moisture loss **(El-Sayed and El-Sayed, 2021; Zheng et al., 2021)**. Additionally, the high-water activity, near-neutral pH, and nutrient-rich composition of white soft cheese provide favorable conditions for the growth of spoilage microorganisms and foodborne pathogens **(Santiago-López et al., 2018)**, thereby compromising product quality and safety (**Falardeau et al., 2021**). Although chemical preservatives such as potassium sorbate are commonly used to control spoilage in dairy products, rising consumer concerns about synthetic additives and the demand for clean label foods have spurred the development of natural preservation strategies **(Díaz et al., 2016; Salama et al., 2022).**

Synthetic additives are commonly employed in the food industry for preservation, extending the shelf life and improving overall safety and quality. Synthetic additives have been increasingly substituted with natural ones in recent years (**Nikmaram et al., 2018).**

In the cheese industry, clean-label technologies aim to extend the shelf life of products without using artificial preservatives, and many of these strategies have already been explained reducing the harmful microbial content; using preservatives obtained from natural sources, such as plants; essential oils (EOs) provide significant benefits compared to synthetic preservatives by integrating strong antioxidant and antibacterial properties with clean-label attributes, consumer attractiveness, and regulatory simplicity **(Nieto 2020)**

The food preservation potential of essential oils (EOs) stems from their unique antimicrobial, chemical, and physical properties. Although synthetic preservatives have traditionally dominated the food industry, their long-term intake has been associated with adverse health effects, including allergic reactions, toxicity, carcinogenesis, and various degenerative disorders (**Bhavaniramya et al. 2019).** Essential Oils (EOs) and their extracts from aromatic plants were found to have significant antibacterial and antioxidant activity, so they can be employed as alternative natural food preservatives (**Varghese et al., 2020).**

Rosemary(*Rosmarinus officinalis* L.) shows strong potential as a natural source for antimicrobial chemotherapy (**Alvarez et al., 2019; Garcia-Sotelo et al., 2019**). Phytochemical profiling of its essential oils reveals key bioactive compounds—including 1,8-cineole, camphor, pinenes, camphene, and 3-carene—which primarily drive its antimicrobial potency (**Cutillas et al., 2018; Yeddes et al., 2021)**. Its antimicrobial activity has been reported against several notable Gram-positive and Gram-negative bacteria (**Alvarez et al., 2019; Garcia-Sotelo et al., 2019; Eid et al., 2022**), such as *L. monocytogenes* ,*K. pneumoniae* ,*P. aeruginosa* ,*S. aureus* ,and *S. Typhimurium*.

2. Materials and Methods

2.1. Materials

Fresh buffalo milk was collected from a private farm in the Sharika Governorate (Egypt) and processed within 1 h of milking for soft cheese production. Commercial fine-grade NaCl was obtained from El-Nasr Salines Company, Egypt. Commercial powder rennet (CHY-MAX® Powder Extra NB, Activity ≈ 2200 IMCU/g, Dosage: 0.027 g ≈ 60 IMCU/ L milk, Chr. Hansen©, Horsholm, Denmark) was reconstituted in distilled water to prepare a standard rennet solution, which served as the coagulating enzyme for milk clotting. Grindsted® Mono-Diglyceride HP 60 emulsifier was obtained from Danisco, DuPont Nutrition Biosciences ASP, Grindsted, Denmark. All chemicals and reagents used in the analytical procedures were of analytical grade. We purchased natural plant essential oils, including thyme oil (Thymus vulgaris) and black seed oil used in this study, from a certified supplier (Organic Company for Natural Oils, Assiut, Egypt) and stored them in dark glass bottles until use.

2.2. Methods

2.2.1. Manufacture of low-salt white soft cheese

To manufacture full-fat, low-salt soft cheese, specific processing modifications were introduced to ensure homogeneous distribution of rosemary essential oil (RMEO) in the milk. To ensure efficient blending, the mixture of full-fat buffalo milk (7%fat), RMEO, and the emulsifier (0.2%) was heated to 45°C. Following this, the pre-warmed mixture was homogenized using a blender. After homogenization, the mixture was pasteurized (65°C for 30 min), cooled (40°C), and calcium chloride (0.02%) and rennet were added to the milk at a dosage of 0.027 g/L (providing ~60 IMCU/L of milk). Five treatments of white soft cheese, control without additives, PSC (Potassium sorbate cheese), (RMEOC1, RMEOC2, RMEOC3), and mixed with 0.1, 0.2, and 0.3 % RMEO, respectively. The resulting cheese was packaged in plastic bags and stored at 4 ±1 °C for 60 days in a whey solution modified to 3% salt. Three replicates were carried out for each treatment. The cheese was analyzed when fresh and then after 15,30,45 and 60 days of cold storage.

2.2.2. Gas chromatography–mass spectrometry analysis (GC-MS) for RMEO

The GC-MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt.

2.2.3 Determination of peroxide value (pv) for RMEO

Peroxide value was determined according to **Magu et al., 2017.** The peroxide number was calculated by the following equation:

Peroxide number (meq/kg) = (V Na2S2O3 (mL) × M $Na_2S_2O_3$ × 1000)/ (Mass of the sample, g)

2.2.4. Determination of acid value (AV) for RMEO

Acid value was determined according to **Shimamoto et al., 2016.** Acid value was used to calculate (mg KOH/g oil) of AV. The following equation was used.

Acid value (mg/g): V KOH (mL) × M KOH × 56.1 / Mass of the sample (g)

where, V = volume of KOH, and M (molarity) = concentration of KOH solution.

2.2.5. Determination of radical ABTS scavenging activity of RMEO and cheese Samples

The stock solutions of ABTS reagent were prepared according to **Hwang and Do Thi (2014)**

2.2.6. Analysis of the Total Phenolic Content (TPC) of RMEO and SOFT cheese Samples

was measured using the Folin-Ciocalteu method, as proposed by **Singleton et al. (1999),** The results were presented as milligrams of gallic acid equivalents per liter.

2.2.7. Antimicrobial activity: Agar well diffusion method for RMEO

The agar-well diffusion method was conducted for the determination of antimicrobial activity of essential oil extracts as described by **Schillinger & Lucke (1989**). A semi-solid (0.7% agar w/v) nutrient medium was used to perform the test against Staphylococcus aureus as an indicator bacterial strain. The semi-solid media were molten, cooled down to 48°C, inoculated with 0.1 mL of fresh overnight culture of the indicator strains (approximately $\mathbf{10^5}$ CFU /ml), and poured into a Petri dish. Wells of 6 mm in diameter were cut into the agar and filled with 100 µL of the essential oil extract. After holding the plates at room temperature for 2 hr. to allow diffusion of the oil into the agar, the plates were incubated at 37°C for 24 hr. Then, they were examined for inhibition of the bacteria, and the diameters of the inhibition zones were measured. All tests were performed in triplicate.

2.2.8. Microbiological analyses of soft cheese

The total bacterial count of all low white soft cheese samples was determined according to **Marshal (1992).** Coliform bacterial, yeast, and mould counts were enumerated as described by **APHA (1994).** All samples were evaluated when fresh and then after 15, 30,45, and 60 days. The results were recorded as log number of colony-forming units per g **($\log_{10}$ CFU/g).**

2.2.9. Sensory evaluation

All samples of low-salt white soft cheese enhanced with RMEO were sensory evaluated when fresh and then after 15, 30, 45, and 60 days of storage by ten staff members of the Dairy and Food Science Departments, Faculty of Agriculture, Zagazig University, Egypt. They were selected for their interest and experience in sensory evaluation. Panelists judged the cheese samples using the following points: flavor (50 points), Body and texture (35 points), and colour & appearance (15 points) according to **Hassan et al. (1983).**

2.2.10. Statistical analysis

A factorial split-plot design with three replications was used to evaluate the effects of RMEO treatments and storage periods on the evaluated parameters. The main plots were assigned to the different treatment formulations (Control and treatments fortified with RMEO at concentrations of 0.1%, 0.2%, and 0.3%), while the sub-plots consisted of the storage periods (0, 15, 30, 45, and 60 days). The obtained data were subjected to Analysis of Variance (ANOVA) using Statistix 9.0 software package. Means were compared using the Least Significant Difference (LSD) test at a significance level of 0.05.

3. Results and Discussion

3.1. Determination of Physicochemical Attributes and Bioactive Components of Rosemary Essential Oil

The results presented in **Table 1** highlight the high quality and strong bioactivity of **RMEO**. The oil showed a remarkably high total antioxidant capacity of 9717.0 ppm (ascorbic acid equivalent), strongly linked to its rich total phenolic content of 959.0 ppm (gallic acid equivalent**) (Yazici et al. 2020).** Furthermore, the physicochemical parameters indicate excellent oil stability and freshness, as shown by the low peroxide value (8.49 meq O_2/kg oil) and minimal acid value (0.68 mg KOH/g oil), confirming the absence of significant oxidation or hydrolytic degradation.

Table 1. Total antioxidant capacity, total phenols, peroxide value, and acid value of rosemary essential oil

Parameter	rosemary essential oil (RMEO)
Total Antioxidant Capacity	9717.0 ppm (ascorbic acid equivalent)
Total Phenols	959.0 ppm gallic acid equivalent)
Peroxide Value	8.49 meq O_2 / Kg oil
Acid value	0.68 mg KOH /g Oil

3.2. Evaluation of Crude Rosemary Essential Oil (REO) as a Natural Bio-Preservative: Disc Diffusion Analysis

Antimicrobial effect of REO against *Staphylococcus aureus*: As REO concentration increased, zones of inhibition increased significantly ($p < 0.05$), with the inhibition zone (13.33± 0.66 mm) (**Figure 1)**. Recent studies attribute REO's antimicrobial efficacy to its rich phytochemical composition, including 1,8-cineole, camphor, α-pinene, borneol, and phenolic diterpenes such as carnosic acid and carnosol (**Micić et al., 2021)**. These constituents are known to destabilize bacterial cell membranes, interfere with enzymatic systems, and promote leakage of intracellular contents, ultimately leading to cell lysis (**Yang et al., 2023).**

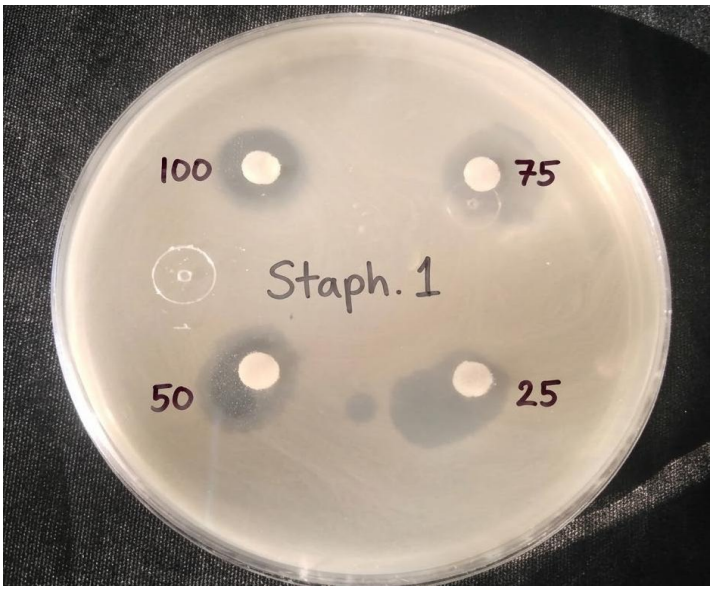


Figure 1. Inhibitory activity (zone of inhibition) of REO against *Staphylococcus aureus.*

3.3. Volatile Compounds Profile and Functional Constituents of Rosemary Essential Oil (REMO)

The GC-MS spectral dataset in **Table 2 & Figure 2** of Rosmarinus officinalis essential oil established a diverse phytochemical fingerprint. The major bioactive bicyclic oxygenated monoterpene, Eucalyptol (cineole-1,8) (54.7%), alongside Camphor (11.91%), the antimicrobial monoterpenes 1,8-cineole and camphor, represent the master constituents of rosemary essential oils and are widely applied as bio preservatives **(Shaaban 2020; Al-Maqtari et al. 2021)**, and endo-Borneol (4.47%) formed the fundamental active matrix of the oil. The therapeutic and antioxidant profile was further supported by Bornyl acetate (0.4%), alpha-Terpineol (1.03%), and Terpinen-4-ol (0.39%). These oxygenated species were synergistic with bicyclic monoterpenes such as alpha-Pinene (15.13%), Camphene (3.52%), and beta-Pinene (1.71%). On the other hand, the presence of bicyclic monoterpenes, specifically α-pinene and β-pinene, accounts for the strong, resinous odour characteristic of rosemary oil, as well as acyclic monocyclic terpenes including beta-Myrcene (0.72%), gamma-Terpinene (0.33%), p-Cymene (0.29%), and Linalool (0.33%). The presence of sesquiterpenes such as Caryophyllene (4.12%) and Humulene (0.37%) completes the chemical core responsible for the oil's biological efficiency. Essential oil extracted from rosemary is a commercially prominent plant product traditionally applied as a spice. In food science applications, it is extensively leveraged for its antioxidant and antimicrobial efficacy, operating as an eco-friendly biopreservative to enhance food quality and prolong shelf stability **(Sirocchi et al., 2017).**

Table 2. Gas Chromatography-Mass Spectrometry (GC-MS) Chemical Composition of rosemary essential oil (RMEO)

Peak	RT (min)	Components	Formula	Area	Area Sum %
1	1.77	.alpha.-Pinene	C10H16	17889587.5	15.13
2	1.898	Camphene	C10H16	4157638.75	3.52
3	2.214	.beta.-Pinene	C10H16	2019080.27	1.71
4	2.478	.beta.-Myrcene	C10H16	852288.54	0.72
5	2.574	.alpha.-Phellandrene	C10H16	199659.83	0.17
6	2.639	3-Carene	C10H16	163748.5	0.14
7	2.735	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	C10H16	341828.29	0.29
8	2.96	1,8 Cineole	C10H18O	63936043.5	**54.7**
9	3.295	.gamma.-Terpinene	C10H16	395740.87	0.33
10	3.681	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	C10H16	219789.74	0.19
11	3.932	Linalool	C10H18O	387630.78	0.33
12	4.396	Camphor	C10H16O	14082205	11.91
13	4.705	endo-Borneol	C10H18O	5288558.24	4.47
14	4.859	Terpinen-4-ol	C10H18O	459722.74	0.39
15	5.065	.alpha.-Terpineol	C10H18O	1212865.84	1.03
16	6.314	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	C12H20O2	474663.81	0.4
17	7.402	Copaene	C15H24	313336.28	0.26
18	7.917	Caryophyllene	C15H24	4866649.22	4.12
19	8.316	Humulene	C15H24	443049.79	0.37
20	8.65	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	C15H24	208576.49	0.18
21	9.223	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	C15H24	334667.05	0.28

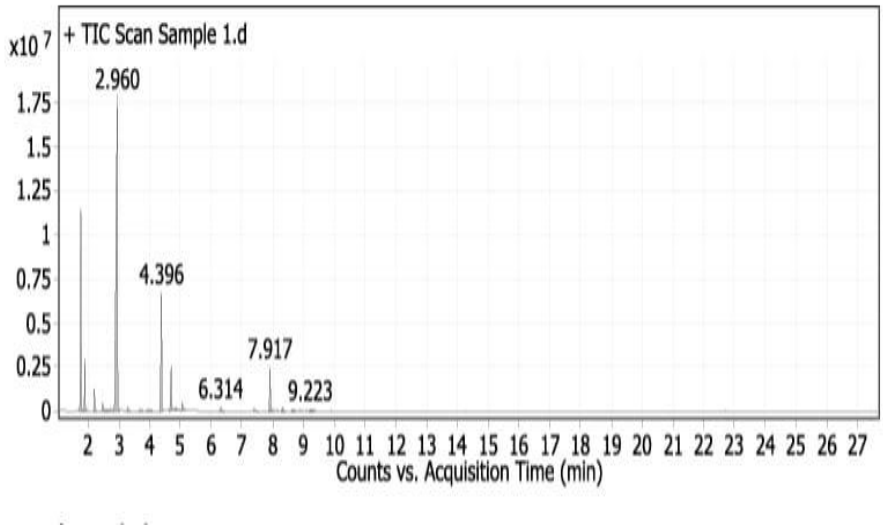
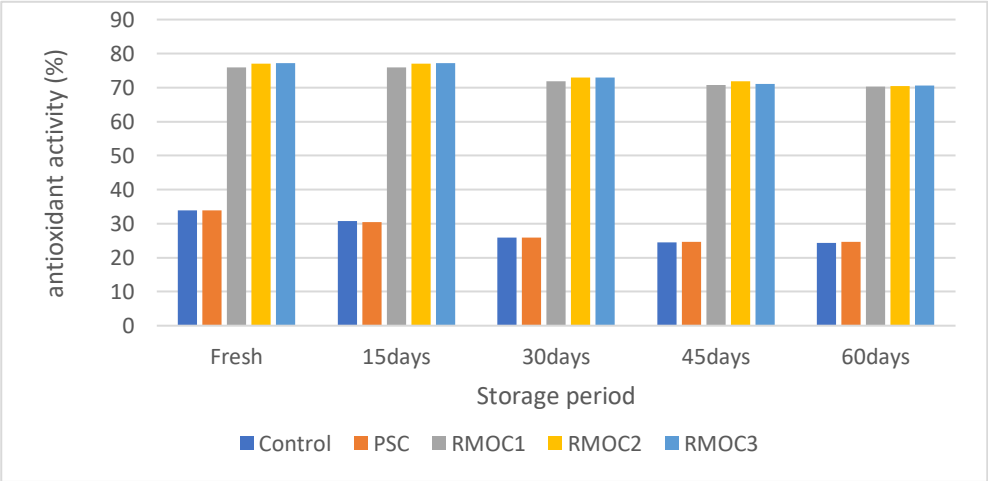


Fig. (2). Gas chromatography-mass spectrometry (GC-MS) of rosemary essential oil

3.5. Antioxidant properties in low salt -soft cheese

Fig. (3) illustrates changes in antioxidant activity during refrigerated storage of low-salt soft cheese samples containing varying concentrations of rosemary essential oil (RMEO). The addition of essential oil raised the antioxidant activity in soft cheese when compared to the control cheese. The fresh cheese's antioxidant activity was 33.65, 33.84, 76, 77.11, and 77.23% for the control cheese and the cheese samples that contained essential oil and rosemary, respectively. All the cheese samples exhibited the same pattern during chilled storage, with the antioxidant activity progressively declining over time. When fresh and during storage, the antioxidant activity of soft cheese with essential oil was often higher than that of the control cheese. **Olmedo et al. (2013**) found that rosemary essential oil demonstrated a protective effect against lipid oxidation and fermentation in flavoured cheese.

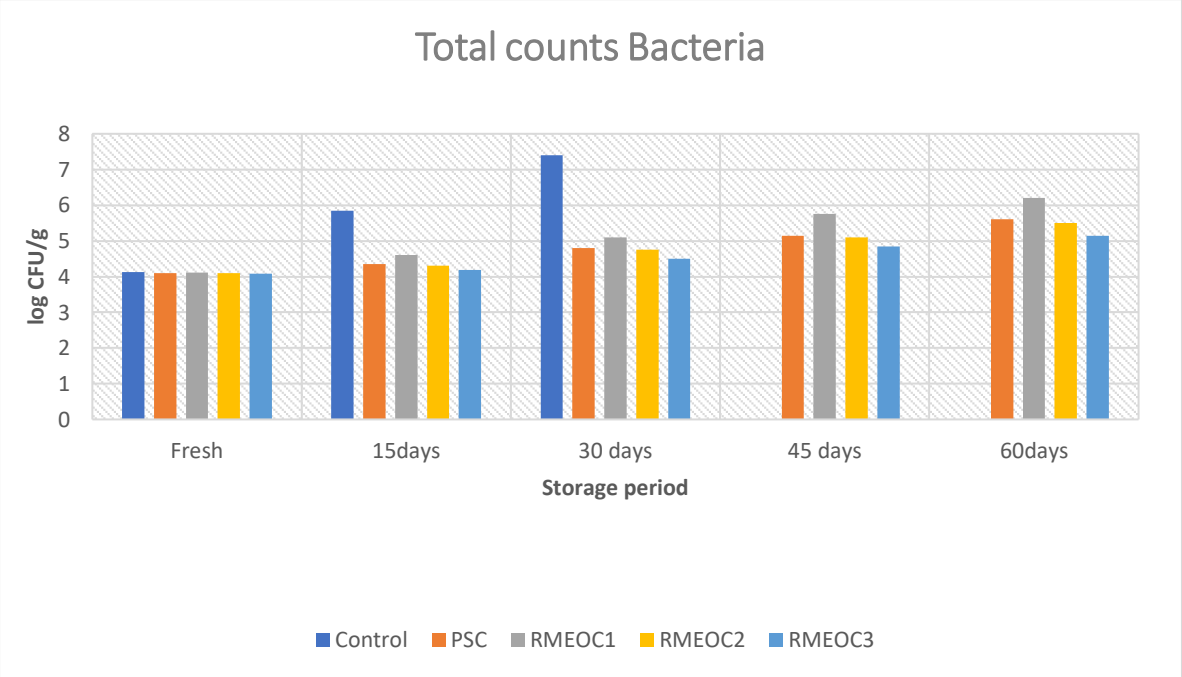


C: Control Sample**, PSC**: potassium sorbate cheese, **RMEOC1**: cheese containing 0.1% rosemary essential oil, **RMEOC2**: cheese containing 0.2% rosemary essential oil, **RMEOC3**: cheese containing 0.3% rosemary essential oil,

Figure 3. Changes in antioxidant capacity of low-salt soft white cheese treatments (Control vs. RMEO-treated cheese) during 60 days of refrigerated storage

3.4. Total bacterial counts (TBC) of low-salt soft cheese samples

Microbial evaluation of low-salt soft cheese stored at refrigeration temperature (4 ± 1 °C) for 60 days. **Fig. 4** showed clear differences among the control, the chemical preservative (potassium sorbate, PSC), and the cheese samples treated with rosemary essential oil. In the control samples, the total bacterial count increased quickly from 4.12 log $_{10}$ CFU/g} at the fresh state to 7.40 log $_{10}$ CFU/g after 30 days. After that, the control samples spoiled completely and could not be measured at 45 and 60 days. This shows that low-salt soft cheese spoils very fast without a preservative. In contrast, adding essential oil successfully stopped the fast growth of bacteria over the 60 days. Its preservative effect was very close to—and at higher levels even better than—potassium sorbate cheese (PSC). While PSC kept the bacterial count at 5.60 log $_{10}$ CFU/g at 60 days, the highest concentration of rosemary oil (0.3%) gave the best result by keeping the count at 5.15 log $_{10}$ CFU/g. Also, the medium concentration (0.2%) gave results similar to PSC (5.50 log $_{10}$ CFU/g at 60 days), and even the lowest concentration (0.1%) successfully prevented spoilage and kept the cheese safe up to 60 days (6.20 log $_{10}$ CFU/g. Antimicrobial activity is likely attributed to REO's phenolic components, such as 1,8-cineole, camphor, and α-pinene, which are known to disrupt microbial membranes and interfere with cellular processes (**Kontogianni et al., 2022).** These results prove that rosemary essential oil can be successfully used as a natural, safe alternative to chemical preservatives to extend the shelf life of low-salt soft cheese up to 60 days.



C: Control Sample**, PSC**: potassium sorbate cheese, **RMEOC1**: cheese containing 0.1% rosemary essential oil, **RMEOC2**: cheese containing 0.2% rosemary essential oil, **RMEOC3**: cheese containing 0.3% rosemary essential oil,

Fig. 4. Total bacterial counts (TBC) of low-salt soft cheese containing rosemary essential oil during refrigerated storage at 4℃ for 60 days

The results presented in **Table (3)** indicate that the addition of rosemary essential oil effectively inhibits the growth of yeast and molds in low-fat white soft cheese throughout the 60-day refrigerated storage period **(Carvalhinho et al., 2012).** Yeast and mold counts were initially not detected (ND) in all fresh samples. They progressively increased in the control group over time. In contrast, the treatments containing rosemary essential oil (RMEOC1, RMEOC2, and RMEOC3) showed significantly lower or delayed microbial counts, demonstrating its strong antimicrobial potential as a natural preservative to extend the shelf life and safety of the cheese.

Table (3). Effect of rosemary Essential Oil (RMEO) on yeast & mould counts in low-salt white soft cheese during refrigerated storage at (±4℃) for 60 days

Treatment	Yeast &moulds (log_{10} CFU /g)				
	Storage period				
	Fresh	15days	30 days	45 days	60days
Control	ND	1.85	3.42	S	S
PSC	ND	ND	ND	5.15	5.60
RMEOC1	ND	ND	1.45	2.30	2.95
RMEOC2	ND	ND	ND	1.30	1.83
RMEOC3	ND	ND	ND	ND	1.10

C: Control Sample**, PSC**: potassium sorbate cheese, **RMEOC1**: cheese containing 0.1% rosemary essential oil, **RMEOC2**: cheese containing 0.2% rosemary essential oil, **RMEOC3**: cheese containing 0.3% rosemary essential oil, **ND:（Not Determinde）＜10 CFU/g.**

3.6. Sensory evaluation of low salt soft cheese containing RMEO

The results in **Table (4)** indicated that all the cheese samples treated with essential oil maintained acceptable flavor scores over the course of the 60-day storage period, according to the sensory evaluations. Adding rosemary essential oil effectively increased the product's shelf life and prevented microbiological deterioration, while the control sample showed signs of spoilage during the later storage intervals (at 45 and 60 days). The product's shelf life was successfully increased, and microbiological deterioration was avoided by adding RMEO. The treatments showed that these natural antibacterial extracts maintained the product's freshness during prolonged storage while offering good customer acceptability without introducing an overpowering off-flavour.

Table 4. Sensory properties of low-salt soft cheese containing different concentrations of RMEO during refrigerated storage at (4±1 ̊C) for 60 days

Treatment	**parameter**	**Storage Period (days)**				
		Fresh	**15**	**30**	**45**	**60**
Control	**Flavour Score (50)**	**37**	**38**	**40**	**Spoiled**	**Spoiled**
PSC		**36**	**36**	**38**	**39**	**39**
REOC1		**31**	**32**	**33**	**34**	**36**
REOC2		**33**	**34**	**35**	**36**	**38**
REOC3		**34**	**35**	**36**	**37**	**39**
Control	**Body & Texture (35)**	**35**	**30**	**23**	**Spoiled**	**Spoiled**
PSC		**34**	**34**	**30**	**32**	**33**
REOC1		**28**	**30**	**31**	**31**	**32**
REOC2		**30**	**30**	**32**	**32**	**33**
REOC3		**30**	**31**	**32**	**33**	**33**
Control	**colour & appearance (15)**	**14**	**14**	**10**	**Spoiled**	**spoiled**
PSC		**13**	**13**	**13**	**14**	**10**
REOC1		**12**	**12**	**14**	**13**	**14**
REOC2		**10**	**10**	**12**	**12**	**12**
REOC3		**9**	**8**	**8**	**8**	**7**

C: Control Sample**, PSC**: potassium sorbate cheese, **RMEOC1**: cheese containing 0.1% rosemary essential oil, **RMEOC2**: cheese containing 0.2% rosemary essential oil, **RMEOC3**: cheese containing 0.3% rosemary essential oil.

It has been demonstrated that RMEO and other antimicrobial plant oils can increase the antioxidant capacity of dairy products without overpowering their natural flavours. **(Fernandes et al., 2017).** Regarding body and texture, the cheese samples treated with varying concentrations of rosemary essential oil (RMEO) (0.1%, 0.2%, and 0.3%) exhibited relatively stable and comparable scores during storage relative to the control group. The essential oil did not adversely disrupt the soft cheese's structural protein matrix, allowing the texture to remain cohesive, smooth, and commercially acceptable through the end of the 60-day storage period. Colour scores remained generally acceptable across the lower and moderate concentrations. However, a gradual and noticeable decrease was observed at the highest concentration (0.3%) of RMEO. This minor reduction in panelist scores is directly attributed to the inherent natural yellowish-green pigmentation and intense aromatic extract characteristics of the rosemary essential oil itself. At elevated application levels, the oil's natural color becomes more pronounced within the cheese matrix, slightly altering the characteristic uniform appearance expected by the panelists, which naturally resulted in lower scores compared to the control and lower concentration treatments.

4. Conclusion

Rosemary essential oil (RMEO), particularly at 0.3% concentration in cheese, serves as a highly effective natural bio-preservative agent, offering a promising solution to extend the shelf life and enhance the functional quality of soft white cheese without relying on synthetic additives.

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