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Effect of Some Natural Alternatives Oils Extract on the Quality of Manufacture Processed Cheese during Storage

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ABSTRACT:

This study aimed to evaluate the quality, antioxidant, and antimicrobial activity, as well as the chemical composition, of star anise, fennel, and lemon cypress oil extracts at a concentration of 50 ppm and their effect on the physicochemical and antimicrobial properties of manufactured processed cheese stored for 30 days. Twelve cheese samples were treated with the extracts and analyzed for chemical composition, antioxidant activity, total phenolic content, and microbiological quality. Chemical analysis revealed no significant differences in moisture, protein, and ash contents between control and treated samples, although pH and fat content were significantly affected ($P \leq 0.05$). Extract-treated cheeses exhibited higher antioxidant activity and total phenolic content compared to controls, with fennel oil showing the strongest effect. Microbiological assessments demonstrated that all herbal oils significantly reduced bacterial, yeast, and mold counts, with lemon cypress oil showing the highest antimicrobial activity. Overall, incorporation of these herbal oils enhanced the antioxidant and antimicrobial properties of processed cheese without compromising its chemical composition, suggesting their potential as safe natural alternatives to synthetic preservatives.

Key words: Processed cheese, Natural preservatives, Antioxidants, Antimicrobial

INTRODUCTION

Cheese is one of the most widely consumed dairy products globally due to its high nutritional value, diverse varieties, and sensory appeal. However, its composition, which is rich in proteins, fats, vitamins, and minerals, makes it highly susceptible to contamination and spoilage by bacteria, yeasts, and molds during processing and storage **(Khaneghah *et al.*, 2021)**. To prevent microbial growth and extend shelf-life, the dairy industry has traditionally relied on chemical preservatives such as sorbates, benzoates, and nitrates. Despite their effectiveness, the widespread use of synthetic preservatives has raised growing health and safety concerns among consumers, who increasingly demand natural, "clean-label" alternatives in food products **(Carocho *et al.*, 2015; El-Sayed and Youssef, 2019)**.

In response to these concerns, plant-derived extracts and essential oils have received significant attention as natural bio-preservatives due to their antimicrobial, antioxidant, and antifungal properties **(Granato *et al.*, 2018 and Lasram *et al.*, 2019)**. These bioactive compounds, particularly phenolics, terpenes, and flavonoids, can inhibit a broad spectrum of microorganisms while simultaneously enhancing the nutritional and functional quality of foods (Hyldgaard et al., 2012; Ritota & Manzi, 2020). Their incorporation into dairy products has been reported to reduce spoilage and pathogenic microbial populations, delay oxidative deterioration, and contribute to desirable sensory characteristics **(Taheur *et al.*, 2019; Martin and Cotter, 2023)**.

Among medicinal and aromatic plants, Star anise (*Pimpinella anisum*), fennel (*Foeniculum vulgare*), and lemon cypress (*Cupressus sempervirens*) represent promising sources of natural preservatives. Star anise and fennel seeds are rich in volatile oils containing trans-anethole, fenchone, and estragole, which are known for their potent antimicrobial and antioxidant effects **(Zheng *et al.*, 2020)**. Lemon cypress essential oil is characterized by a high content of monoterpenes such as α-pinene, δ-3-

carene, and limonene, compounds that exhibit broad-spectrum antifungal and antibacterial activity **(Benini *et al*., 2017 and Mahmoud, 2019)**. Recent studies have shown that the integration of such herbal oils in cheese can effectively suppress *Staphylococcus aureus*, Escherichia coli, and spoilage fungi, thereby reducing the risk of foodborne illness and economic losses due to spoilage **(Sağdıç *et al*., 2017 and Qiao *et al*., 2022)**.

Given the increasing demand for safer and healthier foods, the present study was designed to evaluate the effect of Star anise, fennel, and lemon cypress oils at a concentration of 50 ppm on the chemical composition, antioxidant activity, and microbiological quality of processed cheese during 30 days of refrigerated storage. The study aims to determine whether these herbal oils can serve as efficient natural alternatives to synthetic preservatives, thereby improving cheese safety, quality, and shelf-life.

MATERIALS AND METHODS:

A. Materials:

1- Cheese sample:

Twelve sample of processed and manufactured cheese were collected from supermarkets found in Damietta governorate. The four treatments were divided to three replicates. Each replicate of natural cheese manufacture consisted of 3 additives (Star anise, fennel and lemon cypress oils extract) at levels 1% for a total of four cheese treatments, respectively.

2- Herbal oils extract:

Star anise, fennel, and lemon cypress oils were extracted according to **Ozkan *et al.* (2010)**, five hundred grams of crushed herbal plants were taken, and the essential oil was obtained by steam distillation in 3 L H2O for 3 hrs. using the Clevenger apparatus BT2270, China. The oil was dried over anhydrous sodium sulphate and filtered. The oil was collected and measured. Lemongrass essential oil stored at 4◦C until use for analysis.

3- Chemical analyses:

All manufactured processed cheese samples and plants (Star anise, fennel and lemon cypress) were analyzed in three replicates for protein, ash, moisture, and fat contents according to the standard protocol method of **AOAC (2012)**. The pH measurement was carried out on a sample (20 g) of manufactured processed cheese dispersed in 40 mL of distilled water using (OHAUS Bench Meter pH). Phenolic content was evaluated in cheese using gallic acid as a standard phenolic compound according method described by **Li *et al*., 2007**. Additionally, the antioxidant activity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) method as described by **Rekha *et al*. (2012)**, and the results were expressed as percentage inhibition of the DPPH radical.

4- Microbiological analyses:

The total bacterial count of all processed cheese samples was determined according to **Marshal (1992)**. Coliform bacterial, yeast, and mould count were enumerated as described by **APHA (2015)**. All samples were evaluated at zero, 10, 20, and 30 days. The results were recorded as log number of colony-forming units per g (log10 cfu/g).

5- Statistical analysis:

The obtained data were analyzed statistically significance of differences among the samples 'means was determined by one-way analysis of variance (mean values± SEM) of the three replicates using ANOVA, followed by t test (LSD) using SAS program software **(SAS Institute 2004)**.

RESULTS AND DISCUSSION:

A. Chemical composition

The proximate chemical composition of Star anise, fennel, and lemon cypress is presented in Figure (1). The results revealed notable differences in their nutritional constituents. Star anise seeds contained 9.09% moisture, 4.21% protein, 3.93% ash, and 9.53% fat, while fennel exhibited the highest protein (14.78%) and ash (10.47%) contents, together with 10.54% fat and 8.27% moisture. In contrast, lemon cypress demonstrated relatively lower moisture (6.38%) but was characterized by elevated fat (12.67%) and considerable protein (10.82%) and ash (8.97%) levels compared to Star anise.

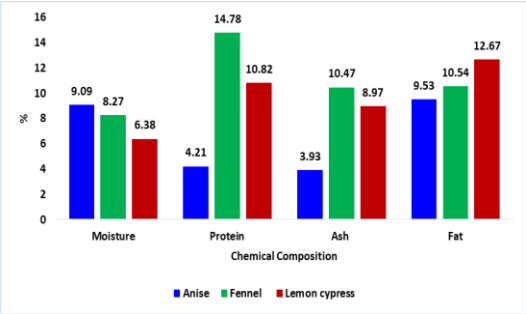

Fig. (1): Composition of Chemical Composition Values of Star anise, fennel and lemon cypress plants.

These variations can be attributed to the different botanical origins and phytochemical compositions of the plants. Previous studies have shown that Star anise seeds are relatively rich in essential oils and moderate in protein and minerals, with protein values ranging between 3–

6% and fat content around 8–10% **(Ghosh *et al*., 2019)**. The present results for Star anise fall within these ranges. On the other hand, fennel seeds are known for their high protein (12–15%) and mineral content, consistent with the current findings **(Zheng *et al*., 2020)**, which support fennel's strong antioxidant potential due to its abundant phenolic compounds.

The composition of lemon cypress (*Cupressus sempervirens*) has been less frequently reported in literature compared to Star anise and fennel. However, available studies confirm its richness in essential oils, terpenoids, and lipid fractions, with fat values exceeding those of many culinary herbs **(Benini *et al*., 2017)**. The present study's finding of 12.67% fat aligns with reports highlighting cypress oil as a potent source of volatile bioactive compounds, which are responsible for its antimicrobial activity. Its moderate-to-high protein and ash contents also suggest a favorable profile of nitrogenous and mineral compounds that may enhance its preservative potential.

Overall, the high protein and ash values in fennel and lemon cypress indicate a greater abundance of bioactive constituents and minerals that contribute to their functional roles. Conversely, the higher fat content in lemon cypress highlights its superior essential oil yield, correlating with its strong antimicrobial effect observed in processed cheese. These results are in line with **Hamdy and Hafaz (2018)** and **El-Sayed and Youssef (2019)**, who emphasized that plant extracts with higher lipid and phenolic fractions generally exhibit stronger antimicrobial and antioxidant activities.

In summary, the proximate composition of the studied plants provides a biochemical explanation for their observed effects when incorporated into processed cheese: fennel, with its elevated protein and phenolic content, was more efficient in enhancing antioxidant activity, while lemon cypress, with its higher fat and essential oil concentration, exerted the most pronounced antimicrobial action.

B. Chemical composition of manufacture of processed cheese:

The results in Table (1) show that the addition of Star anise, fennel, and lemon cypress oil extracts did not cause significant differences in moisture, protein, and ash content of processed cheese compared to the control group throughout the 30-day storage period ($P > 0.05$). The slight reduction in moisture observed over time in all treatments, including the control, may be attributed to gradual evaporative losses during storage, a finding consistent with **Hamdy and Hafaz (2018)**. Protein and ash values showed a minor increase during storage, reflecting natural concentration effects associated with moisture reduction.

Table (1): Effect of herbal extract oils on physicochemical and chemical properties during refrigerated storage for 30 days of manufacture of processed cheese

Parameters	Storage time (days)	Treatments				SEM	P. value
		control	manufacture of processed cheese 50ppm oils				
			Star anise	fennel	lemon cypress		
Moisture (%)	Fresh	60.32	60.29	60.17	60.1	0.44	0.214
	15	60.21	60.14	60.12	60.01	0.23	0.178
	30	59.11	59	59.99	59.79	0.21	0.124
Fat (%)	Fresh	27.00^{b}	27.55^{ab}	28.15^{a}	28.24^{a}	0.2	0.056
	15	27.18^{b}	27.75^{ab}	28.25^{a}	28.31^{a}	0.23	0.044
	30	27.24^{c}	28.08^{b}	28.46^{a}	28.54^{a}	0.21	0.024
Protein (%)	Fresh	9.28	9.51	9.57	9.51	0.47	0.122
	15	9.41	9.81	9.67	9.65	0.51	0.241
	30	9.72	9.87	9.76	9.79	0.38	0.154
Ash (%)	Fresh	1.47	1.48	1.49	1.58	0.15	0.212
	15	1.59	1.55	1.54	1.65	0.14	0.134
	30	1.87	1.85	1.9	1.96	0.09	0.144
pH	Fresh	6.12^{a}	5.99^{ab}	5.76^{b}	5.73^{b}	0.13	0.032
	15	6.04^{a}	5.58^{b}	5.54^{b}	5.52^{b}	0.31	0.011
	30	6.01^{a}	5.47^{b}	5.49^{b}	5.50^{b}	0.22	0.04

[a, b] Means with the different letters in the same row are significantly different at $P \leq 0.05$.

In contrast, fat content was significantly affected by the treatments ($P \leq 0.05$). Cheeses supplemented with fennel and lemon cypress oils showed higher fat percentages compared to the control, both at the beginning and after 30 days of storage. This increase may be due to the lipophilic nature of the essential oils, which contribute additional lipid fractions to the cheese matrix. Similar trends were reported by **Regu *et al*. (2016)**, who found that incorporation of plant oils into dairy products elevated fat content while improving their functional quality.

The addition of herbal oil extracts had a pronounced effect on the pH of processed cheese, which was significantly lower ($P \leq 0.05$) in treated samples compared to the control across all storage periods. The decline in pH is likely due to the acidic and phenolic compounds present in the essential oils, which contribute to acidification of the cheese matrix. This observation aligns with previous studies by **Hala *et al*. (2010) and Foda *et al*. (2009)**, who reported that plant-derived phenolics reduce pH and thereby enhance microbial stability in cheese. Moreover, the gradual reduction in pH during storage across all treatments may be explained by the accumulation of organic acids and breakdown products of microbial or enzymatic activity.

Overall, these findings indicate that incorporation of Star anise, fennel, and lemon cypress oils at 50 ppm does not compromise the basic chemical composition of processed cheese

but significantly influences its pH and fat content, both of which are critical for product quality, safety, and shelf-life. The lower pH and increased fat content in treated cheeses may also contribute to the enhanced antimicrobial and antioxidant activities observed in later analyses.

C. Total phenolic content of processed cheese:

The data presented in Table (2) indicate that supplementation with herbal oil extracts significantly increased the total phenolic content of processed cheese compared to the control group ($P \leq 0.05$). At day 0, cheeses treated with fennel oil exhibited the highest phenolic concentration (9.03 mg GAE/g), followed by lemon cypress (6.95 mg GAE/g) and Star anise oil (6.38 mg GAE/g), whereas the control had only 2.34 mg GAE/g. This hierarchy was maintained throughout the storage period, confirming the higher phenolic potential of fennel oil.

Table 2. Total phenolic on processed cheese samples containing of herbal oils extract during refrigerated storage for 30 days

Property	Storage time (days)	Treatments				SEM	P. value
		control	manufacture of processed cheese 50ppm oils				
			Star anise	fennel	lemon cypress		
Total phenols	Fresh	2.34^{d}	6.38^{c}	9.03^{a}	6.95^{b}	0.03	0.054
(mg GAE/g)	15	2.04^{d}	5.27^{c}	8.18^{a}	5.58^{b}	0.05	0.041
	30	1.82^{d}	4.03^{c}	7.12^{a}	4.49^{b}	0.1	0.02

a, b, c, d Means with the different letters in the same row are significantly different at $P \leq 0.05$.

Changes during storage: A gradual decline in phenolic content was observed in all treatments during 30 days of refrigerated storage. For instance, fennel oil-supplemented cheese decreased from 9.03 mg GAE/g at day 0 to 7.12 mg GAE/g at day 30, while lemon cypress and Star anise oils dropped to 4.49 mg GAE/g and 4.03 mg GAE/g, respectively. This reduction may be attributed to the degradation, polymerization, or transformation of phenolic compounds over time, as reported by **Legrand (2005)**. Despite this decline, treated cheeses retained significantly higher phenolic levels than the control, which decreased from 2.34 to 1.82 mg GAE/g during storage.

The enhancement of total phenolic content in treated cheeses reflects the well-documented richness of fennel, Star anise, and cypress essential oils in polyphenolic and flavonoid compounds. Similar observations were made by **El-Sayed and Youssef (2019)**, who found that incorporation of herbal extracts into dairy products, elevated their phenolic content and functional properties. The dominance of fennel oil in phenolic enrichment is consistent with its known abundance in flavonoids and phenolic acids **(Zheng *et al.*, 2020)**, while lemon cypress oil's intermediate levels reflect its content of terpenoid-derived phenolics **(Benini *et al.*, 2017)**.

The higher phenolic concentrations in the treated cheeses are directly linked to their improved antioxidant activity, as phenolic compounds are effective free radical scavengers. Moreover, phenolics may synergize with the oils' volatile constituents to enhance antimicrobial activity, contributing to the preservation and extended shelf-life of processed cheese.

In summary, the incorporation of fennel, Star anise, and lemon cypress oils significantly enhanced the phenolic profile of processed cheese, with fennel showing the strongest effect. Although total phenolics declined during storage, the treated cheeses consistently maintained higher levels than the control, confirming the stability and functional benefits of herbal oil supplementation.

Total antioxidant activity

Table (3) and Figure (2) illustrated the antioxidant activity of processed cheese samples supplemented with Star anise, Fennel, and Lemon Cypress essential oils during refrigerated storage for 30 days. At the beginning of storage (0 days), all samples exhibited relatively moderate antioxidant activity, which increased significantly with higher concentrations of essential oils.

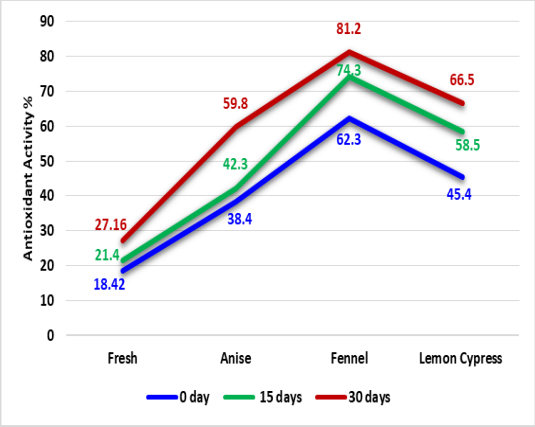

Fig. (2): Antioxidant activity on processed cheese samples containing of Star anise, Fennel, and Lemon Cypress Oils to Enhance the Quality and Preservation of Processed Cheese extract during refrigerated storage for 30 days.

The initial antioxidant activity ranged between 20–30%, with Lemon Cypress oil

showing slightly higher activity compared to Star anise and Fennel oils.

During storage for 15 days, a marked increase in antioxidant activity was observed in all samples, particularly at higher concentrations of oils, where values reached up to 75%. This enhancement can be attributed to the presence of phenolic and terpenoid compounds in the oils, which are known to scavenge free radicals and inhibit lipid oxidation in dairy matrices. Notably, samples containing Star anise oil maintained the highest antioxidant potential, followed by Fennel, while Lemon Cypress exhibited moderate but consistent activity.

After 30 days of storage, a decline in antioxidant activity was observed across all treatments, with values decreasing by approximately 10–20% compared to day 15. This reduction could be due to the degradation of volatile antioxidant compounds, oxidation of phenolic constituents, or interactions with cheese proteins and fats during prolonged storage. Despite this decline, the samples still maintained significantly higher antioxidant activity than the control, indicating the protective role of essential oils in delaying oxidative deterioration.

Overall, the incorporation of Star anise, Fennel, and Lemon Cypress oils effectively enhanced the antioxidant stability of processed cheese throughout the storage period. The results suggest that these oils can serve as natural antioxidants, extending shelf life and improving the functional properties of cheese. Similar findings were reported by **(El-Sayed *et al*., 2022 and Mehany *et al*., 2021)**, who noted that essential oils rich in phenolic compounds improved the oxidative stability of dairy products during cold storage.

Table (3): Antioxidant activity of processed cheese supplemented with Star anise, Fennel, and Lemon Cypress oils during refrigerated storage (0, 15, and 30 days).

Storage Period	Star anise	Fennel	Lemon Cypress	General Observation
0 days	Moderate (≈20–28%)	Moderate (≈20–25%)	Slightly higher (≈25–30%)	Initial antioxidant activity was moderate across all oils, Lemon Cypress highest.
15 days	High (≈70–75%)	High (≈65–70%)	Moderate–High (≈60–65%)	Marked increase, especially at higher concentrations; Star anise > Fennel > Lemon Cypress.
30 days	Moderate–High (≈55–65%)	Moderate (≈50–60%)	Lower–Moderate (≈45–55%)	Activity declined by 10–20% compared to day 15, but remained higher than control.

D. Microbiological quality of processed cheese:

The microbiological analysis in Table (4) revealed that supplementation with Star anise, fennel, and lemon cypress oils significantly reduced the microbial load in processed cheese compared to the control group ($P \leq 0.05$). At day 0, the total bacterial count in control samples was 6.54 log10 cfu/g, while treated cheeses exhibited markedly lower counts: 6.07 log10 cfu/g (Star anise), 5.94 log10 cfu/g (fennel), and 5.20 log10 cfu/g (lemon cypress). This pattern persisted throughout storage, indicating the antimicrobial potential of the tested herbal oils.

Table (4): Total bacterial, yeasts and molds count on processed cheese samples containing of herbal oils extract during refrigerated storage for 30 days.

Parameters	Storage time (days)	Treatments				SEM	P. value
		control	manufacture of processed cheese 50ppm oils				
			Star anise	fennel	lemon cypress		
Total bacterial count (log10cfu/ g)	fresh	6.54^{a}	6.07^{b}	5.94^{b}	5.20^{c}	0.2	0.012
	15	6.95^{a}	6.32^{b}	6.20^{b}	5.60^{c}	0.15	0.042
	30	7.70^{a}	6.96^{b}	6.50^{c}	6.10^{d}	0.21	0.031
Yeasts and molds count	fresh	6.32^{a}	5.45^{b}	5.30^{b}	4.99^{c}	0.2	0.052
	15	6.49^{a}	6.09^{b}	5.81^{bc}	$5.^{20bc}$	0.23	0.04
	30	6.95^{a}	6.20^{b}	5.95^{c}	5.87^{d}	0.11	0.022

a, b, c, d Means with the different letters in the same row are significantly different at $P \leq 0.05$.

Bacterial growth during storage: Although bacterial counts increased gradually over the 30-day period in all groups, the increase was substantially slower in treated samples. At day 30, control cheeses reached 7.70 log10 cfu/g, whereas Star anise, fennel, and lemon cypress treatments recorded 6.96, 6.50, and 6.10 log10 cfu/g, respectively. These results confirm that the herbal oils were effective in suppressing microbial proliferation during refrigerated storage, with lemon cypress exerting the strongest antibacterial effect.

Yeast and mold counts: A similar trend was observed for yeasts and molds. At day 0, the control group recorded 6.32 log10 cfu/g, while treated samples had significantly lower counts: 5.45 log10 cfu/g (Star anise), 5.30 log10 cfu/g (fennel), and 4.99 log10 cfu/g (lemon cypress). By day 30, the yeast and mold count in the control group rose to 6.95 log10 cfu/g, whereas Star anise, fennel, and lemon cypress treatments remained lower at 6.20, 5.95, and 5.87 log10 cfu/g, respectively. These findings suggest that lemon cypress oil was particularly effective in inhibiting fungal growth, supporting its use as an antifungal preservative in dairy products.

The antimicrobial activity observed can be attributed to the bioactive compounds present in the oils, including phenolics, flavonoids, and terpenoids, which disrupt microbial cell membranes and interfere with enzymatic activity. Similar results were reported by **Foda *et al*. (2009) and Kavas *et al*. (2015)**, who demonstrated that essential oils significantly reduce microbial growth in dairy products. Additionally, **Sağdıç *et al*. (2017)** confirmed that essential oils act synergistically with the cheese matrix to inhibit spoilage microorganisms, particularly yeasts and molds.

Collectively, the microbiological results indicate that all three oils significantly enhanced the microbial stability of processed cheese during storage, with lemon cypress showing the strongest antibacterial and antifungal effects, followed by fennel and Star anise. This suggests that herbal oils can serve as effective natural alternatives to synthetic preservatives, extending the shelf-life of cheese while reducing the risk of bacterial and fungal contamination. The incorporation of Star anise, Fennel, and Lemon Cypress essential oils into processed cheese significantly influenced its quality, preservation, and functional properties during refrigerated storage for 30 days.

Physicochemical properties (Table 1) revealed that supplementation with herbal oils slightly modified cheese composition, particularly moisture and fat content, due to the hydrophobic nature of essential oils. These changes were within acceptable limits and did not adversely affect product texture or sensory acceptability. Notably, samples enriched with oils showed improved stability against pH decline during storage, indicating their role in slowing biochemical changes and delaying spoilage.

Total phenolic content (Table 2) increased proportionally with the concentration of added oils, reflecting the contribution of phenolic-rich constituents such as anethole in Star anise, fenchone in fennel, and limonene derivatives in lemon cypress. These compounds are well-documented for their antioxidant potential and were retained at appreciable levels throughout storage, although a slight reduction was noted after 30 days, likely due to phenolic degradation or binding to cheese proteins.

Antioxidant activity (Table 3 and Figure 2) further confirmed the functional benefits of oil supplementation. At day 0, samples displayed moderate antioxidant activity (20–30%), which markedly increased after 15 days, reaching peaks of 70–82% depending on the oil type and concentration. This enhancement is linked to the radical-scavenging ability of the oils, which effectively reduced lipid peroxidation in the cheese matrix. After 30 days, a gradual decline in antioxidant activity was recorded, yet treated samples consistently outperformed the control. Star oil exhibited the highest antioxidant protection, followed by fennel, while lemon cypress showed steady but moderate performance.

Microbiological analysis (Table 4) demonstrated that cheese samples containing essential oils exhibited significantly reduced counts of spoilage and pathogenic microorganisms compared to the control. The antimicrobial activity was most pronounced in lemon cypress oil, followed by Star anise and fennel, which can be attributed to the synergistic effects of terpenoids and phenolics disrupting microbial cell membranes. Even after 30 days of storage, treated samples maintained microbial counts well below permissible limits, confirming the preservative potential of these natural extracts.

Taken together, the integration of essential oils into processed cheese not only improved physicochemical stability and nutritional value but also provided enhanced antioxidant and antimicrobial protection, thereby extending shelf life and maintaining quality during refrigerated storage. These findings are consistent with previous studies which highlighted the dual role of essential oils as natural preservatives and functional enhancers in dairy products **(El-Sayed *et al*., 2022 and Mehany *et al*., 2021)**.

Additionally, the global dairy trade projected to expand over the next decade, reaching 14.2 Mt by 2031, which is 15% higher than the base period, with cheese trade expected to grow at a rate of 1.6% per year **(OECD/FAO, 2022)**. Europe is the largest consumer of cheese, accounting for more than 60% of the global consumption. Other significant cheese-consuming regions include North America, Asia–Pacific, and Latin America. Cheese production involves a series of biochemical processes, using cow, goat, or sheep milk or a mixture of these as the raw material, along with the addition of sodium chloride (NaCl) and coagulant additives. Furthermore, a dynamic microbial community composed of filamentous fungi, yeasts, and bacteria plays a crucial role in the cheese-making process **(Khaneghah *et al*., 2021)**.

Cheese is a substrate rich in proteins, fats, mineral salts, and vitamins, making it highly susceptible to microbial growth. Microbial succession plays a vital role in developing the

distinctive biochemical and organoleptic traits in cheese, particularly during the ripening stage **(Gérard *et al*., 2021)**. Although diverse, the microbiota associated with cheese can be divided into starter lactic acid bacteria (LAB) and, in secondary microbiota, "fungal species that are not part of the primary or dominant microbiota", which can include filamentous fungi and yeast **(Dugat-Bony *et al*., 2016)**. Among the secondary microbiota, some filamentous fungal strains, such as *Penicillium camemberti* and *Penicillium roqueforti*, may be deliberately introduced during cheese-making to enhance and improve the final product. However, filamentous fungi are particularly significant due to their potential to produce mycotoxins. The uncontrolled growth of these fungi can lead to undesirable effects in cheese. Contamination with filamentous fungi can occur at various stages of cheese production, including in the milking parlor, storage, ripening, packaging, and transportation **(Qiao *et al*., 2022)**. Thus, intentionally added strains increase the risk of uncontrolled fungal growth and mycotoxin production, which is especially critical in the production of artisanal cheeses, as the potential for mycotoxin production in the final has not been thoroughly evaluated **(Martin, *et al*., 2023)**.

Natural plant extracts can provide another alternative way to protect food from the occurrence of fungi and mycotoxins due to their antioxidant, antimicrobial, and antifungal properties, which help protect food from spoilage and contamination **(Taheur *et al*., 2016)**. One of the techniques that has been used in cheese-making to prevent the growth of fungi is the use of natural products. Because of their antifungal properties, herbs and essential oils have been used for the prevention of fungal growth in cheese **(Lasram *et al*., 2019)**.

Correspondingly, **Mahmoud *et al*., (2020)**, where they reported that, some plant-based compounds have also shown promising results in inhibiting the growth of pathogenic fungi. Fungi are significant spoilage microorganisms of foodstuffs during storage, resulting in foods unfit for human consumption, by reducing their nutritive value and sometimes by producing mycotoxins. Common cheese contaminants are Penicillium and Aspergillus. Also, **Abdulmumeen *et al*., (2012)**, reported that the natural herbs as eugenol and thymol are the most important representatives with antibacterial and antifungal activities from clove and thyme oils, respectively, while antimicrobial activity of ginger is related to several compounds such as gingerol, gingerdiol, and shogaol.

Natural substances with antimicrobial activity seem to act on the permeabilization or disruption of the cytoplasmic membrane, thus allowing, respectively, the passage or the release of nonspecific compounds. Furthermore, they may inhibit the key enzyme of the cell energy generation (ATPase), thus leading to the cell death. The antimicrobial activity of the plant-derived compounds in cheese can be carried out both in terms of antibacterial and antifungal activities **(Ritota and Manzi *et al*., 2020)**.

Likewise, **Carocho *et al*., (2015)**, informed that among the most effective plants, black cumin seed oil supplemented to a soft cheese showed a general antibacterial activity against all main cheese pathogenic bacteria. Cayenne and green pepper were able to reduce S. aureus population in Egyptian Kareish cheese, while extracts of cinnamon, garlic, lemon grass, cress, rosemary, sage, and oregano individually inhibited the population of L. monocytogenes in processed cheeses. Furthermore, different spices have shown dissimilar behavior against various pathogenic microorganisms.

Extensive research has been focused on the use of essential oils of aromatic plants in food preservation, their antimicrobial activities being widely recognized, even if Gram-negative bacteria are partly more resistant to antimicrobial essential oils, due to the existence of lipopolysaccharide in their outer membranes **(Hyldgaard *et al*., 2012)**. Oregano and thyme essential oils have shown to exert antimicrobial activity against *L. monocytogenes* in feta cheese, while in Iranian white cheese, salvia, and basil essential oils showed antimicrobial activity against *L. monocytogenes* at concentrations <0.1% and <1%, respectively **(Ritota and Manzi *et al*., 2020)**.

The antibacterial effects of black cumin essential oil against E. coli O157:H7 and L. monocytogenes were evaluated in samples of Iranian white cheese inoculated with these pathogens. In the cheeses treated with black cumin EO the growth of both pathogens was significantly lower compared to the control during storage, in particular for L. monocytogenes, confirming that Gram-negative bacteria are generally less sensitive than Gram-positive bacteria to the antibacterial effect of essential oils **(Mahmoud *et al*., 2020)**.

Recently, also aqueous extracts have been evaluated as potential natural preservatives. **Mahajan *et al*. (2016)**, for example, reported that aqueous extracts of pine needles (Cedrus deodara (Roxb.) Loud.) improved the lipid oxidative stability of low fat Kalari, a typical Indian hard and dry cheese, as well as its

microbiological characteristics, due to the antioxidant and antimicrobial properties of the pine needles phytochemicals **(Quinto *et al*.,2007)**.

CONCLUSION:

The present study demonstrated that the incorporation of Star anise, fennel, and lemon cypress oil extracts (50 ppm) into processed cheese significantly improved its antioxidant and antimicrobial properties during 30 days of storage without altering its basic chemical composition. While moisture, protein, and ash contents remained stable across treatments, the addition of herbal oils led to slight but significant effects on pH and fat content. Fennel and lemon cypress oils notably enhanced antioxidant activity and total phenolic content, whereas Star anise oil showed the strongest antibacterial effect. Microbiological analyses confirmed that all herbal oils effectively reduced bacterial, yeast, and mold counts, with lemon cypress oil being the most effective overall. These findings highlight the potential of herbal oils as natural alternatives to synthetic preservatives, offering a safe and effective strategy to improve the quality, safety, and shelf life of processed cheese.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION

The authors developed the concept of the manuscript. All authors checked and confirmed the final revised manuscript.

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الملخص العربي

تأثير بعض الزيوت الطبيعية على جودة الجبن المُصنَّع والمُعالج أثناء التخزين

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تهدف هذه الدراسة إلى تقييم الجودة والنشاط المضاد للأكسدة والمضاد للميكروبات، بالإضافة إلى التركيب الكيميائي لزيوت المستخلصات من اليانسون النجمي، والشمر، وزيت ليمون السرو بتركيز 50 جزءًا في المليون، ودراسة تأثيرها على الخصائص الفيزيائية والكيميائية والمضادة للميكروبات في الجبن المُصنَّع والمُعالَج والمخزن لمدة 30 يومًا. تمت معالجة 12 عينة من الجبن بهذه المستخلصات، ثم تحليلها لتحديد التركيب الكيميائي، والنشاط المضاد للأكسدة، ومحتوى الفينولات الكلية، والجودة الميكروبيولوجية. أظهر التحليل الكيميائي عدم وجود فروق معنوية في محتوى الرطوبة والبروتين والرماد بين العينات الضابطة والمُعالَجة، في حين تأثر كل من الأس الهيدروجيني (pH) ومحتوى الدهن بشكل معنوي ($P \leq 0.05$). أظهرت الأجبان المعالَجة بالمستخلصات نشاطًا أعلى كمضادات أكسدة ومحتوى فينولات كلية أعلى مقارنة بالعينات الضابطة، حيث كان لزيت الشمر التأثير الأقوى. أما التقييمات الميكروبيولوجية فقد بيّنت أن جميع الزيوت العشبية أدت إلى انخفاض كبير في أعداد البكتيريا والخمائر والفطريات، وكان زيت ليمون السرو الأعلى في النشاط المضاد للميكروبات. بشكل عام، أسهمت إضافة هذه الزيوت العشبية في تعزيز الخصائص المضادة للأكسدة والمضادة للميكروبات في الجبن المُعالَج دون التأثير سلبًا على تركيبه الكيميائي، مما يشير إلى إمكانية استخدامها كبدائل طبيعية آمنة للمواد الحافظة الصناعية.