

Effect of Rosemary Leaf Essential Oil Addition on Chemical Properties of Raw Milk During Storge

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Abstract

The aim was to extract essential oil from rosemary leaves and use it to improve the properties of raw milk and extend the shelf life of raw milk during low-temperature storage. This study provided a detailed evaluation of the chemical composition of rosemary (*Salvia rosmarinus*) leaf essential oil, providing important baseline data for its structural integrity, biochemical stability, and functional efficacy.

The extracted essential oil showed excellent physical and chemical quality indicators, along with strong antioxidant activity, with a total oil production of a significant 39.20%, with a relative density of 0.86 g/cm³. The Acid value of essential oil was set at 1.22 mg KOH/g, with a corresponding free fatty acid ratio of 0.65%. Furthermore, the initial peroxide value was recorded at a low level of 4.75 mmEq/ kg oil, indicating the absence of extensive early oxidation in oil samples. The essential oil showed strong antioxidant activity, manifested in a high ascorbic acid content of 20.40% and a high radical-scavenging activity free radical removal activity of 6.70 mmEO₂/kg oil.

Rosemary oil demonstrated a clear dose-dependent antioxidant activity to inhibit lipid peroxidation and oxidative damage in raw milk during extended cold storage. For DPPH, the 1.00% concentration produced the highest antioxidant capacity, with an average mean inhibition of 51.73% and a significant peak of 68.40% on the first day of storage. At the same time, the untreated control sample rapidly deteriorated from its initial state to a mean inhibition of only 2.10% on the eighth day. Fat oxidation was suppressed, measured via the peroxide (PV) value, effectively through 1.00% processing, which maintained a significantly lower PV of 1.36 mEq/ kg milk even on the last day of storage. However, the control group experienced a rapid oxidative acceleration, reaching 5.42 mEq/kg milk on the eighth day, indicating an advanced stage of spoilage. Similarly, the value of thiobarbituric acid (TBA)), which measures oxidation byproducts such as malondialdehyde (MDA) responsible for rancidation, were severely limited by extracts. The 1.00% essential oil treatment restricted the TBA value to only 0.50 mg malondialdehyde/kg milk on day eight, while the untreated control group increased to of 2.20 mg malondialdehyde/kg milk, well above acceptable sensory and regulatory thresholds.

Keywords: rosemary, oil, milk.

Introduction

Rosemary, one of the most prominent plants of the Lamiaceae family, has evolved from a traditional medicinal herb to a cornerstone of modern bioconservation techniques [1]. It reflects its journey through human history and its evolution into a high-tech food additive with versatile biopotency [2].

The aromatic part of rosemary is composed of volatile organic compounds that are mainly extracted by hydrodistillation [3]. It is 1,8-Cineole (Eucalyptol) which is often the predominant ingredient in many species of rosemary, and is appreciated for its powerful antibacterial and antifungal properties [2]. This compound works by disrupting the cellular respiration chain and the integrity of the plasma membrane of bacteria [3]. α -Pinene

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monoterpene bicyclic shows significant antibacterial activity, especially against Gram-positive bacteria such as *Staphylococcus aureus* [1]. This bicyclic monoterpene ketone contributes to the characteristic pungent odor of rosemary and has been shown to promote the penetration of other bioactive molecules through bacterial cell walls [3, 4].

Raw milk is one of the most chemically complex and fast-perishing agricultural products, with its high nutrient density and favorable physical environment making it a prime target for rapid biological and chemical spoilage [5].

Raw milk consists of a multiphasic system, which is an emulsion of fats, a colloidal suspension of proteins, and a solution of lactose and minerals [6]. Thanks to its high-water activity ($a_w \approx 0.99$) and its near-neutral pH (6.6–6.8), raw milk provides an ideal environment for microbial reproduction [5]. The high concentration of lactose (about 4.8%) is an easy-to-ferment energy source, while complex proteins (caseins and whey) and emulsified fats provide the building blocks of microbial metabolism [6, 7]. The milk fat globule membrane forms a natural barrier against oxidation, but mechanical shear or temperature fluctuations may rupture this membrane, exposing unsaturated fatty acids to oxidative and enzymatic attack [1, 8]. Lipid autooxidation involves a non-enzymatic chemical reaction through a free radical chain mechanism [1]. Free radicals attack the double bonds of unsaturated fatty acids, forming primary oxidation products called hydroperoxides, which are measured by peroxide value (PV) [8]. These hydroperoxides are degraded into volatile byproducts such as malondialdehyde (MDA), which is quantitatively measured by the TBARS (Thiobarbituric acid reactants), is responsible for the distinct undesirable flavors "Rancid" and "Cardboard" [1, 7]. The degradation of lipids and proteins by endogenous or bacterial enzymes leads to irreversible loss

of quality [6]. Lipases stimulate the release of free fatty acids from triglycerides, and short-chain free fatty acids (e.g., butyric acid) are particularly potent, causing pungent odors that resemble that of goat or soap [9]. Proteases (especially plasmin or bacterial proteases) break down caseins into bitter-tasting peptides, and this decomposition destroys the stability of milk curds, and may cause premature coagulation or clumping cream [6, 9].

The global dairy sector is undergoing a radical shift in conservation strategies, driven by the dual pressures of food safety requirements and changing consumer behavior (Jalloul *et al.*, 2022). Traditionally, the sector has relied heavily on manufactured chemical preservatives, such as Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), and Tert-Butylhydroquinone (TBHQ), to inhibit fat oxidation and extend the shelf life of dairy products [1, 4]. Despite the effectiveness and low cost of these processed antioxidants, some recent studies have raised serious concerns about their long-term health effects, including their potential for cancer and endocrine disorders [10, 11]. As a result, modern consumers are increasingly turning to products with natural ingredients, which are characterized by being free of artificial additives and containing known natural ingredients [12]. This consumer trend towards environmentally friendly products has led dairy manufacturers to look for bioactive alternatives that are comparable to the effectiveness of processed chemicals without compromising the nutritional value or sensory properties of raw milk [13]. Due to its complex composition of proteins, lactose, and trans fatty acids, raw milk is exceptionally susceptible to rapid microbial spoilage and oxidative rancidity, making the search for effective natural stabilizers a top priority for food scientists [6, 14].

Plants of the family Lamiaceae have emerged from diverse plant sources, notably rosemary (*Rosmarinus officinalis*

L.), as one of the most prominent in this field [15]. Rosemary extracts are unique in preservation, providing a multifaceted effect, as they contain a high concentration of phenolic diterpenes (Carnosic acid) and phenolic acids (Rosmarinic acid) that provide strong antioxidant protection and broad-spectrum antimicrobial activity [3, 4].

The regulatory status of rosemary supports its use in the preservation of raw milk, with the European Food Safety Authority (EFSA) and the U.S. Food and Drug Administration (FDA) granting rosemary extracts the food additive code "E392", recognizing them as "generally recognized as safe (GRAS)" substances for food uses [1, 16, 17]. Furthermore, the incorporation of these plant extracts is consistent with the principles of circular economy and sustainable intensification, as bioactive compounds can often be extracted from agricultural by-products or aromatic crops grown sustainably [18, 19]. Therefore, the use of rosemary in food preservation is compatible with consumer safety and environmental sustainability in

2. Hydro-distillation extraction of essential oil from rosemary leaves

The essential oil was extracted from the leaves of rosemary by the method of hydro-distillation by the Clevenger apparatus [20], which was done by cutting the plant material into small pieces and then subjecting it to hydro-distillation with an essential oil extraction device called Clevenger, as the water distillation depends on the ability of water vapor to carry the essential oil of the plant, by immersing 500 g of the plant's soft leaves in distilled water, which is inside a glass balloon (liter capacity, provided that the latter is not fully full, It works at most two-thirds of the volume of the decanter, in order to avoid exceeding the boiling zone and the rush of the mixture, and after boiling under the influence of the heat source), the water vapor is saturated with the essential oil of the plant and travels with it through vertical

the dairy supply chain [5, 10]. The objective of the present study is to extract essential oil from rosemary leaves and use it to improve milk quality and prolong the shelf life of raw milk and its oxidative stability during storage at low temperatures.

Materials and Methods

1. Rosemary seedlings

Rosemary seedlings were obtained from agricultural nurseries in Hilla/Babylon, and left in the garden of the house for a whole month and fertilized with a one-time balanced NPK fertilizer and sprayed daily with water for the purpose of giving the plant enough time to get rid of the residues of fertilizers and pesticides that were previously treated in the agricultural nurseries. The leaves were then taken, washed thoroughly several times and dried in an air dryer at a constant temperature (60 °C) for 48 hours until they were stable the weighing was then ground by an electric grinder to obtain a fine, homogeneous powder that was kept in sterile and opaque glass bottles for the purpose of estimating its chemical content.

pipes that pass through a cooling device, where the process of condensation of steam occurs and small droplets are formed that accumulate in a pipe containing distilled water, and due to the difference between the density of distilled water and essential oil, the oil remains floating on the surface of the distilled water to the bottom, and the distillation process takes three hours after boiling. The essential oil is collected in tightly closed and opaque glass bottles. The amount of water that may remain at the bottom of the bottle is eliminated by anhydrous sodium sulfate, and the bottles are kept away from light and at a temperature between (4-6) °C.

3. Essential Oil Yield (%)

The essential oil yield of rosemary was expressed in grams per 100 g of dry plant matter (leaves) according to the equation of Ban *et al.* [21]:

$$\text{Oil yield \%} = \frac{\text{amount of extracted oil (g)}}{\text{dry plant material (g)}} \times 100$$

4. Relative density of essential oil (g/cm³)
The relative density of essential oil was estimated using a 25 ml Pycnometer at

$$\text{Relative density} = \frac{\text{weight of density bottle with oil} - \text{weight of empty bottle}}{\text{volume of density bottle}}$$

5. Estimation of the Acid value of essential oil (KOH mg/g)

The acidity value of the essential oil was estimated using the soaking method by dissolving 5 g of oil in 50 ml of ethanol

$$\text{Acidic value} = \frac{(\text{NaOH sample} - \text{NaOH control}) \times \text{NaOH normality} \times 56.1}{\text{sample weight}}$$

6. Estimation of Free Fatty Acid (FFA) Content in Essential Oil (%)

Free fatty acids were estimated by carefully weighing 2 g of rosemary extract in conical flask, dissolving it completely in 50 mL of 95% ethanol, adding 3 drops of

$$\text{FAA} = \frac{(\text{sample solution volume} - \text{blank titration solution volume}) \times \text{KOH normality} \times 28.2}{\text{sample weight}}$$

7. Estimation of the value of peroxide in essential oil (mmEq/kg oil)

The peroxide value was estimated according to the method mentioned in AOAC [24] by dissolving 5 g of the essential oil sample in a mixture of chloroform and glacial acetic acid in a ratio of 2:3 and then adding 0.5 g of KI with shaking for 2 minutes and leaving it in a dark place for 10 minutes, then adding 30 ml of water and titrating with sodium

$$\text{Peroxide value} = \frac{(\text{sample of Na}_2\text{S}_2\text{O}_3 \text{ volume} - \text{control of Na}_2\text{S}_2\text{O}_3 \text{ volume}) \times \text{Na}_2\text{S}_2\text{O}_3 \text{ normality} \times 1000}{\text{sample weight}}$$

8. Estimation of DPPH value in essential oil (mmEq/kg oil)

The DPPH radical scavenging activity of rosemary oil extract was estimated by mixing 1 mL of extract in different concentrations with 3 mL of 0.1 mM methanolic DPPH solution, after incubating the mixture in total darkness at 30 °C for 30 min, and its absorption was measured at 517 nm using an ultraviolet spectrophotometer. The percentage of free

20 °C according to the method mentioned by IUPAC [22] according to the equation:

alcohol, adding 2-3 drops of Phenolphthalein indicator (1%) and then titration with 0.1 molar NaOH solution until pink color appeared according to AOCS [23] using the following equation:

Phenolphthalein guide (1%) and titrating the solution with 0.1 molar of KOH until the light pink color persists for at least 15 seconds. The FFA is then calculated according to AOCS [23] using the equation:

thiosulfate with continuous shaking until the yellow color disappears. Drops of starch indicator were added with shaking until it turns blue and soaked once Another with sodium thiosulfate until the blue color disappears, then the experiment was repeated on the blank sample by replacing the oil sample with distilled water in the same amount, and the value of peroxide was calculated using the equation:

radical scavenging is then calculated for the titration sample prepared without the plant extract [25].

9. Estimation of Ascorbic Acid Content in Essential Oil (%)

The ascorbic acid content of rosemary oil extract was estimated by spectral method by mixing a fine sample size with 5% trichloroacetic acid (C₂ HCl₃ O₂) to stabilize the vitamin complex and precipitate the overlapping proteins. After

centrifugation, the clear supernatant was reacted with the iron- phenanthroline complex reagent, which undergoes colorimetric reduction the presence of ascorbic acid. The absorption of the mixture was measured at 520 nm using an ultraviolet spectrophotometer, and the final concentration was measured by Comparison of results with standard ascorbic acid calibration curve [26].

10. Add rosemary essential oil to milk

Table (1) Cow's milk with rosemary essential oil

Details	Symbol	Treatment
No substance was added to cow's milk, and the comparison sample represented Control (0%).	T1	The first
Adding 0.25 ml of rosemary essential oil to each 100 ml of cow's milk, and the sample was represented (0.25%).	T2	The second
Add 0.5 ml of rosemary essential oil per 100 ml of cow's milk, representing the sample (0.50%).	T3	The third
Add 1 ml of rosemary essential oil to every 100 ml of cow's milk, representing the sample (1.00%).	T4	Fourth

11. Oxidation Tests for Raw Milk During Refrigerated Storage

- I. Preparation of the Dilutions: Take 1 ml of milk and put it in a tube containing 9 ml of water and mix well to obtain a dilution of 1/10 of the milk, then the other solutions were prepared by transferring 1 ml of the first dilution to the other tubes containing 9 ml of water, then the required tests were performed using these scales in the laboratories of the Health and Medical Education Authority of the Holy Shrine of Husseinia in Karbala, Iraq.
- II. Estimation of Free Radical Inhibition Percentage (DPPH) in Raw Milk during Refrigerated Storage: The DPPH radical scavenging activity of milk was estimated by mixing 1 mL of milk in different concentrations with 3 mL of 0.1 mM methanolic DPPH solution, after incubating the mixture in total darkness at room

Whole cow's milk was used from evening milking and non-heat treated by local cow breeders in Babylon Governorate, and the milk was stored in the refrigerator in Stomacher bags until use, and the following treatments were performed on it as mentioned in Table (1):

temperature for 30 minutes, and its absorption was measured at 517 nm using an ultraviolet spectrophotometer. The percentage of free radical scavenging was then calculated for a sample The calibration was prepared without milk [25].

- III. Estimation of peroxide (PV) value of raw milk (mmO₂/kg milk) during refrigerated storage: The peroxide value was estimated according to the method mentioned in AOAC [24] by dissolving 5 g of the milk sample in a mixture of chloroform and ice acetic acid in a ratio of 2:3, then adding 0.5 g of KI, shaking for 2 minutes, leaving it in a dark place for 10 minutes, then adding 30 ml of water and titration with sodium thiosulfate with continuous shaking until the color disappears Yellow. Drops of starch indicator were added with shaking until it turned blue and

rubbing again with sodium thiosulfate until the blue color disappeared, then the experiment was repeated on the blank sample by replacing the milk

$$\text{Peroxide value} = \frac{(\text{sample of Na}_2\text{S}_2\text{O}_3 \text{ volume} - \text{control of Na}_2\text{S}_2\text{O}_3 \text{ volume}) \times \text{Na}_2\text{S}_2\text{O}_3 \text{ normality} \times 1000}{\text{sample weight}}$$

IV. Estimation of thiobarbituric acid (TBA) values of raw milk (mg MDA/kg milk) during cryogenic storage: The thiobarbituric acid (mg MDA/kg milk) in chilled milk was determined by mixing 10 ml of milk sample with 10 ml of trichloroacetic acid (TCA) at a concentration of 20% for protein deposition and Malondialdehyde extraction (MDA). The mixture is separated by centrifuge at 3000 rpm for 15 minutes at 4°C, then the clear floating liquid is collected and 2 ml of this supernatant is transferred to a glass tube, 2 ml of Thiobarbituric acid aqueous solution at a concentration of 0.67% is added, and incubated in a boiling water bath (95 °C) for 20 minutes until pink appears. The tubes were immediately cooled in an ice bath to stop the reaction, and the absorption was measured at 532 nm using a

sample with distilled water in the same amount, and the value of peroxide was calculated using the equation:

spectrophoto. The final concentration was calculated using a standard calibration curve prepared from malondialdehyde and expressed in units of MDA/kg milk [27].

12. Statistical Analysis

The laboratory experiment was carried out as a factorial experiment to study the effect of the factors of adding rosemary oil at four levels (0, 0.25, 0.50, 1.00) ml oil/100 ml milk, and the storage period factor at six levels, i.e. for storage days (0, 1, 2, 4, 6, 8) days. Full randomization was used in the execution of the transactions, and each treatment was repeated three times. Data were statistically analyzed according to ANOVA analysis of variance, and the averages were compared using the least significant difference LSD test. At a probability level of 0.05. The data analysis process was performed using the Data Analysis tool in Microsoft Excel [28].

Results and Discussion

1. Physical and chemical properties of volatile essential oil of rosemary leaves

Table (2) shows the physical, chemical and antioxidant properties of volatile essential oil of rosemary leaves, including oil yield 39.20%, relative density 0.86%, acid value 1.22 mg KOH/g, free fatty acid content 0.65%, peroxide value 4.75 [29] in a similar study, they noted that rosemary oil is colorless to pale yellow, has a distinct flavor and taste. The specific density is 0.894-0.912, the refractive index is 1.464-1.476, and the optical rotation is 5-10 degrees. The oil is also insoluble in water, but is soluble at 10 times its volume of alcohol at a concentration of 80%. The pH value does not exceed 1.0, using the acidity value (ACV) and free fatty acids

(FFA). Analyze the level of fatty acids in the fat sample to determine its quality. The peroxide value (PV) measures the degree of fat oxidation in fats and oils, but does not measure their stability. When the peroxide value measures a transient (temporary) oxidation product, a low value may indicate early or advanced oxidation, which can be distinguished over time, and a low peroxide value indicates the absence of oxidation in oil samples [30].

Antioxidant activity was evaluated based on ascorbic acid content, DPPH and hydrogen peroxide removal activity (Table 2). Rosemary leaf oil extract showed a high ascorbic acid content (20.40%) and a high DPPH free radical removal activity (6.70%). As phenolic compounds increase in concentration or hydroxylation, their activity in removing DPPH free radicals,

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known as their antioxidant activity increases [31]. The therapeutic effect of many plants and herbs is due to the presence of antioxidant compounds, such as flavonoids [32], 2004), phenolic acids, and biphenolic terpenes [33]. A strong association has been found between antioxidant activity and total phenol content in the extracts. All rosemary extracts showed high free radical removal activity [34]. In another study, Kuhlmann and Röhl [35] were able to demonstrate that the DPPH free radical scavenging activity of rosemary extracts depends on the amount of the three phytochemical compounds: Carnosic acid, Carnosol and Rosmarinic acid, especially the latter. The anti-inflammatory activity of the extracts is mainly based on their content of Carnosic acid. The chemical composition and quality of oils generally vary according to the source of the plant materials used, cultivation conditions, environmental conditions, extraction method, and other factors, and for fatty acids, the pH value, in addition to the saponification value, can be used to measure the amount of neutral fats present, and free fatty acids are usually prepared by oleic acid. When free fatty acids are crossed through oleic acid, the acidity value is practically twice that of free fatty acids [36]. Unlike most of its biological activities, rosemary's antioxidant activity is directly attributable to the chemical compounds found in its essential oils and extracts, and while synergistic

mechanisms between many of the oil's components are likely to contribute to this efficacy, biphenolic terpenes, such as carnosic acid, carnosol, and rosmarinic acid, have been identified as the strongest antioxidants found in rosemary essential oil [37].

Rosemary exerts its antioxidant effect through several mechanisms' pathways, for example, rosemary essential oil and extract have been shown to scavenge free radicals and reduce oxidative damage [38]. Rosemary is also able to inhibit lipid peroxidation, a destructive process caused by oxidative stress [39]. In addition to reducing the number of reactive species in the body, rosemary has been found to increase the activity of antioxidant enzymes [40]. Taken together, these effects strengthen the body's defenses against harmful reactive species and oxidative damage, and oxidative stress contributes to the development of many diseases, and a lack of antioxidant defenses and the resulting oxidative damage have been shown to cause both diabetes and cancer [41]. By reducing oxidative stress in the body, rosemary helps prevent diseases such as free radical accumulation and other reactive species, so the powerful antioxidant activity of rosemary is fundamental to its therapeutic potential, and in fact its antioxidant activity is fundamental to almost all other therapeutic applications of rosemary [42].

Table (2) Physical and chemical properties of volatile essential oil of rosemary leaves

Value	Indicators	No.
39.20	Oil Yield (%)	1
0.86	Relative density of essential oil (g/cm ³)	2
1.22	Acidic value of essential oil (KOH mg/g)	3
0.65	Free fatty acids (FFA) in essential oil (%)	4
4.75	Peroxide value in essential oil (mmEq/ kg oil)	5
6.70	Estimation of DPPH value in essential oil (mmEq/kg oil)	6

20.40	Ascorbic acid content in essential oil (%)	8
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2. Effect of rosemary leaf essential oil addition on free radical inhibition percentage (DPPH) in raw milk during 8-day refrigerated storage

Table (3) shows the effect of adding different concentrations of rosemary essential oil (0, 0.25, 0.50, and 1.00%) on the effectiveness of DPPH radical-scavenging activity in milk during a chilled storage period of 8 days, as the statistical analysis data indicate a significant increase based on an increased concentration in the antioxidant capacity of the milk samples. The control sample (0%) showed the lowest overall significant mean of DPPH inhibition by 7.65%. As the concentration of rosemary oil increased, the free radical removal efficiency increased accordingly, recording averages of 20.43%, 33.27%, and 51.73% for concentrations of 0.25%, 0.50%, respectively. It was also observed that the highest mean of antioxidant power in the treatment was 1.00%, reaching 51.73%, significantly exceeding all other treatments.

For the storage period, the pre-treatment baseline value for all samples was 12.30%. After the addition of essential oil on the first day, the mean DPPH inhibition significantly increased to 38.28%. However, as the refrigerated storage progressed, there was a significant decrease in antioxidant activity during all subsequent days, and the mean values gradually decreased from 36.25% on the second day to 23.40% by the eighth day.

The interaction between oil concentration and storage period was significant, as on the first day, the 1.00% concentration treatment achieved a peak inhibition of 68.40%, and although this value decreased significantly to 49.20% by the eighth day, it remained significantly superior to the comparison sample, which rapidly deteriorated from an already significantly low percentage of 11.00% on the first day to a low percentage of 2.10%

by the end of the storage period (the eighth day).

The significant improvement in the effectiveness of DPPH free radical removal in milk treated with rosemary essential oil is directly attributed to the rich phytochemical composition of the extract, as rosemary oil is exceptionally rich in natural polyphenols and diterpenes, especially carnosic acid, carnosol and rosmarinic acid [43]. These bioactive compounds act as highly efficient primary antioxidants, containing structural hydroxyl groups that act as hydrogen donors, effectively equivalent to unstable DPPH free radicals and convert them into stable, inactive molecules [1]. The dose-dependent response (concentration), observed in the results, is consistent with basic chemical principles: the higher the concentrations of the essential oil, the greater the number of phenolic compounds available for the donation of hydrogen atoms, resulting in higher rates of free radical removal [44].

The gradual deterioration of antioxidant capacity during the eight-day storage period is a well-documented phenomenon in food chemistry, and as milk is stored in the refrigerator, it remains susceptible to autooxidation, particularly the peroxidation of unsaturated fats, and the phenolic compounds in rosemary oil are gradually consumed over time, inhibiting reactive oxygen species (ROS) and stopping chain oxidation reactions within the milk structure [45]. Furthermore, potential interactions between polyphenols in essential oil and milk proteins (e.g., Casein) may lead to the formation of protein-polyphenol complexes, which may partially mask antioxidant groups and reduce their measurable efficiency in eliminating free radicals over time [46]. The use of natural antioxidants, such as rosemary oil, is highly in line with the Iraqi Quality Standards (IQS) and the guidelines of the Central Organization for

Standardization and Quality Control (COSQC), which call for the maintenance of chemical stability and sensory freshness of dairy products, and the comparison samples approached oxidative exhaustion by day 8 (2.10% inhibition), which is usually associated with the appearance of sensory defects, such as unwanted flavors and rancidity. In contrast, fortified milk

with a special concentration of 1.00% maintained a large store of antioxidants throughout the storage period, suggesting that rosemary essential oil can be used as a natural and highly effective alternative to synthetic antioxidants to curb oxidative damage, preserve nutritional value, and prolong the shelf life of chilled milk [47].

Table (3) Effect of adding essential oil to rosemary leaves for raw milk treatments on free radical inhibition percentage (DPPH) during 8-day refrigerated storage

Average effect of oil concentrations	Refrigerated storage duration (days)						Treatments
	8	6	4	2	1	0	
7.65	2.10	4.20	6.60	9.70	11.00	12.30	T1
20.43	14.60	18.60	22.30	26.60	28.20	12.30	T2
33.27	27.70	32.50	38.40	43.20	45.50	12.30	T3
51.73	49.20	54.70	60.30	65.50	68.40	12.30	T4
	23.40	27.50	31.90	36.25	38.28	12.30	Average Storage Duration Effect
For oil = 1.736	For interaction = 2.744			For days = 2.241			LSD

T1: 0% Essential Oil/Milk, T2: 0.25% Essential Oil/Milk, T3: 0.50% Essential Oil/Milk, T4: 1.00% Essential Oil/Milk.

3. Effect of adding essential oil to rosemary leaves on the peroxide (PV) value of raw milk (mEq/kg milk) during chilled storage for 8 days

Table (4) shows the effect of adding different concentrations of rosemary essential oil (0, 0.25, 0.50, and 1.00%) on the peroxide (PV) value of milk, expressed in mEq/kg of milk during a chilled storage period for 8 days. Statistical analysis revealed an inverse relationship with a high significance between the concentration of rosemary essential oil and the composition of primary oxidation products. The control group (0%) showed the highest total significance of peroxide value at 2.05 mEq/kg of milk the increase in the concentration of essential oil, the average value of peroxide (PV) decreased significantly, recording values of 1.53, 1.06 and 0.60 mEq/kg of milk for treatment

concentrations of 0.25%, 0.50% and 1.00% respectively, in addition to the highest degree of significant oxidative stability at the concentration of 1.00%, which recorded the lowest mean significance of the peroxide value (PV) of 0.60 mEq/kg of milk.

Regardless of the treatment, the duration of cryogenic storage had a significant effect on the rate of fat oxidation, and all samples shared an initial base peroxide value of 0.14 mEq/kg of milk before treatment, during the 8-day storage period, the average significant PV value gradually increased, from 0.27 mEq/kg milk on the first day to the highest significant value of 3.26 mEq/kg milk on the eighth day.

The overlap between oil concentration and storage duration was significant, as the control group (0%) witnessed a rapid and accelerated rate of fat

oxidation, with the peroxide value significantly increasing from 0.34 mEq/kg of milk on the first day to 5.42 mEq/kg of milk by the eighth day. In stark contrast, higher concentrations of rosemary oil significantly inhibited this oxidative acceleration, with treatment at a concentration of 1.00% showing exceptional stability, maintaining the peroxide value Significantly lower 1.36 mEq/kg milk even on the last day of storage, effectively stopping the lipid oxidation diffusion phase compared to the unfortified control group.

The peroxide value (PV) is a very important physicochemical indicator used to quantify the amount of primary lipid oxidation products, specifically hydroperoxides, that are formed during the early stages of autooxidation in dairy fat [48]. The significant and rapid rise in the peroxide value of the comparison samples during the 8-day storage period reflects the susceptibility of the unsaturated fatty acids in milk fat to react with free radicals when exposed to dissolved oxygen, light, or Enzymatic activity, even under refrigeration conditions [49]. The observed inhibition of hydroperoxides in milk samples treated with 1.00% rosemary essential oil is directly due to the strong antioxidant capacity of the bioactive phytochemical compounds in the extract, and rosemary oil is characterized by a high concentration of phenolic diterpenes, especially Carnosic acid and Carnosol, as well as Rosmarinic acid [43]. These phenolic compounds act as powerful antioxidants that interrupt the chain of reactions, intervening in the phase of fat peroxidation diffusion by giving hydrogen atoms from their hydroxyl groups

to the alkyl or peroxy radicals in the lipids, effectively neutralizing them and preventing the continuous formation of primary hydroperoxides [1].

The concentration-dependent efficacy, observed in the data by achieving a concentration of 1.00% of the lowest significance value of PV (1.36 mEq/kg of milk on day 8), is consistent with the principle that a higher concentration of essential oil provides a denser composition of electron donor compounds, and this high concentration ensures that antioxidant stores are not fully depleted during the long storage period, allowing the milk lippocyte membrane to be sustainably protected [45]. From a commercial and regulatory quality perspective, adherence to Iraqi Quality Standards (IQS) and guidelines issued by the Central Organization for Standardization and Quality Control (COSQC) is critical for the safety of dairy products. Exceeding the peroxide (PV) value of 1.0 to 2.0 mEq/kg of milk in dairy products indicates the onset of significant primary oxidation, which quickly turns into secondary oxidation (resulting in undesirable flavors and rancidity) [48]. By day 8, the comparison sample (5.42 mEq/kg of milk) and the treated sample exceeded by 0.25% (3.80 mEq/kg of milk) is far the acceptable limit, indicating advanced chemical deterioration. In contrast, the 1.00% treatment maintained a peroxide value of 1.36 mEq/kg of milk throughout the storage period. This confirms that rosemary essential oil is a highly potent natural technical additive capable of reducing fat autooxidation, thereby preserving the organoleptic properties and nutritional value of chilled raw milk [47].

Table (4) Effect of adding rosemary leaves essential oil on the peroxide (PV) value of raw milk (mEq/kg of milk) during chilled storage for 8 days

Average effect of oil concentrations	Refrigerated storage duration (days)						Treatments
	8	6	4	2	1	0	
2.05	5.42	3.6	1.94	0.83	0.34	0.14	T1

1.53	3.80	2.70	1.52	0.71	0.30	0.14	T2
1.06	2.45	1.71	1.20	0.57	0.26	0.14	T3
0.60	1.36	0.94	0.64	0.32	0.17	0.14	T4
	3.26	2.24	1.33	0.61	0.27	0.14	Average Storage Duration Effect
For oil = 0.709	For interaction = 1.121			For days = 0.916		LSD	

T1: 0% Essential Oil/Milk, T2: 0.25% Essential Oil/Milk, T3: 0.50% Essential Oil/Milk, T4: 1.00% Essential Oil/Milk.

4. Effect of adding rosemary leaf essential oil to thiobarbituric acid (TBA) values of raw milk (MDA mg/kg milk) during 8-day refrigerated storage

Table (5) shows the effect of adding different concentrations of rosemary essential oil (0, 0.25, 0.50, and 1.00%) on the values of thiobarbituric acid (TBA) in milk measured in MDA mg/kg milk during a chilled storage period of 8 days, as the data indicate a significant decrease in dose dependent (concentration) in the formation of secondary oxidation products with an increase in the concentration of rosemary oil, as the control group (0%) recorded the highest mean TBA value At 0.65 mg MDA/kg milk, the use of rosemary oil significantly reduced the mean TBA values to 0.53, 0.29, and 0.17 mg MDA/kg milk for treatments at concentrations of 0.25%, 0.50%, and 1.00%, respectively, and the concentration of 1.00% proved to be highly significant, inhibiting the formation of malondialdehyde to its lowest observed levels.

The duration of refrigerated storage significantly affected TBA values in all pre-treatment/primary stage samples, with all samples showing an identical baseline TBA value of 0.03 mg MDA/kg milk. As the storage period progressed, the average TBA value showed a significant effect, reaching 0.18 mg MDA/kg milk on day 4 and peaking at 1.31 mg MDA/kg milk on day 8.

The overlap between essential oil concentration and storage duration had a significant effect, with the comparison

sample showing a sharp oxidative deterioration, with TBA values significantly increasing to 1.40 mg MDA/kg milk on day 6, and then to 2.20 mg malondialdehyde/kg milk on day eight. In contrast, treatments with higher concentrations of rosemary oil were successful in maintaining exceptional oxidative stability. In particular, the treatment was limited to a concentration of 1.00% of TBA to 0.50 mg malondialdehyde/kg milk only on the last day of storage, which prevented the rapid chemical damage observed in the comparison group.

The thiobarbituric acid (TBA) test is a very important and globally recognized biochemical measure, and it is used to quantify the byproducts of secondary lipid oxidation, the most important of which is malondialdehyde (MDA), as MDA is formed through the degradation of unstable primary hydroperoxides, which are generated during the autooxidation of polyunsaturated fatty acids in dairy fat [48]. Because MDA Highly volatile aldehyde is responsible for undesirable flavors such as, oxidation, and heating, and the TBA value is a direct indicator of deterioration of sensory properties and unacceptability of the product in foods [50]. The significant increase in the TBA value of the comparison samples of 2.20 mg MDA/kg milk on day eight reflects severe oxidation of secondary fats. From a quality assurance perspective, based on international guidelines and Iraqi quality standards for raw and refrigerated dairy products, a TBA value exceeding the threshold of 1.0 to 2.0

mg MDA/kg milk is strongly associated with a significant deterioration in sensory properties and consumer rejection [51]. By day six, the value of malondialdehyde in untreated milk (1.40 mg MDA/kg milk) exceeded the threshold of oxidative rancidity, resulting in a deterioration of its chemical and sensory properties.

The significant decrease in malondialdehyde composition in milk samples is attributed to Fortified with rosemary essential oil, especially at a concentration of 1.00%, to the powerful antioxidant properties of this oil, rosemary essential oil is very rich in diterpene phenolic compounds, specifically carnosol, carnosic acid, and rosmarinic acid [43]. These bioactive phytochemical compounds inhibit the chain reaction of free radicals [1]. By donating hydrogen atoms, these

compounds stabilize the primary lipid peroxides and prevent them from degrading into malondialdehyde and other volatile aldehydes [45].

The dose-dependent efficacy of rosemary oil, which was observed in this study, confirms that its high concentrations provide a more effective antioxidant barrier, maintaining the integrity of the milk lipid membrane structure throughout storage [46]. Maintaining a TBA value of 0.50 mg MDA/kg milk only after 8 days of refrigerated storage, the treatment of 1.00% rosemary oil has proven to be highly effective, demonstrating its commercial viability as a natural preservative. It is safe to significantly reduce fat peroxidation, thus prolonging the shelf life of milk and maintaining its sensory purity in accordance with stringent food safety standards [47].

Table (5) Effect of adding aqueous extract oil to rosemary leaves on thiobarbituric acid (TBA) values of raw milk (MDA mg/kg milk) during chilled storage for 8 days

Average effect of oil concentrations	Refrigerated storage duration (days)						Treatments
	8	6	4	2	1	0	
0.65	2.20	1.40	0.06	0.17	0.06	0.03	T1
0.53	1.60	0.89	0.50	0.11	0.05	0.03	T2
0.29	0.95	0.60	0.02	0.09	0.04	0.03	T3
0.17	0.50	0.27	0.14	0.06	0.03	0.03	T4
	1.31	0.79	0.18	0.11	0.05	0.03	Average Storage Duration Effect
For oil = 0.345	For interaction = 0.545			For days = 0.445			LSD

T1: 0% Essential Oil/Milk, T2: 0.25% Essential Oil/Milk, T3: 0.50% Essential Oil/Milk, T4: 1.00% Essential Oil/Milk.

Conclusions

The 1.00% essential oil concentrate strongly stopped the primary lipid peroxidation process, maintaining the lowest peroxide value (1.36 mmO₂/ kg milk) for eight days thanks to its high free radical elimination capacity. Thanks to its ability to reduce secondary lipid oxidation, the 1.00% concentration of essential oil

significantly reduced the formation of malondialdehyde to only 0.50 mg MDA/kg milk (TBA value), significantly preventing the appearance of unwanted flavors and biochemical rancidity.

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