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Extraction and GC-MS Characterization of Cardamom Essential Oil and its Antimicrobial Activity Against Dairy Microflora

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ABSTRACT *Green cardamom oil (Elettaria cardamomum L.) is a good source of bioactive volatile compounds that have antioxidant and antimicrobial properties and is therefore a prospective natural preservative in the food industry. The purpose of this study was to obtain a cardamom oil by means of hydro distillation, identify its chemical properties according to the GC-MS technique, and test its antimicrobial properties against microorganisms related to dairy products (soft cheese). According to GC-MS, the predominant compounds in the oil were 1,8-cineole (28.2) and 33.51 0 -terpinyl acetate, with other minor compounds being linalool, terpinen-4-ol, and 0 -terpineol. Agar diffusion in vitro tests showed that the oil could inhibit the growth of Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli with an increasing activity at higher concentrations. When cardamom oil was added to soft white cheese at concentrations of 0.25%, 0.50%, and 0.75%, a significant decrease in the growth of total bacteria, E. coli, yeasts, and molds was observed during 21 days of storage at 4°C, without any adverse effect on sensory qualities at low and medium concentrations. These results suggest that cardamom oil can be an effective natural antimicrobial agent in dairy products, achieving a suitable balance between microbial safety and sensory acceptability.*

Keywords: Cardamom oil, GC-MS, antimicrobial activity, soft white cheese, natural preservatives, sensory evaluation.

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INTRODUCTION

Essential oils extracted from plants are important bioactive compounds in the food and pharmaceutical industries due to their active compounds with antioxidant and antimicrobial properties. This makes them promising alternatives to synthetic preservatives, which, while effective in extending food shelf life, remain subject to scientific debate regarding their long-term safety (1). With the growing interest in healthier and more natural diets, the need to explore safe plant-based sources to improve food microbial safety has become increasingly apparent, especially given the growing problem of microbial resistance resulting from antibiotic overuse (2), Plants produce a wide range of secondary metabolites, most notably essential oils, which are characterized by their complex chemical structure and richness in volatile compounds such as terpenes and their derivatives. These oils have demonstrated broad potential in multiple fields, possessing antioxidant, antibacterial, antifungal, and antiviral properties, in addition to anti-inflammatory effects. This depends on the type and concentration of the active compounds they contain (3).

Green cardamom (Elettariacardamomum L.) (Maton) is an important aromatic plant belonging to the ginger family. Its essential oil is characterized by a chemical composition rich in volatile compounds, particularly oxidized monoterpenes such as α-terpinyl acetate (38.4%), 1,8-cineole (28.71%), linalool acetate (8.42%), sabinene (5.21%), and linalool (3.97%). These compounds are responsible for their biological properties, including antibacterial, antifungal, and antioxidant activity (4). Recent studies have shown that Cardamom oil has wide-ranging applications in the food and pharmaceutical industries. It is also used as a natural flavoring agent and a functional compound in numerous food preservation research projects (5).

confirmed that the biological activity of green cardamom oil is not limited to bacterial inhibition but extends to affecting quorum sensing mechanisms between bacterial cells. This increases its effectiveness in controlling microbial contamination within complex food systems, such as dairy products (4).

Despite these promising results, most research on the biological activity of essential oils has focused on in vitro evaluation in simple environments like microbial media, without considering their effect within complex food matrices such as dairy products. Food components like fat, protein, and starch can interact with the active compounds and limit their antimicrobial activity.

(6) pointed out that the properties of the food matrix are a crucial factor in reducing the efficacy of essential oils within food. A recent study by (7) confirmed that the antibacterial efficacy of essential oil components is highly dependent on the surrounding food components, leading to a clear discrepancy between laboratory test results and food applications. Furthermore, the use of these oils in food systems often requires higher concentration than those determined in laboratory tests, which can cause undesirable sensory effects such as altered taste and smell. This poses a major challenge to their use in the food industry (8).

Studies investigating the effect of these oils on the sensory properties of food products remain limited, even though sensory acceptability is a crucial factor in determining the feasibility of using these substances in economically viable products (9). Accordingly, this study aims to evaluate the effectiveness of locally extracted cardamom fruit essential oil using GC-MS, and to study its integrated effect on microbial growth and sensory properties in a real diet represented by soft white cheese, as well as to identify the optimal concentrations that achieve a balance between inhibitory activity and sensory acceptability, thus providing a solid scientific basis for its use as a promising natural preservative in dairy products.

MATERIALS AND METHODS

Raw material preparation

The ripe Indian-origin cardamom fruits imported for local consumption in the city of Mosul were produced in June 2025.

Extraction of essential oils

The volatile oils were extracted from cardamom fruits by hydro distillation using a Clevenger type apparatus, according to the method mentioned in [10]. The water was then removed from the oil, and it was dried using sodium sulfate. It was then placed in 50 ml colored glass bottles without leaving a vertical space and stored in the refrigerator (+4°C) until use.

Estimation of the active compounds of cardamom fruit oil using GC-MC technology

We used a gas chromatography (GC) instrument (HP6890 Series II G.C.) with an HP5973 mass-selective detector (made by Hewlett-Packard in Palo Alto, USA) to find out what volatile oils are made of and how much of each one there is. The instrument had HP-5MS capillary columns (0.25×30 m, Hewlett Packard i.d., film thickness 0.25µm) on it. The column was heated to 50°C, then raised by 3°C/min until it reached 150°C. It stayed at this temperature for 10 minutes, then raised by 10°C/min until it reached 250°C. The carrier gas was helium, which flowed at a rate of 2 mL/min. The injector was at 250°C. Hexane was used to dilute the oil samples by a factor of 1/100, and 1 microliter of each sample was put into a gas chromatography-mass spectrometer [11].

Bacterial isolates

The identified bacterial isolates were obtained from the College of Science, University of Mosul: Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, and Staphylococcus aureus. The isolates were activated on nutrient broth medium and kept refrigerated until needed.

Evaluating the effectiveness of cardamom oil in inhibiting microorganisms

The microbial inhibitory activity of cardamom oil against selected microorganisms was evaluated using the agar well diffusion method as described by [12]. Bacterial strains were activated in Nutrient Broth medium and incubated at 37°C for 24 hours, and then the bacterial inoculum density was adjusted to be equivalent to 0.5 McFarland Standard, which is approximately equivalent to 1.5×10^8 colony units/ml (CFU/mL), 20 mL of Mueller-Hinton agar was poured into sterile Petri dishes and allowed to solidify. 5 mm diameter wells were then prepared aseptically. Cardamom oil concentrations (25, 50, and 100 µL/mL) were prepared using dimethyl sulfoxide (DMSO) as a solvent, and 100 µL of each concentration was added to the wells, with DMSO used as a control,The dishes were incubated at 37°C for

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24 hours, and the diameters of the inhibition zones (mm) were then measured. All experiments were performed in triplicate, and results are expressed as mean ± standard deviation.

Cheese making

Soft white cheese was manufactured according to the method of [13][14], using whole milk. Microbial rennet was added to the milk according to the manufacturer's instructions, and it was incubated at 40°C for a sufficient time to complete coagulation. The curd was then separated from the whey using a clean cheesecloth. Salt was added at a concentration of 2% (w/w), followed by cardamom oil at concentrations of 0.25%, 0.50%, and 0.75% (w/w) of the curd. A control sample (0%) was prepared free of oil. The cheese was molded in sterile moulids, and gentle pressure was applied to push all the whey out and get a smooth and uniform soft feeling. The samples were then refrigerated at 4 o C until they could be analyzed in various manners.

Estimating the microbial content of cheese

Microbiological tests of cheese samples were conducted to determine the total bacterial count, Escherichia coli, yeasts and molds by the international ISO standards. Total bacterial count was done using ISO 4833-1:2013, Escherichia coli detection and quantification was done using ISO 16649-2:2001, and the quantification of yeast and molds was done using ISO 21527-1/2:2008. Sequential decimal dilutions were carried out, and samples were incubated on suitable selective media of each group of microbes as dictated by each standard. Results are expressed in CFU/g [15].

sensory assessment

A sensory evaluation of the cheese samples was conducted by 10 evaluators from the College of Agriculture and Forestry at the University of Mosul, aged 40-50 years, using an evaluation form that included flavor, texture, color, and bitterness. A scoring system of 1 to 10 was adopted, based on the method described in reference [13], where 10 represents the highest level of sensory acceptance and 1 the lowest. The evaluation was carried out by a panel of trained judges under standardized and controlled conditions to ensure the accuracy of the results.

Statistical analysis

The data was analyzed using the SAS Statistical Analysis System's fully randomized design (CRD). At a significance level of $P<0.05$, Duncan's multiple range test indicates that significant differences between the treatment means are indicated by different letters [27].

Results and discussion

Detection of active compounds in cardamom fruit oil using GC-MS technology

Table(1) shows the biologically active compounds that are part of the composition of the volatile oil of cardamom fruits, which were identified through GC-MS analysis and represent about 90% of it. The table had determined 21 compounds in cardamom fruit oil. The main bioactive components were found to be α-terpinyl acetate (33.5%), 1,8-cineole (28.2%), sabinene (5.11%), and linalool (1.99%). These findings correspond to other researchers, including [4] [5] and [16] which showed that cardamom oil is rich in alpha-terpinyl acetate and 1,8-cineole as primary compounds, with different proportions of secondary compounds, including linalool and terpinen-4-ol, based on the heterogeneous chemical structure of the oil.

A similar study by [17] showed that GC-MS recorded a high percentage of these compounds.

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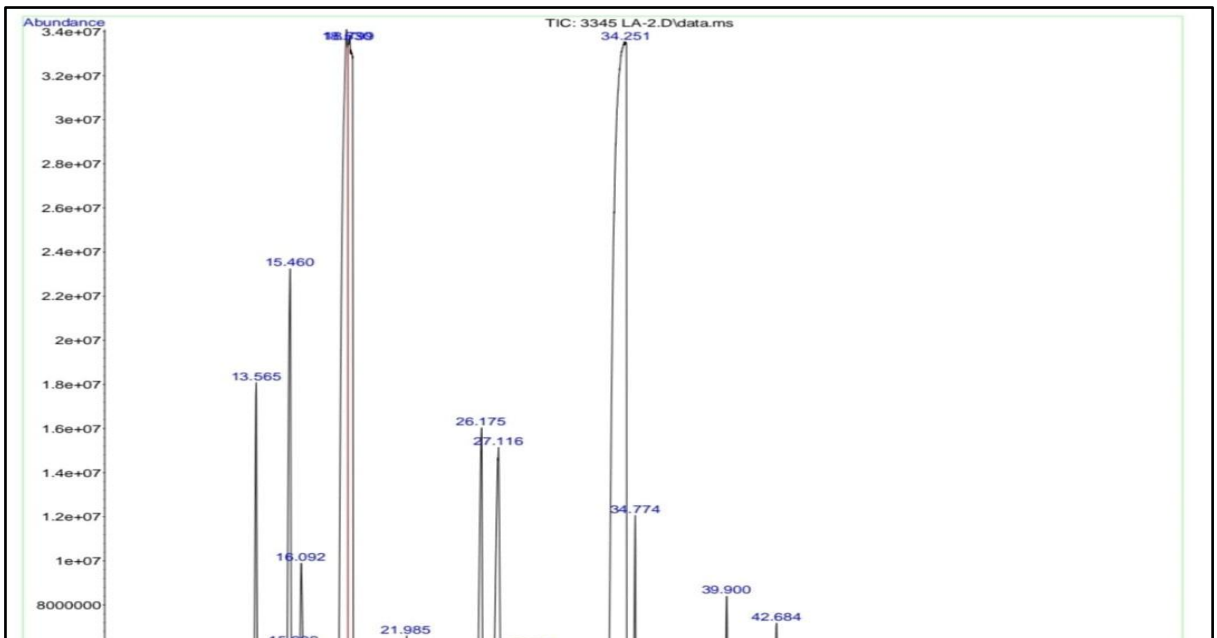
The volatile oxygens in cardamom oil, with high concentrations of α-terpinyl acetate and 1,8-cineole, confirm that these compounds are responsible for most of the oil's biological activities, including its antioxidant and antimicrobial properties, as documented in other experimental studies.

As shown in the table, some compounds, such as methyl-thiourea and cinnamaldehyde, are present in low concentrations. These represent secondary compounds that contribute to the oil's flavor and unique chemical properties. The distribution of compounds shown in the table reflects the natural chemical composition of cardamom extracted by hydro distillation, and our results are consistent with previous studies in understanding the relationship between chemical composition and the biological activity of the oil [4][16] [5][17].

Table(1) GC-MS Identified Compounds and Relative Concentrations

No.	Time. Retation (min)	Compound Name	Concentration %
1	13.14	α-Thujene	0.5
2	13.563	α-Pinene	3.03
3	15.461	Sabinene	5.11
4	15.689	β-Pinene	0.93
5	16.09	Myrcene	2.43
6	16.81	Methyl thiourea	0.54
7	18.799	1,8-Cineole	28.2
8	19.627	γ-Terpinene	0.13
9	20.57	Thujanol	0.46
10	20.919	Limonene	0.17
11	21.982	Linalool	1.99
12	23.319	Terpinen-4-ol (isomer)	0.33
13	26.177	Terpinen-4-ol	4.28
14	27.114	α-Terpineol	5.38
15	28.532	Neral	0.21
16	28.715	Linalyl acetate	1.01
17	29.663	Geraniol	1.79
18	29.892	Geranial	0.64
19	31.212	Cinnamaldehyde	0.74
20	31.881	Terpinyl acetate (isomer)	0.65
21	34.253	α-Terpinyl acetate	33.51



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Figure(1) shows the peaks of the most important biologically active compounds in cardamom fruit oil analyzed by GC-MS.

The inhibitory effect of cardamom oil on bacteria

The agar diffusion technique Table(2) shows that cardamom fruit oil has an inhibitory effect on the growth of the tested bacteria. The oil showed a distinct inhibitory effect which was directly proportional to concentration. The greatest sensitivity was found in Bacillus cereus with diameters of inhibition zones of 13.00, 19.00, and 23.00 mm at 2.5%, 5 and 10% concentrations respectively. The values of Staphylococcus aureus were between 11.00 and 19.00 mm, whereas Pseudomonas aeruginosa had the lowest response (9.00 to 16.00 mm), and Escherichia coli was among the average value of the response (8.00 to 14.00 mm). Such a gradient in response indicates a direct relationship between the rising oil concentration on the one hand and the inhibitory efficacy on the other hand.

These findings are in line with the recent research findings that show Gram-positive bacteria are more susceptible to essential oils as compared to Gram-negative bacteria. The reason is that the former does not have a lipopolysaccharide rich outer membrane enabling the diffusion of hydrophobic compounds through the plasma membrane [2]. Conversely, the comparatively low sensitivity of Pseudomonas aeruginosa is attributed to the complicated outer membrane and effective efflux pumps that decreases the intracellular concentration of active compounds and restricts their inhibitory impact.

We also find that the effectiveness of cardamom oil varies with concentration, in line with [16] and [18], which support the finding that the oil has the ability to inhibit Staphylococcus aureus and Escherichia coli even at relatively low concentration, which is indicative of a potent bioactivity of its components. Cardamom oil has an antibacterial effect due to the presence of active compounds in it, and 1,8-cineole and α-terpinyl acetate are capable of disrupting cell membrane integrity and raising the permeability. This causes leaks of important cellual components and interruption of intracellular metabolic activities, which ultimately leads to cell demise. This interpretation is supported by a recent study by [19] which shows that 1,8- cineole has a direct influence on enzyme and structural systems in bacterial cells.

Moreover, [20] study revealed that cardamom oil has an exceptional power to suppress the formation of biofilms, which makes it more effective in the control of pathogenic bacteria, particularly in complex food systems. Recent studies have confirmed that the efficacy of essential oils depends not only on the type and concentration of active compounds, but also on the synergistic effect between these compounds and their interaction with the surrounding

environment. This synergistic effect can either enhance or limit their antimicrobial activity [21][22].

It should be noted that the agar diffusion method may reduce the accuracy of assessing the antibacterial activity of hydrophobic compounds due to their limited diffusion in a solid medium, potentially leading to a reduction in the diameter of the inhibition zones. However, overall, the results showed concentration-dependent antibacterial activity, with higher activity against Gram-positive bacteria, supporting the potential use of cardamom oil as a natural preservative.

Table(2) Inhibitory effect of cardamom oil on selected bacteria by well diffusion method (mm)

Bacteria	25µL/mL	50µL/mL	100µL/mL
Bacillus cereus	13.00 e	19.00 ab	23.00 a
Staphylococcus aureus	11.00 f	17.00 bc	19.00 ab
Pseudomonas aeruginosa	9.00 g	14.00 de	16.00 cd
Escherichia coli	8.00 g	12.00 ef	14.00 de

Discussion of the sensory evaluation of cheese

Table (3) shows the effect of adding cardamom oil at different concentrations (0.25%, 0.50%, 0.75%) on the sensory characteristics of soft white cheese during 21 days of storage at refrigeration. The findings show that the addition of the cardamom oil at low- to medium-levels did not have any adverse impact on the sensory attributes during the initial storage conditions but that the effects were more pronounced in high levels and with the duration of the storage period. It was found that bitterness was minimal during the initial days of all treatments, then rose slowly over time, peaking at a concentration of 0.75% on day 21 (6.1). This is a cumulative effect of the phenolic and terpene constituents present in the oil and it is in agreement with [23] observation of increased perception of bitterness when essential oils are applied in high concentration.

In terms of color, all samples were high in the initial stages of storage, and the levels have slightly and gradually decreased. This reduction was greater at the highest concentration, which was 7.7 on day 21. This is based on potential oxidation of certain volatile compounds or reaction with cheese constituents, which is in line with the results of [24].

As for texture, the results did not show significant changes in the first few days. But with the extension of time of storage, especially at the concentration of 0.75, there was a little decrease in the value. This could reflect on the impact of the oil on the moisture equilibrium or the casein system of the cheese as documented in prior studies about the impacts of essential oils in dairy products.

The findings about flavor indicated that low and middle concentrations (0.25% and 0.50) were both highly sensory acceptable up to the expiry date. But the flavor scores went down at the concentration of 0.75 to 7.5 on day 21. This also shows that adding to the level of concentration of oil can have a negative impact on the overall acceptability of the product,

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which agrees with [19] who proposed that higher concentration of essential oils, although they have antimicrobial properties, can reduce sensory acceptability.
Overall, the results suggest that using cardamom oil at concentrations between 0.25% and the right degree of addition is 0.50%; this is an appropriate compromise to add to the cheese to improve antimicrobial properties without affecting the sensory attributes. This leads to its possible application as a natural preservative in the food industry, though the concentration has to be kept under control in order to prevent unwanted sensory properties.

Table(3) Sensory Evaluation of Soft White Cheese Fortified with Cardamom Oil

Concentration (%)	Time (day)	Bitterness	Color	Texture	Flavor
0	1	0.0 f	10.0 a	10.0 a	10.0 a
	7	0.0 f	10.0 a	10.0 a	9.5 ab
	14	4.5 c	9.5 ab	9.5 ab	9.2 bc
	21	6.0 a	8.5 c	8.6 c	7.8 d
0.25	1	0.0 f	10.0 a	10.0 a	10.0 a
	7	0.0 f	10.0 a	10.0 a	10.0 a
	14	4.1 cd	9.5 ab	9.5 ab	9.7 ab
	21	5.8 ab	8.5 c	8.6 c	8.8 c
0.50	1	0.0 f	9.75 ab	10.0 a	10.0 a
	7	0.0 f	9.5 ab	9.75 a	9.6 ab
	14	4.0 d	9.0 b	9.0 b	9.3 bc
	21	5.9 a	8.25 c	8.5 c	8.5 c
0.75	1	0.0 f	9.8 ab	10.0 a	9.8 ab
	7	1.5 e	9.5 ab	10.0 a	9.1 bc
	14	3.0 d	8.0 c	9.0 b	8.2 cd
	21	6.1 a	7.7 d	7.7 d	7.5 d

Discussion of the microbial content of cheese

Table(4) shows the effect of adding Elettaria cardamomum oil at different concentrations on the growth of E. coli bacteria, yeasts and mold in cheese during a storage period of up to 21 days at a temperature of 5°C. The data show a clear inhibition of all microorganisms in the samples to which cardamom oil was added compared to the control sample, with the intensity of the effect increasing with increasing oil concentration. On the twenty-first day, the control sample recorded the highest value for the total bacterial count 50×10^3 CFU/g, while this value decreased to 29×10^3 CFU/g at the highest concentration of 0.75% of the oil. The growth of E. E. coli levels was low during storage, ranging from 0 to 4×10^3 CFU/g, with a significant decrease as oil concentration increased. Yeasts and molds also showed slower

growth compared to the control sample, with a marked decrease in numbers in samples with higher concentrations. The antimicrobial activity is attributed to the active compounds in cardamom oil, such as α-terpinyl acetate, 1,8-cineole, and linalool acetate, which are known for their antibacterial and antifungal properties. This is supported by recent studies. A study by [20] demonstrated cardamom oil's ability to inhibit biofilm formation and reduce the activity of E. coli and Salmonella Typhimurium in food media at low concentrations, consistent with the decrease in E. coli numbers in cheese as oil concentration increased. A study by [25] showed that cardamom oil has MIC values against E. coli of 0.5–1 mg/mL, further supporting the evidence for its antibacterial activity against gram-negative bacteria in food. The trend of the decline in the total number of bacteria as the oil concentration increases is supported by the stored oil. We also agree with the research carried out by [26], who verified that the oil is effective against yeasts and fungi as well by inhibiting the formation of biofilms. This is consistent with our observations about the decreased level of yeasts and molds in the cheese samples. These outcomes suggest that cardamom oil addition can improve the safety and quality of cheese during storage without affecting the sensory characteristics adversely, which proves its possible application as a natural antimicrobial agent in food.

Table(4) Effect of adding cardamom oil to cheese on microbial growth in cheese

Storage period (days)	Oil concentration %	Total bacterial count (CFU × 10^3/g)	E. coli (CFU ×10^3/g)	Yeasts and molds (CFU ×10^3/g)
1	0	3×10^3	0×10^3	0×10^3
	0.25	3×10^3	0×10^3	0×10^3
	0.50	2×10^3	0×10^3	0×10^3
	0.75	2×10^3	0×10^3	0×10^3
7	0	15×10^3	0×10^3	2×10^3
	0.25	13×10^3	0×10^3	1×10^3
	0.50	10×10^3	0×10^3	0×10^3
	0.75	3×10^3	0×10^3	0×10^3
14	0	25×10^3	2×10^3	4×10^3
	0.25	20×10^3	1×10^3	2×10^3
	0.50	18×10^3	1×10^3	1×10^3
	0.75	16×10^3	1×10^3	0×10^3
21	0	50×10^3	4×10^3	7×10^3
	0.25	45×10^3	3×10^3	3×10^3
	0.50	33×10^3	2×10^3	2×10^3
	0.75	29×10^3	2×10^3	2×10^3

Conclusion

The experiment has shown that green cardamom oil harbors volatile substances (i.e., 1,8-cineole and α-terpinyl acetate) that provide it with a definite capacity to prevent the growth of bacteria, yeasts, and fungi in soft white cheese. It was also revealed that low to moderate concentrations (0.25-0.50) give an optimal balance between antimicrobial effect and

maintenance of sensory properties, thus cardamom oil is a potential natural preservative in dairy products.

Conflict of interest

None of the authors will claim a conflict of interest.

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