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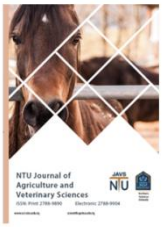

Extraction of oregano oil, identification of its active compounds and its effect on the sensory evaluation of cheese

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A B S T R A C T

The study results showed that the percentage of oregano oil extracted by hydrodistillation technique reached 1.6%, as every 50 g. of oregano herbs gave 0.8 ml oil. The physical properties of the extracted oil were refractive index (1.338) and relative density (0.9236). The oil color was light yellow. The chemical properties of the oregano oil included a peroxide value of 7.2, and an acidity 1.683. The results of the analysis of the components of the oil in the GC-Maas device showed the identification of 30 compounds, and the carvacrol compound was the highest as its percentage reached 51.51%, which is attributed to the inhibitory effect of the oil in addition to other compounds. The evaluation results of the quality of cheese added to oregano oil at a concentration of 0.25 of the weight of the clot with cheese not treated with oil and the treated cheese were identified by smell and taste due to the clear effect of the aromatic oils. No significant differences were observed on the first day between the control treatment and the treatment containing 0.25% oil. In comparison, there were significant differences in all characteristics except the texture characteristic with the cheese containing 0.75% oil on the same day. The sensory evaluation also showed that the control cheese became unacceptable on the fourteenth day of storage at the refrigerator temperature. As for the 0.75 treatment, the results showed from the first week for the two characteristics of flavor and bitterness that they were unacceptable by the evaluation committee members, while the cheese treated with 0.25% oil showed an acceptable flavor and good taste even after twenty-one days.

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Introduction

Cheese is one of the oldest processed foods, and cheesemaking is believed to date back to 10,000 BC when sheep and goats were domesticated in the Middle East to produce milk in warm climates where the milk has a short shelf life due to spoilage microorganisms. Therefore, milk is converted into several cheese products, produced worldwide, and its variety and consumption have increased over the years [1]. Cheese is produced as a result of the curdling of milk, where it changes from the known liquid state to the cohesive gelatinous state known as cheese curd as a result of the precipitation of casein or its curdling by the action of the enzyme rennet (animal cheeses) or vegetable cheeses or by the acidity formed from the fermentation of lactose and its conversion to lactic acid with the separation of a large amount of whey, which contains dissolved milk compounds, when the curd is cut, stirred, and thermally treated at either high or moderate temperatures, then placed in the press. All of these processes combined help in the curd curdling process, and in this way, the milk turns into a more concentrated food with its components, with an increased ability to be preserved for a longer period [2]. Oregano is a type of plant belonging to *Lamiaceae* family. Its original homeland is the Mediterranean region and western Eurasia. It is a dense perennial herb, reaching a height of 90 cm. Its stem has hairs and is characterized by ribbed stems that gradually turn into woody stems as they age. Its leaves are oval, dark green, with smooth edges. The shape of the leaf tips ranges from sharp (pointed) to obtuse. As for the flowers, they are small and clustered in a bouquet at the top of the stem, with a blue or purple color. They bloom in midsummer [3]. Oregano grows wild worldwide, especially in the mountainous regions of Greece and Turkey, where high-quality oregano is found. Oregano is rich in minerals and vitamin C, vitamin A, and niacin [4]. Oregano contains a number of chemical compounds ranging from 30 to 60 chemical compounds that differ in terms of concentrations and biological activities [5]. Oregano is an abundant source of anthocyanins and natural phytochemicals with medicinal properties. The main compounds in oregano extract include phenolics (e.g., carvacrol and thymol), monoterpene hydrocarbons, cymene, and terpinene.

Carvacrol and thymol constitute the major antibacterial content of oregano. Other potential antioxidant phenolics have also been obtained from the extract. The potential synergistic effects of this plant are attributed to the presence of oregano's herbaceous terpenes, the most abundant of which is Rosmarinus acid. Oregano has antioxidant capacity and also exhibits antimicrobial activity against pathogenic microorganisms such as Salmonella typhimurium, Escherichia coli, Staphylococcus aureus, and Staphylococcus epidermidis, which enhances its properties by its use as an additive in the food industry [6]. Oregano oil is extracted from the plant through processes such as steam distillation It is known for its antibacterial activity and has been found have inhibitory effects against various bacterial strains, including E. coli and Staphylococcus aureus [6,7]. and [8] mention that the oil is a powerful antioxidant, anti-inflammatory, antidiabetic, and anticancer agent. In addition, oregano oil has been shown to have potential benefits in terms of growth performance, disease resistance, immunity, and gut health. Oregano oil contains more than 30 components, most of which are phenolic compounds with different bioactivities. Adding olive oil can improve the quality of some foods. Chemical analysis of oregano oil has identified the main components as carvacrol, thymol, p-cymene, and g-terpinene. The composition of essential oils may vary between harvest seasons, geographical locations, and plant parts [9]. In a study [10] oregano extract was used as a natural compound to improve the shelf life of yogurt at refrigerated temperatures for 30 days. The best inhibition of yeasts and molds was found at 1.5%. The descending order of the in vitro antibacterial effect of oregano extract was L. monocytogenes, S. aureus, B. subtilis, B. cereus, S. typhimurium, and E. coli O157:H7. [11] found that adding a blend of oregano and copaiba essential oils (0.025, 0.025%) added to meat slices had a superior effect compared with meat containing the synthetic preservative BHT, or meat treated with 0.05% copaiba essential oil alone or 0.05% oregano essential oil, in addition the treated samples maintained acceptable sensory properties over a 21-day storage periodindicating that this essential oil blend could serve as a potential natural alternative to synthetic preservatives in processed foods. The results of the study conducted by [12] showed that the mixture of oregano essential oil (OE) and rosemary extract (RE) has a significant antioxidant effect. The meat pies were individually packed in oxygen-permeable bags and stored at 4°C. They were analyzed for lipid and protein oxidation and evaluated at 0, 4, and 7 days. It was found that all treatments had a significant effect, and the best treatment was composed of 150 ppm of oregano essential oil added to 350 ppm of rosemary extract. In a study [13] when they added 0.05% of oregano oil to quark cheese, they observed a decrease in the number of mold and yeast during the storage period of 35days at 4°C, indicating a positive effect in delaying deterioration. In manufacturing fresh sheep cheese "Toma" [14] reported that cheeses with oregano oil added at 100 and 200 µl/l to milk showed a significant decrease in milk-borne pathogens. Sensory characteristics were not

negatively affected by adding oil, but the highest overall satisfaction values were proven for Toma cheeses with 100 µl/l added. This indicates the possibility of using oregano oil as a natural preservative, and it also led to improving its quality characteristics, including sensory characteristics and antioxidant capacity.

Materials and methods

Extraction of oil by hydrodistillation

The oregano herbs were cleaned of impurities and ground to prepare them for extraction. Oregano oil was extracted using the following method [15] Oregano oil was obtained by hydrodistillation using a Clevenger device. 50 grams of oregano powder was placed in a circular extraction flask and soaked in 500 ml of distilled water at a ratio of (1:10) to immerse the sample completely. The flask was heated using a heater to 90 °C. The water and extracted oregano oil evaporate together. The steam mixture is condensed using a water-return condenser. Since the oil is immiscible with water, it can be easily separated. After separating the oil, it was left for minutes until it became clear. It was collected and stored in a dark glass bottle at a temperature of 4 °C until use.

Identification of active chemical compounds in oregano oil by GC/MS

The chemical composition of the extracted oregano oil was determined using a Gas chromatography-mass spectrometer (GC/MS) (N 5973 Agilent USA). A capillary column of the HP-5MS type was used in the analysis, with dimensions of (30m x 0.25mm x 0.25um). Then, the extracted oregano oil components were determined by comparing the resulting mass spectra for each GC/MS chromatogram peak with the mass spectra in the library available on the device's computer (NIST).

Soft white cheese manufacturing

Soft white cheese was manufactured according to [16] with some modifications. The temperature was raised to 72 for 15 minutes and then cooled to 38 C. Cheese rennet powder was added and stirred for 10 minutes, then the milk was placed in an incubator at 40° to coagulate. After the coagulation process was completed, it was filtered using a gauze cloth to drain the whey. Salt was added at a rate of 2% of the weight of the coagulant, then it was divided into three treatments, two of which were added with oil at a rate of 0.25 and 0.75% of the weight of the coagulant, respectively. Then, the coagulant was placed in molds, and the molds were transferred to the press. The cheese was cut and placed in a refrigerator at a temperature of 5 C until the tests were carried out.

Sensory evaluation of cheese

The sensory evaluation of cheese samples was conducted by a number of professors and students in the Department of Food Sciences according to the sensory evaluation form that included a number of attributes—flavor, texture, color, and bitterness—and using the 10-point system before conducting chemical and microbial analyses on them. A sensory evaluation was conducted on the samples to ensure their suitability for human consumption by cutting the cheese into pieces weighing 20–25 grams for each evaluator [17].

Statistical analysis

One-way ANOVA was used to show differences between treatments; compare means was done by Duncan test [18]

Results and Discussion

Oil extraction ratio

Oregano oil was extracted BY hydro-distillation technique. Oil extraction was calculated as (1.6%), 50g of oregano herbs gave (0.8 ml) oregano oil. The obtained result is consistent with what was found by [19], who found that the yield percentage of oregano oil using the hydro-distillation technique ranged between (0.93% - 1.66%) depending on the source of oregano. While results showed an increase in the percentage of oil yield by [20], which reached 2.3%, this may be due to the difference in the source of herbs and the extraction conditions, as mentioned by [21], as the production and composition of essential oil are the result of various factors, including the genetic type, environment, geographical location, growth stage, and harvest season.

extracted oregano oil Physical properties

The result in Table 1 refractive index (1.338) showed similarity with what [22] reached, which was (1.4774). Asensio et al. (2015) also found a difference in the refractive index when comparing four types of oregano, which ranged between (1.476 and 1.486).

Table 1. physical properties of the oregano oil.

physical properties	Result
refractive index	1.338
relative density	0.9236
Color	Light yellow

The relatively high density (0.9236) may be attributed to the high phenolic content, especially thymol and carvacrol, whose density is close to that of water. This observation was supported by study [23], where the relative density of Mondecino oregano oil was (0.916), in which the percentage of carvacrol and thymol was greater than that of

Mondecino, which had a relative density of (0.898). The result is also implicitly consistent with [24], which reported a density of 0.9132 for oregano essential oil. [25] stated that color is a physical property of volatile oils that includes a large variation depending on the type of oil and the extraction method. In the present study, the oil exhibited a light yellow color, which agrees with the results of study [23], while differing from those reported in study [26], where the oil color was described as golden brown.

extracted oregano oil Chemical properties

Table (2) indicates the peroxide 7.2. The result differed slightly from what [26] reached, as the peroxide value was (6.22). This difference is due to [27], who mentioned that the chemical compositions and concentrations of the components present in the oil give the essential oil its properties.

Table 2. ChemicalProperties of Oregano Oil.

properties	Value
Peroxide	7.2
Acidity	1.683

results in Table (2) showed the acidity is reached (1.683). [28] indicated that the acidity number indicates the quality of fatty acids in the oil. However, these values represent the presence of free fatty acids in oils as an indicator of the presence and extent of hydrolysis by fat-dissolving enzymes and oxidation.

Main components of oregano oil extracted by GC-MS

Table (3) is the results of subjecting the extracted oregano oil sample to GC-MS analysis. 30 compounds were identified, and the carvacrol compound was the highest, reaching 51.51%, attributed to the inhibitory activity in oregano oil and to the thymol compound, which had a percentage of 0.46. The results were close to what [29] found, as the percentage of carvacrol for two oil samples was 57.96% and 59.40%.

Table 3. Components of oregano oil extracted by GC-MS

Compound concentration in %	Retention time	Boat Name
0.83	3.554	Alpha. -The pains
1.47	4.212	beta.-Myrcene
11.99	4.801	Benzene, 4-ethyl-1,2-dimethyl
7.37	5.303	gamma.-Terpinene
0.27	5.735	Cyclohexene, 1-methyl-4-
1.45	6.030	1,6-Octadien-3-ol, 3,7-dimethyl-
3.62	7.527	1,7,7-Trimethylbicyclo
0.14	8.323	alpha.-Terpineol
0.46	9.786	Thymol
51.51	10.643	Carvacrol
0.17	10.859	Phenol, 2-ethyl-4,5-dimethyl-
8.05	11.500	Caryophyllene
0.47	11.751	Naphthalene,1,2,3,5,6,7,8,8a-octa
0.77	12.002	1,4,7,-Cycloundecatriene-1,5,9,9
0.33	12.538	1H-Cycloprop[e]azulene,1a,2,3,5,6
0.28	12.911	Naphthalene,1,2,3,5,6,8a-hexahydrate
3.09	13.992	Caryophyllene oxide
0.13	14.399	2-Naphthalenamine,1,2,4a,5,6,7,8,
0.85	14.797	gamma.-Muurolene
0.37	15.040	Isoaromadendrene opoxide
0.44	15.256	Spiro[4.5]dec-6-en-8-one, 1,7-dime thyl-4-(1-methylethyl)-
0.17	16.693	Urea
1.39	18.493	3,4-Dimethyl-o-phenylenediamine
1.72	19.116	n-Hexadecanoic acid
0.37	19.636	Phenyl-1,2-diamine, N,4,5-trimethy
0.21	21.003	3-Trifluoroacetoxypentadecane
0.75	21.211	9-Octadecenoic acid, (E)-
0.75	21.531	Oleic Acid
0.35	22.250	Podocarp-7-en-3.beta.-ol, 13.beta-methyl-13-vinyl-
0.24	24.102	2-Propen-1-one, 1-(2,6-dihydroxy--methoxyphenyl)-3-phenyl-, (E)-

Sensory evaluation of cheese

Table (4) shows the evaluation of the cheese quality to which oregano oil extracted at a concentration of (0.25 and 0.75% of the weight of the curd) was added, with cheese not treated with oil. The treated cheese was identified by smell and taste due to the clear effect of the aromatic oils. There were no significant differences on the first day between the control treatment and the treatment containing 0.25% oil, while there were significant differences in all characteristics except the texture characteristic with the cheese containing 0.75% oil on the same day. The sensory evaluation also showed that the control cheese became unacceptable on the fourteenth day of storage at the refrigerator temperature. As for the 0.75 treatment,

the results showed from the first week for the flavor and bitterness characteristics that they were unacceptable by the evaluation committee members, while the cheese treated with 0.25% oil showed an acceptable flavor and good taste even after twenty-one days. These differences in the cheese evaluation scores are attributed to the concentration of the extracted oregano oil and the degree of sensitivity of the evaluator.

Table 4. shows the sensory evaluation of cheese with specific concentrations of oregano oil added at storage periods 1, 7, 14 and 21day.

Concentration	Time (day)	Bitterness	Color	Textures	Flavor
0	1	9.75 a	10 a	10 a	10 a
	7	8.37 b	10 a	10 a	9.5 a
	14	0 f	7.25 c	8.75 c	0 e
	21	0 f	5.5 d	7.375 d	0 e
0.25	1	9.66 a	10 a	10 a	9.75 a
	7	9 ab	10 a	10 a	9.375 ab
	14	8.5 b	9.5 ab	9.5 ab	8.5 b
	21	6.25 c	8.5 bc	9 bc	6.25 c
0.75	1	4 d	9.25 ba	10 a	3.5 d
	7	3 ed	9.5 ba	10 a	3.25 d
	14	2.75 e	8 c	9 bc	3 d
	21	2.5 e	7.75 c	7.75 d	3 d

Conclusion

The current study highlights the importance of using natural extracts (oregano oil) in improving the quality of preservation of dairy products without affecting their acceptability by the consumer, as proven by this study.

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