Impact of Marjoram Powder and Alcoholic Extract (Origanum majorana L.) on Quality Attributes and Shelf-Life of Laboratory Yogurt

Heba Abbas Khazal , Eman Ali Hadi

Home Economic Department/College of Education for Women/Baghdad University.

hiba.Abbas2410m@coeduw.uobaghdad.edu.iq emankhafaji2000@coeduw.uobaghdad.edu.iq

Abstract

This study aimed to investigate the impact of using *Origanum majorana* L. (marjoram) powder and alcoholic extract on some qualitative and storage properties of laboratory-produced yogurt. The study evaluated the effects of marjoram powder and alcoholic extract on the chemical, physicochemical, microbiological, sensory, and storage properties of laboratory-produced yogurt. The results showed that marjoram leaf powder has the composition by ingredients 4.58% moisture, 13.58% protein, 3.52% fat, 9.11% ash, 21.65% crude fiber and 47.56% carbohydrates, It further showed high mineral content (1895 mg/100 g calcium and 1498.7 mg/100 g potassium). Marjoram addition improved the physicochemical properties of yogurt. In the optimal treatment, also compared to control, water retention increased from 12.65% to 16.35%, and peroxide value decreased from 4.89 to 4.44 meq/kg leaching of whey was also decreased in the treated yogurt samples compared to the control. Marjoram extracts demonstrated high inhibitory efficacy against fungal growth, particularly the alcoholic extract, confirming its potential to extend the shelf life and improve the quality of manufactured yogurt. Sensory evaluation results showed that adding the alcoholic extract and marjoram powder did not negatively affect the sensory characteristics of the manufactured yogurt; rather, most treatments maintained a high acceptability rating throughout the storage period. The alcoholic extract at concentrations of 0.5% and 1% showed the best results in sensory evaluation, preserving the yogurt's appearance, flavor, and taste. Hence, marjoram powder and alcoholic extract may serve as promising natural additives when producing functional yogurt, as long as the concentration allows to find a balance between storage properties and sensory acceptance.

Keywords: Marjoram, yogurt, alcoholic extract, peroxide value, whey.

1. Introduction

Natural additives, especially constituents from medicinal and aromatic plants, are increasingly considered potential alternatives to synthetic additives in the food industry and have been increasingly incorporated in recent years [1]. This is owing to the presence of a high concentration of bioactive compounds such as phenols, flavonoids, volatile oils, alkaloids, tannins and saponins found in plants, which are based on those measuring antioxidant and antimicrobial activity, and not to mention their potential contribution to improving the nutritional and functional components of food and shelf life.[2-4].

The most common examples of medicinal and aromatic plants are Marjoram (*Origanum majorana* L.), which belong to the family Lamiaceae [5]. This spice, known for its signature scent and flavor, is widely used in culinary cuisine and traditional medicine [6, 7]. Marjoram contains active compounds such as terpinen-4-ol, γ-terpinene, cis-sabinene

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hydrate, carvacrol, and thymol that are closely related to antioxidant, antibacterial and antifungal activity [8, 9]. The composition of essential oil in marjoram is influenced by several parameters such as geographic region, environmental aspects, developmental stage and extraction methods; therefore, the variations among studies on this plant are observed among geographical regions [10].

In contrast, yogurt is one of the most consumed fermented dairy products worldwide due to its excellent nutritional value and many health beneficial effects [11]. Besides containing useful lactic acid bacteria, it is a good source of high-quality protein, as well as calcium, phosphorus, magnesium and zinc [12]. Yogurt is produced by fermenting milk with a starter culture, mainly Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus. These bacteria ferment lactose into lactic acid, lowering the pH and promoting gel formation that forms yogurt's texture [13].But some important plant compounds like phenols, flavonoids, and dietary fiber are naturally absent or present at low concentrations in conventional yogurt [14]. However, more recent international research has exploited plant based bioactive compounds (moringa, propolis, maca, silymarin as well as essential oils or spices extracts) to improve antioxidant and functional properties, in addition to nutritional value and stability of yogurt [15-18]. Tian, Sheng [19] found that modified okra IDF when added to yogurt has better water-holding capacity (WHC), hypoglycemic effects, and in vitro fermentation ability.

Since yogurt is a highly perishable product, affected by storage factors such as acidity changes, whey separation, texture deterioration and sensory acceptance together with the growth of contaminating microorganisms [20], this trend has gotten a special importance. Accordingly, incorporating plant-derived ingredients with antioxidants and antimicrobial potential may improve yogurt quality. The search for safe and natural alternatives to chemical preservatives in yogurt has also contributed to a growing interest in herbal additives. Therefore, the objective of this study was to assess the impact of marjoram powder and alcoholic extract on yogurt produced in the laboratory; while numerically comparing our findings with those found in similar studies on plant-fortified yogurt.

2. Material and methods

2.1 Sample collection and preparation

Marjoram leaves (*Origanum majorana* L.) (from the mint family Lamiaceae) plant samples were collected from some nurseries in Baghdad Governorate in February 2026 and taken to the laboratory in good condition. The impurities, dust and unwanted parts were removed manually, washing was done where required and shade-dried at (25–30°C) avoiding direct sunlight for ten days with continuous turning to avoid rotting or undesirable changes in color and odor. The dried leaves were then powdered using an electric grinder to a fine powder. The powder was preserved in airtight plastic containers, protected from light and moisture prior to use in chemical analyses and yogurt preparation. The plant was identified at the Department of Biology, College of Science, University of Baghdad, the identity *Origanum majorana* L was confirmed.

2.2 Preparation of alcoholic extracts of marjoram leaves

Powdered dried leaves of *Origanum majorana* L. were used to produce alcoholic extracts for the inhibitory activity tests and as an additive in laboratory-made yogurt preparations. The

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plant, ground into a fine powder, was used to facilitate the extraction process. For the preparation ethanol 80% was used as an alcoholic extract. The extracts were subsequently filtered to eliminate plant tissue residues and undissolved substances. Finally, extracts were concentrated and stored in sterile, light-protected vials before analysis. For the inhibitory tests, 1.5 g of the dry extract of each sample was dissolved with 0.5 ml of DMSO, and the volume was then topped up to 10 ml with sterile distilled water to obtain a storage solution of 150 mg/ml and a final DMSO content of 5% v/v. Subsequently, working concentrations of 20, 30, 40 and 50 mg/mL were prepared from this. Solutions were sterilized by filtration with 0.45 µm Millipore filters. After testing for inhibitory activity, 30 mg/ml of the alcoholic extract were selected for yoghurt treatment (based on the previous experiment results), (0.5, 1.0 and 1.5 ml) to investigate their effects using qualitative and storage tests on yoghurt samples.

2.3 Production of laboratory yogurt and addition treatments

Yogurt was made in the laboratory using Raw milk prepared from the dairy plant of the College of Agricultural Engineering Sciences/Abu Ghraib, from the morning batch. The total solids content in the milk was adjusted to 14% using Nido brand powdered milk, then the milk was thoroughly mixed and heat-treated at 90°C for 10 minutes. (to reduce the initial microbial load and modify the structural and functional properties of milk proteins, especially those of whey proteins, which is beneficial to obtain a higher firmness of the curd after fermentation). The milk was gradually cooled to 42°C after heat treatment It was inoculated with Lyofast freeze-dried yogurt starter type Y3550 A 5 UC, consisting of *Streptococcus salivarius* ssp. thermophilus and *Lactobacillus delbrueckii* ssp. bulgaricus, at a rate of 2% v/v. The milk was later divided into seven experimental treatments, one control treatment, and six others containing the addition of marjoram powder and alcoholic extract (table 1).

After making each of the treatments, the samples were incubated at 42 ℃ until coagulation or until the target pH was reached, then cooled and stored at 4–5 °C. The yogurt samples were subjected to chemical, physicochemical, microbiological and sensory tests to determine the influence of type and concentration of additives on the quality and storage characteristics of fermented milk produced in the laboratory.

Table 1. Treatments of Laboratory-Produced Yogurt Fortified with Marjoram Powder and alcoholic extract

Treatments	Description
A	Control Treatment with no additives
B1	Add 0.5 % (w/w) of marjoram powder
B2	Add 1.0 % (w/w) of marjoram powder
B3	Add 1.5 % (w/w) of marjoram powder
C1	Add 0.5 % (v/v) of 30 mg/ml alcoholic extract
C2	Add 1.0 % (v/v) of 30 mg/ml alcoholic extract
C3	Add 1.5 % (v/v) of 30 mg/ml alcoholic extract

2.4 Chemical Analysis of Marjoram Powder and Yogurt

Chemical analysis was performed on both marjoram leaf powder and laboratory-produced yogurt to determine the proximate composition of the samples and to investigate the effect of adding marjoram powder and alcoholic extract on the nutritional value of the final product.

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2.4.1 Moisture Determination

The percentage of moisture in the marjoram powder and yogurt samples was determined using the gravimetric drying method by calculating the weight loss of the sample before and after drying. according to the AOAC 925.10 (2019) method used in the tests for marjoram powder and yogurt.

$$Moisture\ \% = \frac{(Weight\ of\ sample\ before\ drying) - (Weight\ of\ sample\ after\ drying)}{(Weight\ of\ sample\ before\ drying)} \times 100.$$

2.4.2 Protein Estimation

The protein content of marjoram leaf powder and yogurt samples was estimated using the Kjeldahl According to the AOAC 979.09 method, based on the estimation of total nitrogen followed by its conversion to protein using a conversion factor of 6.25.

The protein content of the acid consumed in the titration was then calculated.

$$Protein\ \% = \frac{Consumed\ HCl\ volume\ \ 6.25 \times\ Normality\ \times\ 0.014}{Sample\ weight} \times 100$$

2.4.3 Fat Estimation

The fat content of marjoram powder and yogurt samples was determined using the Soxhlet extraction method, based on the AOAC 920.39 (2019) method.

$$\%\ Fat\ = \left(\frac{Flask\ weight\ after\ extraction\ -\ Flask\ weight\ before\ extraction}{Sample\ weight}\right) \times 100$$

2.4.4 Ash Estimation

The percentage of ash in the marjoram powder and yogurt samples Ash was estimated by dry burning in an incineration furnace according to AOAC 923.03 method. The ash content was calculated as the weight of the mineral residue after burning relative to the original sample weight.

$$Ash\ \% = (Weight\ of\ sample\ /\ Weight\ of\ container\ with\ sample\ after\ burning - Weight\ of\ empty\ container) \times 100$$

2.4.5 Crude Fiber Estimation

Crude fiber content in marjoram powder, as well as in yogurt treatments where needed, The crude fiber was estimated according to the AOAC 978.10 method. Crude fiber is an important component of marjoram powder because it can affect the texture and rheological properties of yogurt, such as water retention and whey permeability. Plant fibers have the ability to bind water and support the curd network.

2.4.6 Carbohydrate Estimation

Carbohydrate content was estimated using the difference method after estimating the other major components: protein, fat, fiber, moisture, and ash. The percentage of carbohydrates was calculated according to the following formula:

$$Carbohydrate\ \% = 100 - (Protein\ \% + Fat\ \% + Fiber\ \% + Moisture\ \% + Ash\ \%)$$

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2.5 Physicochemical Tests of Yogurt Produced in the Laboratory

For stability, texture and storage of yogurt produced in laboratory conditions, physicochemical tests were performed on the samples after adding marjoram powder and alcoholic extract. The pH was measured according to the method presented by [21], which states that 10 g of the sample is taken, 100 ml is added to it, homogenized for one minute, then the sample is filtered and its pH is measured using a pH meter. water holding capacity and whey separation. Water holding capacity was estimated according to the method presented by the [22] it was calculated as the percentage of the weight of the remaining curd after centrifugation to the initial weight of the yogurt sample, according to the following equation:

$$WHC(\%) = \frac{Wp}{Wi} \times 100$$

Where:

Wp = weight of yogurt curd remaining after centrifugation, g.

Wi = weight of the initial yogurt sample before centrifugation, g.

. whey separation was assayed by dispensing 50 g of yogurt into a beaker held at ≈45° for 2 h at 5 °C, then removing the separated whey from the surface with a syringe and re-weighing the sample.

2.6 Yogurt Microbiological Testing

Microbiological tests were conducted to evaluate the effect of marjoram powder and alcoholic extract on the microbial load of yogurt during refrigerated storage, and the total fungal count was estimated. Decimal dilutions of the samples were used, and they were cultured on appropriate nutrient media and then incubated under conditions suitable for each microbial species. After colony formation, countable plates were selected, and the results were calculated in CFU/mL based on the number of colonies and the dilution factor. The antibacterial activity of the alcoholic extract of marjoram leaves was evaluated using the agar diffusion method, and several concentrations of the extract (20, 30, 40, and 50 mg/mL) were tested. The results are presented as mean ± standard deviation.

2.7 Sensory Evaluation

Sensory evaluation of the yogurt samples was conducted in the Department of Home Economics, College of Education for Women, University of Baghdad, by a committee of faculty members and graduate students, according to the Nelson and Trout (1965) model. The evaluation was based on taste, texture, consistency, acidity, and appearance.

2.8 Statistical Analysis:

The SPSS statistical software (2019) was used to analyze the data to study the effect of different coefficients on the studied traits, according to a completely randomized design (CRD). Significant differences between means were compared using the Least Significant Difference (LSD) test.

3. Results and discussion

3.1 Chemical composition of Marjoram leaf powder

a valuable nutritional profile, characterized by moderate protein content (13.58%), low moisture (4.58%), appreciable crude fiber (21.65%), ash (9.11%), and carbohydrates (47.56%), whereas lipid content remained relatively low (3.52%). These findings

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indicate that marjoram powder represents a concentrated source of dietary fiber and mineral-rich dry matter, making it a promising functional ingredient for dairy fortification. The remarkably low moisture content also reflects the efficiency of the drying process and suggests enhanced storage stability by minimizing microbial deterioration and enzymatic degradation.

The protein content of 13.58% in the present study was comparable to Hafez, who reported a value of 12.80 g/100 g in dried marjoram leaves but higher than that obtained by Vishwakarma et al., with a value of 9.72 ± 0.03%. The fat content of our study was 3.52% which is the same result reported by Hafez and Sciences [23]it is 3.75%. The ash content for marjoram powder in our study (9.11%) was lower than the value reported by Khalil, Esoh [24] which was 14.0 %, but falls within range reported by Amer, Hassan [25] where ash content in dried marjoram leaves 10.3%; These comparisons show that the values of this study are well within the range reported in previous studies, which means differences can be explained through factors, such as agricultural environment, harvest time point and drying procedure or analysis in laboratories.

Table 2. Proximate chemical composition of dried marjoram leaf powder

Component	**Percentage (%)**
Moisture	4.58
Protein	13.58
Lipid	3.52
Ash	9.11
Crude fiber	21.65
Carbohydrates	47.56

One of the important results of this study is the high amount of crude fiber (21.65%) which according to studies above can clarify the increased water retention and reduced whey leaching in yogurt produced with marjoram. Plant fibers and carbohydrate compounds can combine within the yogurt protein network to effectively bind water, reducing its movement as free water.

3.2 Mineral composition of marjoram powder

Table No. (3) shows the mineral analysis demonstrated that *Origanum majorana* leaf powder is a rich source of essential macro- and microelements, with calcium (1895 mg/100 g) and potassium (1498.7 mg/100 g) representing the predominant minerals, followed by magnesium (321.4 mg/100 g), phosphorus (289.6 mg/100 g), iron (80.5 mg/100 g), sodium (68.7 mg/100 g), zinc (3.12 mg/100 g), copper (1.18 mg/100 g), manganese (5.25 mg/100 g) and selenium (4.98 mg/100 g). Such a mineral profile confirms the high nutritional value of marjoram and supports its application as a functional ingredient in fermented dairy products.

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Table 3: Table of mineral elements of marjoram leaf powder

no	Name	con
1	Ca (mg/100gm)	1895
2	Fe (mg/100gm)	80.5
3	Mg (mg/100gm)	321.4
4	P(mg/100gm)	289.6
5	k(mg/100gm)	1498.7
6	Na(mg/100gm)	68.7
7	Zn(mg/100gm)	3.12
8	Cu(mg/100gm)	1.18
9	Mn(mg/100gm)	5.25
10	Se (mg/100gm)	4.98

The predominance of calcium and potassium observed in the present study is consistent with previous investigations on different *Origanum* species. Uskutoglu [26] reported calcium concentrations ranging from 1008 to 1582 mg/100 g, potassium from 640 to 1209 mg/100 g, and magnesium from 309 to 436 mg/100 g in *O. vulgare*, *O. onites*, and *O. accutidens* harvested at different flowering stages. Likewise, *O. elongatum* collected from northern Morocco contained approximately 1454 mg/100 g calcium, 2295 mg/100 g potassium, and 304 mg/100 g magnesium, confirming that calcium and potassium are consistently the dominant macrominerals in the genus *Origanum* [27]. The calcium concentration obtained in the present study was slightly higher than those previously reported, whereas potassium remained within the upper range described for Marjoram species.

3.3 Effect of Marjoram on the Chemical Composition of Yogurt

Table No. (4) shows the examination of yogurt samples produced in laboratory showed that the most pronounced changes in chemical composition occurred with the addition of marjoram powder and extract. The protein content rose from 5.36% in the control treatment to 5.85% in treatment C3; fat increased ($P < 0.05$) from7.11% to 7.69%, and ash also continued increasing ($P < 0.05$), ranging from 0.89% at the control and were highest in treatment C3, respectively for both). Moisture dropped from 83.25% in the control treatment to 75.08% in treatment C3, The control treatment recorded the lowest carbohydrate content (3.39%), whereas treatment C3, representing yogurt supplemented with 1.5 mL of alcoholic marjoram leaf extract, showed the highest value (9.91%).This suggests that adding the plant raised the solid content of yogurt and lowered its moisture content.

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Table 4 Effect of marjoram powder and alcoholic extract on the chemical composition of laboratory-manufactured yogurt.

Treatment	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)	Carbohydrates (%)
A	5.36	7.11	0.89	83.25	3.39
B1	5.45	7.20	1.15	80.88	5.32
B2	5.51	7.25	1.30	79.00	6.94
B3	5.59	7.33	1.39	78.25	7.44
C1	5.55	7.39	1.22	77.08	8.76
C2	5.70	7.55	1.40	76.65	8.70
C3	5.85	7.69	1.50	75.08	9.91
L.S.D.	0.448*	0.502*	0.398*	5.190*	3.261*
The means of different letters within the same column differ significantly from one another * ($P \leq 0.05$)					

Hamed, Abd El-Maksoud [28]reported that treatments with 0.6% marjoram or geranium oil had higher viscosity, and less whey seepage than other treatments. It also examined physicochemical properties, pH, chemical composition, antioxidant activity phenols flavonoids antibacterial activity and sensory characteristics.

The highest improvement was consistently observed in yogurt supplemented with 1.5 mL alcoholic extract, whereas the control exhibited the lowest nutritional values.

3.6 Physicochemical properties of yogurt

The incorporation of marjoram powder and alcoholic extract significantly influenced the physicochemical characteristics of yogurt throughout the experimental period. Progressive improvements were observed in pH, water-holding capacity (WHC), peroxide value (PV), titratable acidity, and syneresis as the concentration of marjoram increased. Among all treatments, the alcoholic extract generally produced the greatest improvements, particularly at the highest concentration (1.5 mL).

The pH decreased gradually from 4.52 in the control treatment (A) to 4.44 in treatment B3, while a further decrease was observed in the C treatments, reaching the lowest value of 4.28 in C3. This decline in pH was accompanied by an increase in titratable acidity from 0.85% in the control to 0.98% in B3 and 1.12% in C3, indicating a progressive enhancement of acidification across the treatments. Simultaneously, water-holding capacity (WHC) increased from 12.65% in the control to 14.11% in B3 and reached its highest value of 15.65% in C3, corresponding to an improvement of approximately 23.7% compared with the control. Furthermore, the peroxide value decreased from 4.89 meq/kg in the control to 4.58 meq/kg in B3 and reached its lowest value of 4.50 meq/kg in C3, indicating improved oxidative stability compared with the control treatment. According to the reported L.S.D. values, significant differences ($P \leq 0.05$) were detected among treatments for titratable acidity, pH, WHC, and peroxide value.

The decrease in pH observed during refrigerated storage in the present study agrees with previous reports. Jańczuk, Brodziak [29] reported that the pH of yogurt decreased to 4.41 in the control and 4.10 in lactoferrin-fortified yogurt after 28 days of storage. Similarly, Nakov, Ninova-Nikolova [30] observed a decline in pH from 4.54 to 4.31 in the control yogurt and from 4.58–4.66 to 4.33–4.35 in fortified

yogurts during 24 days of storage. Tang, Zhang [31] also reported initial pH values of 4.62 in the control and 4.46–4.50 in yogurts fortified with mulberry leaf extract, with a continuous decrease throughout 21 days of storage.

These findings are consistent with the study of Hamed, Abd El-Maksoud [28], who evaluated buffalo yogurt supplemented with marjoram essential oil. They reported that incorporation of 0.6% marjoram essential oil significantly reduced lipid oxidation, lowering the peroxide value from approximately 5.31 to 4.12 meq O_2 /kg fat after refrigerated storage. Moreover, Güldane [32] showed that enhancing the biological and antioxidant properties of yogurt with the addition of spice extracts like thyme, cinnamon and ginger was successful but suggested that higher level concentration may be detrimental to sensory acceptability. Thus, we can conclude that the decrease in peroxide value in the current study is consistent with global studies which support the potential of plant-derived phenolic compounds at delaying lipid oxidation process and improving shelf life of dairy products.

Titratable acidity exhibited an inverse relationship with pH. The control yogurt (A) showed the lowest titratable acidity (0.85%), which progressively increased to 0.98% in treatment B3 and reached its highest value of 1.12% in treatment C3. This increase in titratable acidity was accompanied by a gradual decrease in pH from 4.52 in the control to 4.44 in B3 and 4.28 in C3, confirming the inverse relationship between acidity development and pH. Meanwhile, water-holding capacity (WHC) increased from 12.65% in the control to 14.11% in B3 and reached 15.65% in C3, indicating improved water retention in the treated yogurt compared with the control. The differences among treatments were significant ($P \leq 0.05$), with L.S.D. values of 0.194 for titratable acidity, 0.359 for pH, and 2.805 for WHC.

The reduction in whey separation agrees with the observations of Yilmaz-Ersan, Sahin [33], who reported that incorporation of phenolic-rich herbal extracts into probiotic yogurt significantly decreased syneresis while improving gel firmness and viscosity. They suggested that polyphenol–protein interactions reinforce the three-dimensional casein network, thereby reducing serum release and improving structural stability. Similar mechanisms are likely responsible for the improved physicochemical characteristics observed in the present study.

Overall, the physicochemical results clearly demonstrate that marjoram supplementation substantially enhanced yogurt quality by simultaneously improving water retention, reducing oxidative deterioration, strengthening gel stability, and maintaining suitable acidity during refrigerated storage. The superior performance of the 1.5 mL alcoholic extract confirms that hydroalcoholic extraction is more efficient than direct powder addition for delivering antioxidant and functional phytochemicals into fermented dairy products, thereby improving both product stability and technological quality.

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Table5. Effect of marjoram powder and alcoholic extract on syneresis of laboratory-manufactured yogurt during storage

Treatment	Day 1	Day 4	D.S.L.	Significance
A	4.20	4.01	0.277	NS
B1	3.69	3.69	0.265	NS
B2	3.65	3.65	0.217	NS
B3	3.52	3.52	0.108	NS
C1	3.50	3.50	0.114	NS
C2	3.41	3.41	0.085	NS
C3	3.32	3.32	0.072	NS
L.S.D.	0.472*	0.466*	—	—
The means of different letters within the same column differ significantly from one another. * ($P \leq 0.05$)				

The reduction in syneresis observed in the present study is in agreement with previous reports on plant-fortified yogurts. Hamed, Abd El-Maksoud [28] reported that buffalo yogurt supplemented with 0.6% marjoram essential oil or 0.6% geranium essential oil exhibited the lowest syneresis values throughout storage compared with the control and lower supplementation levels. Similarly, Tang, Zhang [31] found that the addition of mulberry leaf extract significantly reduced syneresis, with yogurt containing 0.1% extract showing syneresis values of approximately 14.4–17.7%, compared with 18.4–26.4% in the control during 21 days of refrigerated storage. Likewise, Joung, Lee [34] reported significantly lower whey separation in yogurts supplemented with with Korean traditional plant extracts compared with the control treatment. These findings support the present results and suggest that plant-derived fibers, phenolic compounds, and soluble polysaccharides improve the integrity of the protein gel matrix, thereby reducing whey expulsion and enhancing yogurt stability during storage.

Table 6 Effect of marjoram powder and alcoholic extract on titratable acidity and physicochemical properties of laboratory-manufactured yogurt

Treatment	Titratable acidity (%)	pH	WHC (%)	P.V. (meq/kg)
A	0.85	4.52	12.65	4.89
B1	0.89	4.50	13.00	4.70
B2	0.94	4.47	13.89	4.62
B3	0.98	4.44	14.11	4.58
C1	1.02	4.41	14.25	4.66
C2	1.08	4.32	15.00	4.60
C3	1.12	4.28	15.65	4.50
L.S.D.	0.194*	0.359*	2.805*	0.401*
The means of different letters within the same column differ significantly from one another. * ($P \leq 0.05$)				

3.7 Effect of marjoram powder and alcoholic extract on the fungi stability of yogurt during refrigerated storage

Table 7 revealed differences in the effectiveness of the treatments against fungal growth. In the untreated control, the fungal count increased progressively from 2.80×10^{1} CFU g^{-1} on day 1 to 2.20×10^{2},

3.70×10^2, and 2.80×10^3 CFU g^{-1} on days 3, 6, and 9, respectively. The strongest long-term antifungal effect was achieved with 1.5% marjoram powder, which limited the fungal count to 3.70×10^2 CFU g^{-1} on day 9, corresponding to an approximate reduction of 86.8% compared with the control. The 1.0% powder treatment recorded 4.80×10^2 CFU g^{-1}, representing an approximate reduction of 82.9%, whereas the 0.5% powder treatment reached 1.08×10^3 CFU g^{-1}. Thus, direct incorporation of marjoram powder was more effective than the alcoholic extract in controlling fungal growth during extended storage.s

The alcoholic extract exhibited moderate, concentration-dependent antifungal activity. On day 9, fungal counts were 3.00×10^3, 2.42×10^3, and 1.28×10^3 CFU g^{-1} in yogurt samples containing 0.5, 1.0, and 1.5 mL of the alcoholic extract, respectively. The 0.5 mL treatment did not improve fungal stability because its count slightly exceeded the control value of 2.80×10^3 CFU g^{-1}. In contrast, the 1.5 mL treatment reduced the fungal population by approximately 54.3% relative to the control. These findings demonstrate that a minimum effective concentration of the alcoholic extract was required to inhibit fungal growth. However, under the conditions of this experiment, whole marjoram powder provided stronger antifungal preservation than the alcoholic extract.

The superior antifungal effect of the powder may reflect a broader and more sustained release of antimicrobial constituents. Whole marjoram powder contains volatile terpenes, phenolic acids, flavonoids, fiber-associated phenolics, and other relatively slowly extractable compounds. During refrigerated storage, hydration of the plant particles may permit the gradual diffusion of these compounds into the aqueous and lipid phases of yogurt. In contrast, the alcoholic extract likely delivered a narrower fraction of readily soluble compounds immediately after incorporation. Some of these molecules may subsequently have interacted with casein or fat, reducing their freely available antifungal concentration. Accordingly, the powder may have acted as a slow-release reservoir, which could explain why its advantage became most apparent on day 9.

Table 8. Effect of marjoram powder and alcoholic extract on fungal counts of laboratory manufactured yogurt during storage

Treatments	Day 1	Day 3	Day 6	Day 9	L.S.D.
A	2.80×10^1	2.20×10^2	3.70×10^2	2.80×10^3	72.35*
B1	1.08×10^1	2.50×10^1	2.50×10^1	1.08×10^3	65.44*
B2	4.80×10^0	2.00×10^0	2.00×10^1	4.80×10^2	23.58*
B3	3.70×10^0	5.00×10^0	7.50×10^1	3.70×10^2	19.82*
C1	3.00×10^1	2.30×10^1	2.50×10^1	3.00×10^3	91.36*
C2	2.42×10^1	3.30×10^1	2.00×10^1	2.42×10^3	74.52*
C3	1.28×10^1	2.70×10^1	2.20×10^1	1.28×10^3	61.08*
L.S.D.	106.17*	15.28*	17.86*	106.17*	—

The means of different letters within the same column differ significantly from one another.
* ($P \leq 0.05$)

Microbial counts were transformed to $\log_{1\ 0}$ (CFU/g + 1) prior to statistical analysis to normalize the distribution and accommodate zero-count observations.

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Pascariu, Estevinho [35] showed the elderberry-fortified yogurt exhibited lower fungal growth during storage, where yeast and mold counts in the control increased from 1.72 ± 0.06 log CFU/g at the beginning of storage to 3.32 ± 0.07 log CFU/g after 21 days, whereas fortified treatments maintained substantially lower counts. In another study, yogurt supplemented with Ginger extract was more active against fungi in dairy yoghurt than (0 - 5.09 logCFU/ml) soy yoghurt (0 – 5.29 logCFU/ml). during storage, indicating a strong inhibitory effect against fungal proliferation [36].

Similar observations were reported by Ndhlala, Kavaz Yüksel [37] in Jerusalem artichoke-fortified yogurt, where yeast and mold counts remained within 1.78–2.24 log CFU/g during storage, reflecting improved microbial stability compared with conventional yogurt. Furthermore, studies on yogurts enriched with polyphenol-rich plant materials, including mulberry leaf extract and fruit-derived phenolic extracts, demonstrated delayed fungal growth and prolonged shelf life due to the antimicrobial action of phenolic acids and flavonoids.

3.9 Sensory Evaluation

Table No. (9) shows sensory evaluation performed on the first day demonstrated that marjoram supplementation affected yogurt acceptability in a concentration-dependent manner. The control treatment achieved the highest overall score (**94.6/100**), closely followed by yogurt fortified with **0.5% marjoram powder (94.4/100)**, indicating that low-level fortification preserved the desirable sensory characteristics of conventional yogurt. Increasing the powder concentration reduced the overall score to **91.9** and **88.6/100** for 1.0 and 1.5%, respectively, whereas the alcoholic extract treatments recorded **87.2–90.1/100**. The decline in acceptability was mainly associated with flavor and appearance, while texture and consistency remained statistically unaffected (LSD = 1.89, NS), indicating that marjoram supplementation had little influence on yogurt structure during the first day of storage.

The superiority of the **0.5% powder treatment** agrees with previous studies showing that moderate concentrations of herbal extracts provide the best balance between functional improvement and consumer acceptance. Tang, Zhang [31] reported that yogurt fortified with **0.1% mulberry leaf extract** exhibited the highest sensory acceptance, whereas increasing the concentration to **0.3%** intensified the herbal flavor and green color, resulting in lower sensory scores despite improvements in antioxidant activity and water-holding capacity. The same study concluded that **0.1%** represented the optimum level for balancing physicochemical and sensory quality.

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Table 9. Sensory evaluation of laboratory-manufactured yogurt fortified with marjoram powder and alcoholic extract on day 4 of storage

TREATMENTS	FLAVOR AND TASTE	TEXTURE AND CONSISTENCY	ACIDITY	APPEARANCE	OVERALL
A	42.2	28.2	14.5	9.6	94.6
B1	42.8	28.3	14	9.3	94.4
B2	41	28.1	14	8.8	91.9
B3	39.7	27.5	12.7	8.7	88.6
C1	38.1	27.9	12.4	8.8	87.2
C2	38.7	28.1	13.4	8.9	89.1
C3	39.3	28.9	13.5	8.4	90.1
L.S.D.	3.11 *	1.89 NS	1.85 *	1.064 *	5.176 *

THE MEANS OF DIFFERENT LETTERS WITHIN THE SAME COLUMN DIFFER SIGNIFICANTLY FROM ONE ANOTHER.
* ($P \leq 0.05$)

*NS: non-significant; Significant at P≤0.05.

These findings are consistent with those reported by [28], who found that yogurt fortified with marjoram essential oil maintained high sensory scores throughout storage, with the treatment containing 0.6% marjoram oil receiving overall acceptability scores above 8.0 on a 9-point hedonic scale, corresponding to consumer ratings between “like very much” and “like extremely”. Similarly, Tang, Zhang [31] reported that yogurt supplemented with 0.1% mulberry leaf extract achieved overall sensory scores of approximately 8.1–8.4/9, whereas higher concentrations slightly reduced flavor acceptance because of the stronger herbal taste. Likewise, Turgut and Diler [38] found that coriander-fortified yogurt maintained overall acceptability scores above 7.5/9 throughout refrigerated storage despite significant improvements in microbiological stability.

Conclusions

Marjoram leaf powder has demonstrated favorable nutritional and functional composition in the current study with appropriate proportions of protein, fiber, ash, and carbohydrates along with high calcium and potassium contents. Additionally, the incorporation of marjoram powder and alcoholic extract improved several qualitative and storage properties in yogurt samples, especially water retention, whey permeability and peroxide value. A numerical comparison with similar studies worldwide indicated that our results are directionally generally consistent with research on functional yogurt supplemented with some plant additives, particularly concerning improved WHC and reduced syneresis and improved oxidative stability. The extracts also showed clear activity against *Staphylococcus aureus*, substantiating the possible use of marjoram as a natural source of antimicrobial compounds in fermented dairy products. However,

careful examination of the pH values is necessary before they are published because they are higher than in mature yogurt. This study also suggests the possible utilization of marjoram powder and alcoholic extract in marjoram-fortified yogurt, provided that the addition level is selected to improve storage properties while maintaining sensory acceptability. It also suggests re-evaluating pH and titratable acidity over storage time, to examine the effects of marjoram on starter culture and probiotic counts, and use HPLC to analyse phenolic compounds in yogurt after production and during storage. In this regard, international research showed that marjoram essential oil may have a stronger antibacterial activity against some microorganisms than the corresponding powder, aqueous extract, or alcoholic extract, hence further studies should compare these substances.

References

1. Nieto, G., et al., *Applications of Plant Bioactive Compounds as Replacers of Synthetic Additives in the Food Industry.* Foods, 2023. **13**(1).
2. Jena, N., S. Marndi, and S. Mishra, *Bioactive compounds of Lemon grass (stems) and its flavonoid estimation*. 2025. p. 113-121.
3. Skrovankova, S. and J. Mlcek, *Antioxidant Potential and Its Changes Caused by Various Factors in Lesser-Known Medicinal and Aromatic Plants.* 2025. **11**(1): p. 104.
4. Nasir, N., D.M. Mushfiq, and S. Ateş, *Plant-Derived Antioxidants: Natural Preservation and Health Benefits*, in *Food Additive Innovations - Evolving Consumer Expectations and Industrial Needs*, F. Cosme, F. M. Nunes, and M. Ribeiro, Editors. 2026, IntechOpen: London.
5. Bina, F. and R. Rahimi, *Sweet Marjoram: A Review of Ethnopharmacology, Phytochemistry, and Biological Activities.* J Evid Based Complementary Altern Med, 2017. **22**(1): p. 175-185.
6. Wang, S., et al., *Origanum majorana L.: A Nutritional Supplement With Immunomodulatory Effects.* Front Nutr, 2021. **8**: p. 748031.
7. Bhardwaj, K. and W. Dubey, *Sweet marjoram (Origanum majorana L.) as a magical bio-protective agent against food spoilage: a review.* 2020.
8. Kakouri, E., et al., *Origanum majorana Essential Oil-A Review of Its Chemical Profile and Pesticide Activity.* Life (Basel), 2022. **12**(12).
9. Vera, R.R. and J. Chane-Ming, *Chemical composition of the essential oil of marjoram (Origanum majorana L.) from Reunion Island.* Food Chemistry, 1999. **66**(2): p. 143-145.
10. Attia, F.A.-Z., I. El-Sallami, and O. Ibrahim, *Variability of Essential Oil Content, Composition and Antioxidants of Sweet Marjoram Grown at Different Locations in Egypt.* Assiut Journal of Agricultural Sciences, 2023. **54**: p. 85-101.
11. Hadjimbei, E., G. Botsaris, and S. Chrysostomou, *Beneficial Effects of Yoghurts and Probiotic Fermented Milks and Their Functional Food Potential.* Foods, 2022. **11**(17).
12. Pannerchelvan, S., et al., *Functional yogurt: a comprehensive review of its nutritional composition and health*

benefits. 2024. **15**(22): p. 10927-10955.
13. Ge, Y., et al., *Fermentation characteristics and postacidification of yogurt by Streptococcus thermophilus CICC 6038 and Lactobacillus delbrueckii ssp. bulgaricus CICC 6047 at optimal inoculum ratio.* Journal of Dairy Science, 2024. **107**(1): p. 123-140.
14. Rashwan, A.K., A.I. Osman, and W. Chen, *Natural nutraceuticals for enhancing yogurt properties: a review.* Environmental Chemistry Letters, 2023. **21**(3): p. 1907-1931.
15. Adepoju, F., *Yoghurt fortification with Moringa oleifera: nutritional and production aspects.* Mljekarstvo, 2024. **74**: p. 3-21.
16. Mowafi, I.R., M. Ali, and H.J.S.-I.J.o.A.S. Ahmed, *Incorporation of propolis as a natural additive to enhance rheological, physicochemical and sensorial properties of yoghurt.* 2025. **7**(2): p. 171-181.
17. Korkmaz, I.O., C. Bilici, and S. Korkmaz, *Sensory, pH, synaeresis, water-holding capacity, and microbiological changes in homemade yogurt prepared with maca (Lepidium meyenii) powder and propolis extract.* International Journal of Gastronomy and Food Science, 2021. **23**: p. 100291.
18. Jaffar, H.M., et al., *Antioxidant activity, microbial viability, and sensory attributes of traditional-yogurt enriched with silymarin.* Cogent Food & Agriculture, 2024. **10**(1): p. 2417838.
19. Tian, Y., et al., *Effect of modified okara insoluble dietary fibre on the quality of yoghurt.* Food Chem X, 2024. **21**: p. 101064.
20. Jaafar, F., H.H.M. Al-Rubai'y, and A. Niamah, *Effect of Different Air Oven Temperatures on Chemical, Physical, and Microbial Properties of Dried Bio-Yoghurt Product.* Dairy, 2024. **5**: p. 44-53.
21. Sayre, R., B. Kiernat, and E.J.J.o.f.s. Briskey, *Processing Characteristics of Porcine Muscle Related to pH and Temperature During Rigor Mortis Development and to Gross Morphology 24 hr Post‐Mortem a, b, c.* 1964. **29**(2): p. 175-181.
22. Honikel, K. and R. Hamm, *Measurement of water-holding capacity and juiciness*, in *Quality attributes and their measurement in meat, poultry and fish products*. 1994, Springer. p. 125-161.
23. Hafez, A.A.J.A.J.o.B. and A. Sciences, *Physico-chemical and sensory properties of cakes supplemented with different concentration of marjoram.* 2012. **6**(13): p. 463-470.
24. Khalil, E., et al., *Minerals, proximate composition and their correlations of medicinal plants from Jordan.* 2012. **6**: p. 5757-5762.
25. Amer, H.Y., et al., *Modulation of Immunity, Antioxidant Status, Performance, Blood Hematology, and Intestinal Histomorphometry in Response to Dietary Inclusion of Origanum majorana in Domestic Pigeons' Diet.* Life (Basel), 2023. **13**(3).
26. Uskutoglu, T., *Effect of harvest on the agronomic, mineral and antioxidant profile of three oregano species (Origanum onites L., Origanum vulgare L. ssp. hirtum, and Origanum acutidens (Hand.-Mazz.) Ietswaart).* PeerJ, 2025. **13**: p. e20223.
27. Aarab, I., et al., *Elemental composition and health risk assessment of heavy metals in medicinal plant Origanum elongatum from Northern*

ISSN 2072-3857

Morocco. Journal of Food Composition and Analysis, 2026. **151**: p. 108945.
28. Hamed, A., et al., *Enhancing functional buffalo yogurt: Improving physicochemical properties, biological activities, and shelf life using Marjoram and Geranium essential oils.* Journal of dairy science, 2024. **107**.
29. Jańczuk, A., et al., *Properties of Yoghurt Fortified in Lactoferrin with Effect of Storage Time.* 2023. **13**(10): p. 1610.
30. Nakov, G., et al., *Yogurt Enriched with Chia Seeds: Physicochemical, Microbiological, and Sensory Changes during Storage.* 2024. **10**(8): p. 431.
31. Tang, J., et al., *Fortification of yogurt with mulberry leaf extract: Effects on physicochemical, antioxidant, microbiological and sensory properties during 21-days of storage.* Heliyon, 2024. **10**(18).
32. Güldane, M., *Effect of enrichment with spice extracts on yoghurt quality: Optimizing the extraction process and sensory properties.* Journal of Food Measurement and Characterization, 2025. **19**: p. 4354-4367.
33. Yilmaz-Ersan, L., et al., *Interaction of probiotic activity, antioxidative capacity, and gamma- amino butyric acid (GABA) in chestnut milk-fortified yogurt.* 2022. **46**(12): p. e17266.
34. Joung, J.Y., et al., *Enhanced microbial, functional and sensory properties of herbal yogurt fermented with Korean traditional plant extracts.* 2016. **36**(1): p. 90.
35. Pascariu, O.-E., et al., *Antioxidant Properties and Microbiological Stability of Yogurt Enriched with Elderberry Extract.* Foods, 2025. **14**: p. 1251.
36. Malomo, A., H. Sumbo, and Abiose, *EFFECT OF GINGER EXTRACT ON THE VIABILITY OF LACTIC ACID BACTERIA AND SENSORY CHARACTERISTICS OF DAIRY YOGHURT AND SOY YOGHURT.* 2020.
37. Ndhlala, A.R., A. Kavaz Yüksel, and M. Yüksel, *Nutritional Supplementation of Yogurt with Jerusalem Artichoke Tubers: Organic Acid Profiles and Quality Parameters.* Plants (Basel), 2022. **11**(22).
38. Turgut, T. and A. Diler, *The effect of Coriandrum sativum L. on yogurt: a comprehensive study of physicochemical, microbiological, sensory, and textural properties.* Front Nutr, 2024. **11**: p. 1493602.

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