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Effects of Oregano Methanolic Extract on the Chemical, Microbial, and Sensory Properties of Yogurt

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ABSTRACT

Introduction: The demand for antimicrobial compound alternatives to replace synthetic additives is on the rise. In food commodities, use of synthetic antimicrobials based on herbal extracts is attracted attention. The present study aimed to assess the chemical, microbiological, and sensory properties of the yogurt samples treated with oregano extract (0%, 0.75%, 1%, and 1.5%) during storage for 30 days at refrigerated temperature.

Methods: The in-vitro antibacterial effect of oregano extract was evaluated using agar disk-diffusion assay. Natural yogurt was prepared using a combination mixture of whole milk and 4% skimmed milk powder. Various concentrations of the oregano extract (0%, 0.75%, 1%, and 1.5%) were added to the samples. During 30 days of refrigerated storage, various parameters were evaluated, including titratable acidity, pH, mold and yeast counts, total coliforms, and sensory attributes (overall liking, appearance, and aroma).

Results: The descending order of the in-vitro antibacterial effect of the oregano extract was as follows: Listeria monocytogenes>Staphylococcus aureus>Bacillus subtilis>Bacillus cereus>Salmonella typhimurium>Escherichia coli O157:H7. Differences were observed in the counts of mold and yeast in the samples over time. Accordingly, bioactive yogurt had lower mold and yeast counts (1-1.5 log CFU/g) after 30 days of storage compared to the control ($P<0.05$). In addition, significant differences were observed in the hedonic scores of aroma and appearance between the samples containing the oregano extract compared to the control group ($P<0.05$).

Conclusion: According to the results, oregano extract could be used as a natural compound to improve the shelf life of yogurt at refrigerated temperatures for 30 days. Furthermore, oregano extract is considered to be an effective inhibitory compound against L. monocytogenes, S. aureus, B. subtilis, B. cereus, S. typhimurium, and E. coli O157:H7.

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Introduction

In recent decades, the functional food market has grown rapidly owing to the increased interest of consumers for the purchase of fresh food products with remarkable health benefits [1, 2]. This rising trend has remarkably influenced the food industry, especially in case of milk and dairy products, setting a special goal for the functional food market, which is faced with the challenge of offering novel products with appropriate functional and organoleptic properties to satisfy consumers [3].

Yogurt is considered to be the most consumed dairy product across the world. It is obtained from the fermentation of milk through the combined action of culturing various microorganisms, including *Streptococcus salivarius* ssp. *thermophilus*, *Lactobacillus delbrueckii* ssp., and *Bulgaricus*. These bacteria consume lactose in order to obtain energy, thereby producing the lactic acid required to coagulate milk [4]. Coagulated milk preserves the fat, mineral, and vitamin contents of pure milk despite the low lactose level, resulting in the higher digestibility of the food product compared to milk [5].

A wide variety of commercial yogurts are available on the market, which vary in terms of the composition, texture, and taste [6]. Development of dairy products with new flavors and health benefits could potentially increase the market sales. Some studies have elaborated on the functional products supplemented with fruits, vegetable oils, medicinal plants, and nutrient fortification [7, 8]. Regular consumption of yogurt has positive health effects, including the reduction of cholesterol levels, improving lactose digestion, intestinal syndromes and infections, acute diarrhea, and colon cancer, reduction of the

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nasal colonization of various pathogens (e.g., *S. pneumoniae, S. aureus*, and hemolytic streptococci), and strengthening of the immune defense mechanisms [8]. Meanwhile, the natural antioxidants and antimicrobial agents found in fruits and medicinal plants have attracted the attention of researchers and consumers [6, 9]. Furthermore, reports have confirmed the health benefits of using fresh tropical fruits and plants, which represents a growing body of research [10].

Chemical synthetic preservatives have recently been replaced by natural compounds in food commodities due to their side-effects on the health of consumers [11]. The demand for antimicrobial compound alternatives to replace synthetic additives has risen, and the replacement of synthetic antimicrobials by herbal extracts in food commodities has attracted noticeable attention [12].

Oregano (*Origanum vulgare*) is a native plant that grows in tropical regions, especially in Iran, Pakistan, and Turkey [13]. Oregano is extensively cultivated in several regions in Iran. It is a tropical plant and an abundant source of anthocyanins and natural phytochemical compounds with pharmacological properties [14]. The main compounds found in oregano extract include phenols (e.g., carvacrol and thymol), monoterpene hydrocarbons, cymene, and terpinene. Carvacrol and thymol constitute the major antibacterial content in oregano, while the possible synergistic antimicrobial actions of this plant have been attributed to the presence of terpenes. Other potential antioxidant phenols have also been obtained from the herbal extract of oregano, the most abundant of which has been reported to be rosmarinic acid [15].

To the best of our knowledge, no studies have been published regarding the effects of oregano extract on the shelf life and quality of fresh yogurt. The present study aimed to assess the chemical, microbiological, and sensory properties of the yogurt samples treated with various concentrations of oregano extract (0%, 0.75%, 1%, and 1.5%) during storage for 30 days at refrigerated temperature.

Materials and Methods

Experimental Materials

Milk and oregano were obtained from the local markets in Kermanshah, Iran. The commercial starter culture of yogurt consisting of *Lactobacillus delbrueckii* ssp., *Bulgaricus*, *Streptococcus salivarius* ssp, and *Thermophilus* was also purchased. All the chemicals and microbial cultures in the present study were purchased from Merck, Germany.

Extraction of Oregano

All parts of the oregano plants were dried in a dark place at room temperature. Afterwards, 10 grams of the powdered plant was dissolved in 20 milliliters of methanol and extracted using a shaker at room temperature for 24 hours. The extract was filtered using the Whatman filter paper No. 3, concentrated in a rotary evaporator at the temperature of 40±1ºC, and preserved in chilled condition for further experimentation [16].

In-vitro Antibacterial Effect of Oregano Extract Preparation of Microorganisms

At this stage, a panel of microorganisms was used for the antibacterial examination of the methanolic extract of oregano, which contained *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (ATCC 11774), and *Listeria monocytogenes* (ATCC 19118) as gram-positive bacteria, as well as *Salmonella typhimurium* (ATCC 14028) and *Escherichia coli* O_{157}:H_7 (ATCC 10536) as gram-negative bacteria. The cultures were purchased from the culture collection of the Iranian Research Organization for Science and Technology (IROST) in Tehran, Iran and maintained on slants of brain heart infusion agar (BHI; Merck, Germany). In addition, the bacterial inoculants (8 log CFU/ml) were prepared based on our previously published approach [17].

Agar Disk-diffusion Assay

In order to perform the agar disk-diffusion assay, one milliliter of each bacterial culture (8 log CFU/ml) was uniformly spread on the BHI agar medium using sterile cotton swabs. Following that, the sterile paper discs (diameter: 6 mm), which were dipped in 10 microliters of diluted oregano extract, were placed on the surface of each BHI agar medium. The plates were incubated for at the temperature of 37±1°C for 24 hours, and the inhibition zone was calculated as πr^2 [17].

Yogurt Production

Natural yogurt was prepared using a combination of whole milk and 4% skimmed

milk powder. The mixture was incubated at the temperature of 90±1°C for five minutes and chilled to the temperature of 44±1°C for the incorporation of the commercial starter culture. After the addition of the commercial starter culture, the milk was transferred to sterile beakers aseptically, and various concentrations of the oregano extract (0%, 0.75%, 1%, and 1.5%) were added. Afterwards, all the batches were incubated at the temperature of 44±1°C until reaching the pH of 4.6. At the next stage, the designated products were sealed and stored at the temperature of 4±1°C for 30 days. The control yogurt (without added oregano extract) was also considered in the experiments [18].

Chemical Analysis

On the first day of yogurt production, the levels of protein, fat, ash, and salt in the yogurt samples were measured based on the standards of the Association of Official Analytical Chemist (AOAC) [19]. In addition, the titratable acidity of the samples during refrigerated storage was assessed based on the AOAC method [19]. Changes in the pH of the treated and untreated samples during storage were monitored using a digital pH meter [19].

Microbial Analysis

For the enumeration of molds, yeasts, and total coliforms, potato dextrose agar and violet red bile agar were used, respectively [20].

Sensory Analysis

In order to determine the sensory effects of the addition of oregano extract on the treated and untreated yogurt samples, nine panelists (aged 22-30 years; four females and five males) examined the products based on a nine-point hedonic score (Extremely Dislike=1, Neither Like nor Dislike=5, and Extremely Like=9) for the overall acceptability of the yogurt samples, as well as the acceptability of their appearance and aroma. The samples marked with three-digit random numbers were placed in small, white plastic glasses and served immediately after heat treatment at the approximate temperature of 20±1°C [4].

Statistical Analysis

Data analysis was performed in SPSS version 25, and all the experiments were performed in triplicate. One-way analysis of variance (ANOVA) was used to determine the significant differences between the samples, and the P-value of less than 0.05 was considered statistically significant.

Results and Discussion

In-vitro Antibacterial Effect of Oregano Extract

According to the information in Table 1, the descending order of the *in-vitro* antibacterial effect of the oregano extract was as follows: *L. monocytogenes>S. aureus>B. subtilis>B. cereus>S. typhimurium>E. coli* O_{157}:H_7.

Table 1. Antibacterial Effect of Methanolic Oregano Extract Based on Agar Disk-diffusion Assay

	Inhibition Zone (mm)					
	S. aureus	***B. subtilis***	***B. cereus***	***L. monocytogenes***	***S. typhimurium***	***E. coli* O_{157}:H_7**
Extract	6.32±0.03	5.12±0.05	4.32±0.01	7.06±0.09	3.14±0.01	ND

ND: Not determined

The significant difference in the sensitivity of the bacteria to natural antibacterial compounds could be due to the outer cytoplasmic membrane covering the thin peptidoglycan structure of gram-negative microorganisms, which restricts the diffusion of hydrophobic constituents through its lipopolysaccharide covering [21, 22]. Moreover, the periplasmatic space contains enzymes that could break down the foreign molecules that are introduced from the outer environment [23, 24].

In a study in this regard, Lv et al. [23] investigated the antibacterial effects of some herbal extracts on four food-borne pathogens, including *E. coli*, *S. typhimurium, S. aureus*, and *B. subtilis.* According to the findings, gram-negative bacteria were the most resistant to the evaluated natural compounds, which is consistent with the results of the present study. In another study,

Gilles et al. [24] evaluated the antimicrobial effects of three species of Australian *Eucalyptus* using the agar disc-diffusion method, reporting that gram-positive bacteria were more sensitive compared to gram-negative bacteria. In addition, *S. aureus* was reported to be the most sensitive bacteria, while *P. aeruginosa* was the most resistant strain.

Microbial Analysis of Yogurt

In the current research, the absence of coliforms and bacterial microorganisms (*Salmonella* spp., *L. monocytogenes*, *E. coli* O_{157}:H_7 and *S. aureus*) during storage confirmed the proper sanitary practices of the food production unit in the process of product elaboration in terms of the time and temperature of adequate storage [19].

The counts of molds and yeasts were observed to differ in the yogurt samples over time, and bioactive yogurt samples had lower 1-1.5 log CFU/g after 30 days of storage compared to the control group ($P<0.05$) (Figure 1). In the control group, the initial counts of molds and yeasts were below the detection limit (1 log CFU/g) and significantly reached 3.71 log CFU/g after the study period ($P<0.05$). The decreased counts of yeasts and molds in the treated samples could be attributed to the antimicrobial effect of the oregano extract. According to our previous study, this herbal extract had remarkable effect on extending the shelf life of raw beef meat, while inhibiting the growth of some foodborne pathogenic bacteria [16].

According to the literature, the antibacterial mechanism of herbal extracts is associated with their major compounds, especially polyphenolic compounds, which are able to interact with the cytoplasmic membrane of bacterial cells, thereby leading to the leakage of cellular components [26]. Although some damage in the outer membrane and loss of the cell content are tolerated by bacteria without losing their viability, the extensive loss of cell content or critical molecules and ions could lead to microbial cell death [24]. In the present study, all the treated samples had significantly lower mold and yeast counts compared to the microbiological acceptability limit of 3 log CFU/g throughout the storage period of 30 days ($P<0.05$). Moreover, a significant difference was observed between storage time and treatments ($P<0.05$).

In a research in this regard, Al-Turki et al. [28] evaluated the antimicrobial property of aqueous extracts of oregano, marjoram, sage, and licorice at the concentrations of 1% (v/v) and 5% (w/w) against *E. coli* and *B. subtilis* in milk and labneh, reporting that all the examined aqueous herbal extracts exerted antimicrobial effects against *E. coli* and *B. subtilis*. According to the current research, the addition of various concentrations of the oregano extract significantly maintained the population of *L. bulgaricus* and *S. thermophilus* during the shelf life of the samples ($P<0.05$). In another study, the values obtained by Tabasco et al. [29] were consistent with the results of the present study. In the mentioned research, the findings confirmed the sensitivity of *S. thermophilus* to the grape extracts rich in phenolic compounds, while the deleterious effects on *L. bulgaricus* were not evident.

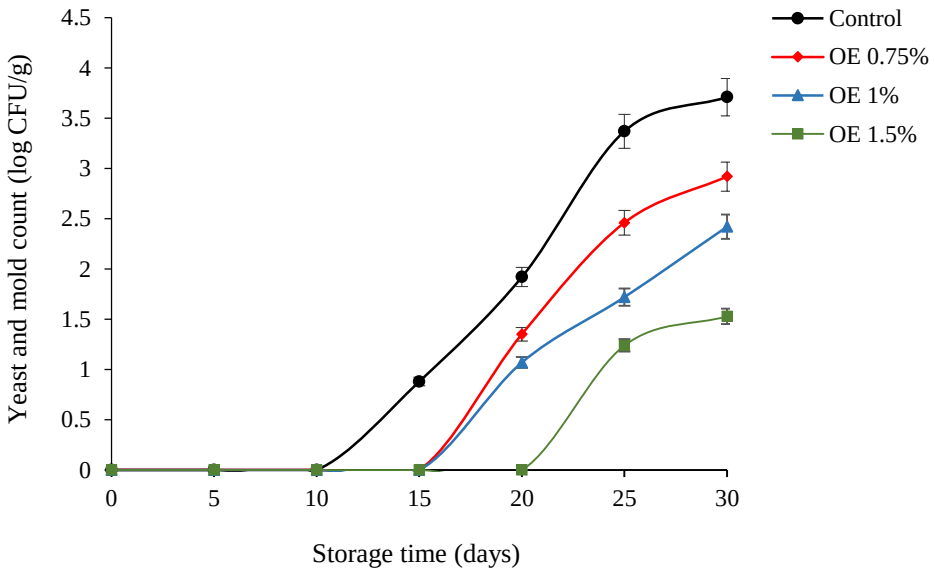

Figure 1. Changes in yeast and mold count of yogurt samples during storage at 4 ± 1 ºC for 30 days.

Chemical Analysis of Yogurt Samples

On the first day of yogurt production, the levels of protein, fat, ash, and salt in the fresh samples were estimated at 4.83±0.08, 4.43±0.13, 0.86±0.01, and 1.01±0.01, respectively. These findings are in line with the previous studies in this regard [3, 4, 29].

In the current research, the milk used to produce yogurt had an average pH of 6.1 and titratable acidity of 0.18 g lactic acid/100 g. During the fermentation process, pH decreased due to the production of lactic acid by the cultured *S. thermophilus* and *L. bulgaricus*. Furthermore, significant interaction were observed between treatment and storage ($P<0.05$); with increased storage time, pH decreased and acidity increased (Figures 2 & 3). The pH of the samples was within the range of 4.52-4.19. Similarly, Thabet et al. [27] reported that the incorporation of essential oils affected the pH and total volatile fatty acid values of the prepared labneh.

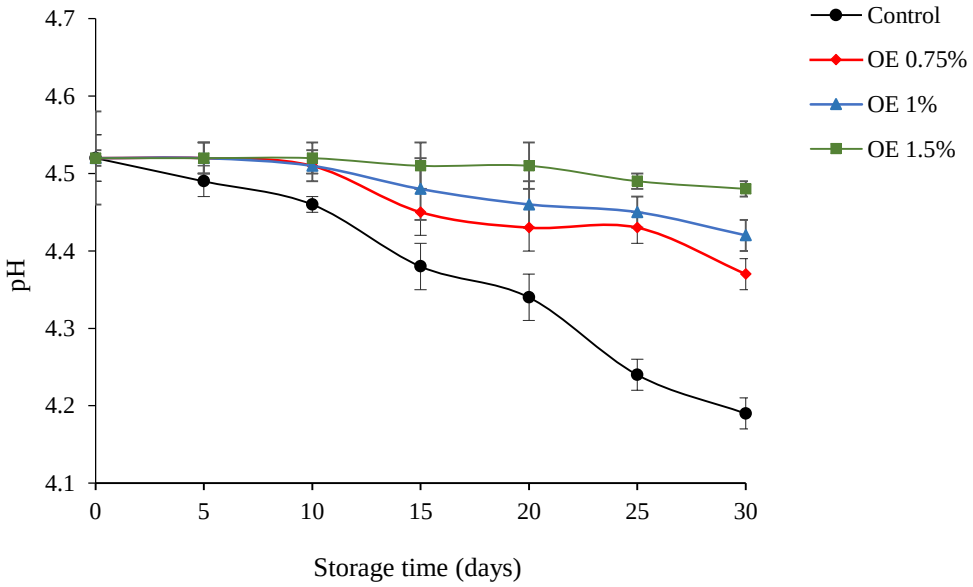

Figure 2. Changes in pH of yogurt samples during storage at 4 ± 1 ºC for 30 days.

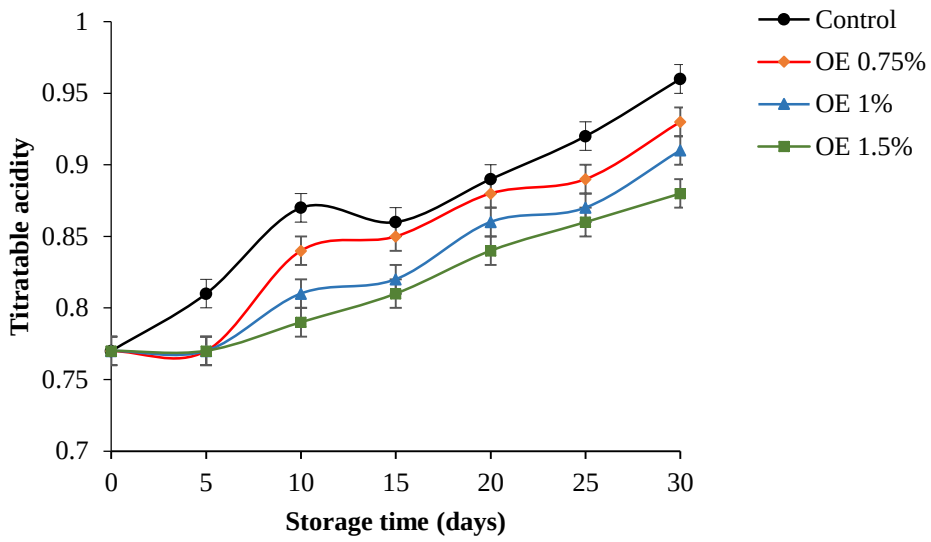

Figure 3. Changes in titratable acidity of yogurt samples during storage at 4 ± 1 ºC for 30 days.

Sensory Analysis of Yogurt Samples

The results of the sensory evaluation of the yogurt samples that were stored at refrigerated temperature indicated no significant differences between the appearance and aroma of the samples treated with the oregano extract at the concentrations of 0.75%, 1%, and 1.5% (P>0.05) (Figure 4). However, significant differences were observed in the hedonic scores of the aroma and appearance of the samples containing the oregano extract at the concentrations of 0.75%, 1%, and 1.5% compared to the control group (P<0.05).

The control and treated samples with 1% oregano extract had the lowest and highest sensory values in terms of all the studied parameters. Some studies have investigated the sensory quality of the food products treated with natural extracts, aiming to predict the applicability of the food products in terms of consumer acceptance [30-32]. The published data in this regard are conflicting in various foodstuffs, and the discrepancy could be due to the organoleptic attributes of the food products, applied concentrations of the extracts, and differences in the storage time and temperature of the products.

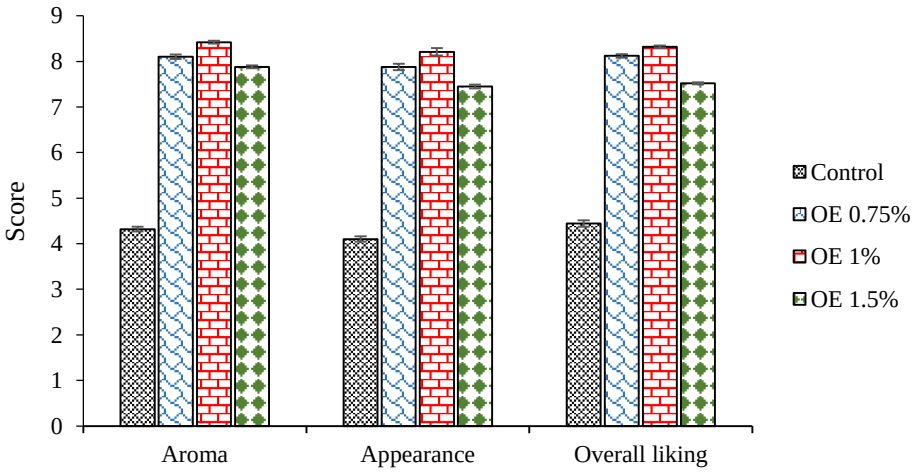

Figure 4. Sensory attributes (aroma, appearance and overall liking) of yogurt samples during storage at 4 ± 1 ºC for 30 days.

Conclusion

According to the results, oregano extract is an appropriate natural compound to extend the shelf life of yogurt during refrigerated condition for 30 days. Moreover, oregano extract has remarkable antibacterial effect against some foodborne pathogenic bacteria, including *L. monocytogenes*, *S. aureus*, *B. subtilis, B. cereus, S. typhimurium*, and *E. coli* O_{157}:H_7. Based on the literature review, this is the first study to assess the *in-vitro* antimicrobial property of oregano extract against the aforementioned pathogens and its application in the production and maintenance of fresh yogurt.

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Conflicts of interest

None declared.

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