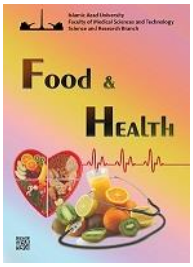

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The effect of adding olive leaves on starter culture and sensory properties of fermented Doogh

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ABSTRACT

Doogh is considered a good carrier for the transfer of nutritious compounds, and also olive leaf is one of the nutritional requirements of experts, which can be used to enrich food products to improve human health and prevent or reduce disease. The effect of adding olive leaves before and after fermentation on pH, acidity, corrosive microorganisms, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and sensory evaluation of the samples were evaluated. The enriched specimens were examined in the refrigerator during the 21-day storage time. The results showed that all samples enriched with different concentrations of olive leaves were acceptable and lower concentrations of treatments had more favorable sensory properties and at higher concentrations due to bitter taste and taste, the herb was diminished from sample acceptance. The number of *L. bulgaricus* colonies were significantly decreased during the maintenance period. The amount of this bacterium on the first day was the highest and did not have a significant effect on the *Lactobacillus* colonies by increasing the concentration of olive leaves. The survival of *S. thermophilus* in the enriched samples were not significantly different from that of the control sample and were not affected by the time and concentration of the olive leaves. Also, the addition of them in both the pre- and post-fermentation process did not have a significant effect in the number of *L. bulgaricus* and *S. thermophilus* colonies. The addition of olive leaf did not affect the viability and growth of starter microorganisms and was similar to the control sample. The microbial condition of the sample was examined for the presence of corrosive and pathogenic microorganisms during the storage of 21 days in a refrigerator. The addition of olive leaves before and after the process did not affect the microbial status of the sample.

1. Introduction

The leaves of the olive tree *Olea europaea* belongs to the *Oleaceae* family. Olive leaves make up about 10% of the wastes of olive packing and oil extraction, and plants are available as evergreen leaves (1). The presence of antimicrobial properties and antioxidant compounds such as oleuropein, phenolic compounds (such as hydroxytyrosol), flavonoids (such as kaempferol) in olive leaves have been proven. Based on the studies, olive leaves are safe for human consumption and do not contain toxic substances (2). Doogh is one of the most popular dairy products, which is widely consumed as useful drinking due to its high nutritional value and the presence of beneficial bacteria. High-quality milk is used to produce Doogh. This milk must be fresh and obtained from healthy livestock (3). In general, the main part of the microbial flora of Doogh is composed of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, and a small part of it is composed of other lactic acid bacteria. One of the basic stages of the Doogh process in industrial units is the inoculation of milk with starter culture in which *L. bulgaricus* and *S. thermophilus* are the predominant bacteria so that the amount and ratio of these two bacteria have a great impact on the quality of produced Doogh (3). The antimicrobial and antioxidant activity of olive leaves was investigated. Oleuropein inhibited the growth and proliferation of

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Escherichia coli and *Bacillus cereus* but did not affect *Staphylococcus aureus* (4). In a study, samples containing olive leaf extract had lower acidity and microbial count than control samples. The acid number of the samples increased during storage (1). The present study was an attempt to include an antioxidant and antimicrobial combination as an effective ingredient and food in people's daily diets.

2. Materials and methods

2.1. Preparation of olive leaves

Olive leaves were prepared from olive trees of oil variety from Rudbar city. Young leaves were selected from the branches. After collection, the leaves were washed and dried in a vacuum oven for 24 h at 45˚C. The dried leaves were crushed by a shredder and passed through a sieve with mesh 80.

2.2. Preparation of starters

Starters containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were used. To prepare them, 200 g of starter was mixed with 1000 ml of distilled water. 1 ml of this solution was added to 1000 ml of milk to prepare Doogh. To examine the effect of olive leaf on the activities of starter microorganisms, olive leaf was added in two stages: one simultaneously with the starter and the other after preparing the Doogh. Prepared olive leaves with concentrations of 0.1, 0.5, 1, and 1.5 mg/ml and concentrations of 0.5, 1, 3, and 5 mg/ml were added to the sample containers simultaneously with starter inoculation. All samples (simple Doogh and enriched Doogh) were incubated for 6 h at 43˚C and were cooled to 4˚C after reaching the desired pH.

2.3. Sensory evaluation

The sensory characteristics of the samples were evaluated based on the National Standard of Iran No. 3-16394 and the evaluators were asked to provide their scoring in accordance with the stated standard and report the desirability of the product. Then, the tests of acidity, pH, microbial status during storage and measurement of the activity of the starters at the beginning of operation and storage time of samples were performed according to Iranian national standards 2406, 2852, 4940, 5234, 6806-1, 7714, 10154, 13965, 4691 (5-13).

3. Results

A sensory evaluation test of samples was performed to determine the usability and acceptability of enriched samples. The results of the sensory evaluation showed that the concentration of 1.5 mg/ml of olive leaves before and after fermentation had unfavorable sensory properties and had a significant difference from the control sample. At this concentration, the bitter taste caused by oleuropein was evident, while the rest of the samples had a good taste according to evaluators. The results are shown in Table 1. Doogh enriched with olive leaves at a concentration of 0.1 mg/ml had better sensory properties than other concentrations. Also, better sensory properties were seen in samples that olive leaves were added after fermentation. The results of the acidity test in Fig. 1 showed that the acidity of the sample increased significantly during 21 days of storage in the refrigerator

Table 1. Results of sensory evaluation of Doogh enriched by olive leaves.

Sample	Concentration mg/ml	[a]A1	[a]A2	[a]A3	[a]A4	[a]A5	[a]A6	[a]A7	[a]A8	[a]A9	[a]A10	Mean±Standard deviation
Adding the olive leaves before fermentation	0.1	5	5	5	5	5	5	5	5	5	5	5.00±0.0
	0.5	4	4	4	4	5	4	4	4	4	4	4.0±1.32
	1	4	3	4	4	3	3	4	4	4	4	7.48±3.0
	1.5	1	2	1	2	1	1	1	1	1	1	2.42±1.0
Adding the olive leaves after fermentation	0.1	5	5	5	5	5	5	5	5	5	5	5.00±0.0
	0.5	4	4	4	4	5	4	4	5	4	1	4.0±2.42
	1	4	3	4	4	4	4	4	4	3	4	8.42±3.0
	1.5	1	2	1	1	1	1	2	1	1	1	2.42±1.0

($p<0.05$). The highest level of acidity was seen in enriched Doogh on day 21 of storage at the refrigerator. With increasing the concentration of olive leaves in Doogh, the acidity also increased and the highest acidity was seen in the concentration of 1 mg/L. The acidity of the control sample also increased during the storage period, but it was lower than that of enriched Doogh. Also, the addition of olive leaves in the two states before and after the fermentation process did not cause a significant difference in the amount of acidity. The pH results in Fig. 2 showed that the enriched samples had a significant decrease ($p<0.05$) and the lowest pH was seen in the enriched

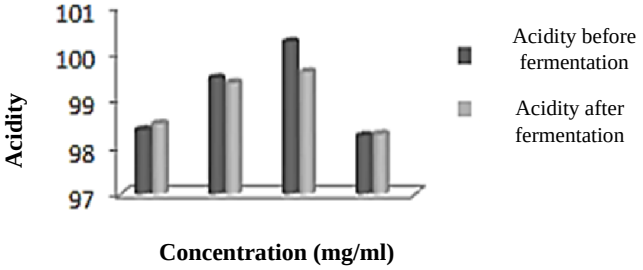

Fig. 1. The acidity of Doogh enriched with olive leaves before and after fermentation during 21-day storage in the refrigerator.

Doogh on day 21 of storage at the refrigerator. With increasing the concentration of olive leaves in Doogh, the pH decreased and the highest pH was seen at a concentration of 0.1 mg/ml.

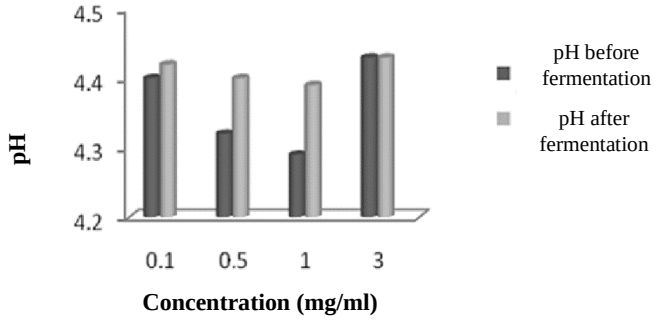

Fig. 2. Results of pH measurement in Doogh enriched with olive leaves before and after fermentation during 21-day storage.

Table 2 also showed that the addition of olive leaves in the two states before and after the fermentation process did not have a significant difference in pH.

Table 2. Compression of the results of the mean effect of adding olive leaves before and after fermentation on acidity and pH.

	Mean	Standard deviation	t	df	Sig.
Acidity	0.10	1.85	0.37	47	0.71
pH	0.00	0.17	1.49	47	0.14

The microbial status of the samples was evaluated in terms of the presence of corrosive and pathogenic microorganisms during storage in the refrigerator for 21 days. The results of Table 3 showed that the addition of olive leaves before and after the process did not affect the microbial status of the samples and the microbial status of the samples during the 21 days of storage at the refrigerator was appropriate.

Table 3. Microbial status of Doogh enriched by olive leaves and control samples during refrigeration storage.

Samples		Coliforms	*Escherichia coli*	*Staphylococcus aureus*	Yeast/ Mold
Enriched Doogh (mg/ml)	0.1	<10	Negative	Negative	<10
	0.5	<10	Negative	Negative	<10
	1	<10	Negative	Negative	<10
	3	<10	Negative	Negative	<10
	Control	<10	Negative	Negative	<10

The effect of olive leaf on the viability of Doogh starters was examined. The results in Table 4 showed that the number of *L. bulgaricus* colonies decreased significantly during the storage period ($p<0.05$). The amount of this bacterium had the highest level on the first day and did not have a significant effect on *L. bulgaricus* colonies by increasing the concentration of olive leaves. Also, the viability of *S. thermophilus* in enriched samples was not significantly different from the control sample and was not affected by time and olive leaf concentration. Also, the addition of olive leaves in the two states before and after the fermentation process did not show a significant difference in the amount of *L. bulgaricus* and *S. thermophilus* colonies in the samples. The addition of olive leaves did not affect the viability and growth of starter microorganisms and was similar to the control sample. The results of comparing adding olive leaves before and after fermentation were similar in both states. The results showed that the number of *Lactobacillus* had a significant decrease under the influence of time and the first day had the highest number of *Lactobacillus* colonies ($p<0.05$).

Table 4. Mean data of *Lactobacillus* and *Streptococcus* in Doogh enriched by olive leaves and control samples during storage.

	Lactobacillus bulgaricus				*Streptococcus thermophilus*			
Sample (mg/ml)	**day 1**	**day 7**	**day 14**	**day 21**	**1 day**	**day 7**	**day 4**	**day 21**
Control	6.45±0.18	6.12±0.32	6.05±0.52	5.74±0.39	6.62±0.48	6.44±0.38	6.23±0.33	6.08±0.62
0.5 before fermentation	6.45±0.41	6.15±0.37	6.08±0.59	5.72±0.69	6.61±0.63	6.41±0.30	6.25±0.29	6.11±0.16
0.5 after fermentation	6.45±0.30	6.12±0.46	6.10±0.42	5.78±0.43	6.62±0.42	6.42±0.14	6.28±0.38	6.12±0.17
1 before fermentation	6.45±0.38	6.15±0.05	6.10±0.99	5.81±0.22	6.63±0.35	6.50±0.64	6.27±0.60	6.12±0.31
1 after fermentation	6.45±0.91	6.17±0.29	6.11±0.25	5.84±0.71	6.64±0.31	6.51±0.65	6.32±0.24	6.14±0.20
3 before fermentation	6.45±0.53	6.20±0.58	6.13±0.25	5.86±0.59	6.64±0.42	6.52±0.59	6.33±0.25	6.16±0.36
3 after fermentation	6.45±0.37	6.22±0.45	6.17±0.61	5.89±0.44	6.63±0.74	6.53±0.45	6.34±0.20	6.20±0.51

4. Discussion

The results of sensory evaluation of the addition of pure oleuropein to yogurt showed that with increasing oleuropein concentration, the popularity of the enriched product decreases, and the product becomes bitter. Also, a similar pattern between the pH of the enriched and control samples was seen, and the addition of oleuropein did not affect the pH of the samples. Oleuropein has no interfering effect on starter microorganisms of yogurt (14). In adding olive leaf extract to probiotic yogurt, the results showed that with the addition of olive leaf extract, the acceptability of the sensory properties of probiotic yogurts increased. Also, with the addition of olive leaf extract, acid production by probiotic microorganisms increased (15). The results of adding mint, basil, and dill to yogurt showed that the addition of vegetables to yogurt changed the activity of microorganisms and the acidity of all samples was higher than the control (16). The results of adding rice bran to yogurt indicate that adding rice bran did not have a significant effect on the acidity of enriched samples (17). The results of adding strawberry fruit to yogurt showed that it had no effect on the acidity of enriched samples and was similar to the control sample (18). The results of adding spices to probiotic yogurt did not have a significant effect on the pH of products (19). The results of adding dragon fruit to yogurt which was done by Zainoldin et al. (20) revealed that adding

dragon fruit in the starter stage had a significant effect on pH and the pH of enriched samples was lower than the control (20). Adding olive leaves did not have an inhibitory effect on starter microorganisms. The number of *Lactobacillus* colonies decreased during the storage period of the samples, which may be due to the lack of food resources in the last days and the high acidity of the environment. The changes in microorganisms in the two states of adding olive leaves before and after the fermentation process were similar. The results of adding olive leaf extract to probiotic yogurt showed that with adding olive extract, the number of probiotic microorganisms increased, which is directly related to the concentration of the extract (15). The results of adding tea to yogurt by Jaziri et al. (21) indicated that adding tea did not have an interfering effect on starter microorganisms. Bertolino et al. (22) added hazelnut peel powder to yogurt and the results indicated no effect on the starter strain.

5. Conclusion

In this study, the results of the sensory evaluation showed that all samples enriched with different concentrations of olive leaves (except the concentration of 1.5 mg / l) were acceptable to the evaluators and lower concentrations had more favorable sensory properties and at higher concentrations, the acceptability of the sample decreased due to its bitter taste and herbal taste. It seems that the bitter taste to be due to the presence of oleuropein in olive leaves. The acceptability of olive Doogh enriched with olive leaf was higher in the post-fermentation process, which could be due to changes in phenolic compounds due to the activity of starter microorganisms in the enriched samples before the fermentation process. The results of adding olive leaves to the samples showed that the pH of the samples and the control decreased during storage due to the activity of starter microorganisms. The results of comparing adding olive leaves before and after fermentation were similar in both states. Acidity is an important factor in the acceptability of the sample and its viability. The results showed that the acidity of enriched and control Doogh increased during storage in the refrigerator due to the activity of initiating microorganisms and it was directly related to increasing the concentration of olive leaves. Adding olive leaves to the samples before and after fermentation had the same results.

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