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Effect of Khalvash (*Mentha piperita*) Extract on the Physicochemical, Sensory and Microbial Characteristics of Doogh During Shelf Life

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ABSTRACT

Background and Objective: Natamycin is generally used to control mold and inhibit yeast growth and some bacteria. However, there is an increased sensitivity for using natamycin. The study aimed to investigate the effect of khalvash (*Mentha piperita*) extract on doogh. **Materials and Methods:** The extracts of khalvash were used at levels of 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 with the control sample as well as the sample containing natamycin in the doogh. Sampling was carried out on the 1st, 15th and 30th day in three replications and its effect on physicochemical properties including pH, acidity, fat, dry matter percentage and microbial properties including mold and yeast count and sensory characteristics were investigated. **Results:** There was a significant difference between the pH factors in the control group and the natamycin three times (days 1, 15 and 30) ($p<0.05$). The level of anti-microbial activity increased in the treatments with kalvash extracts in comparison with the treatment with natamycin and control during the period ($p<0.05$). An increase in acidity helped increase the antimicrobial activity of the extract. The treatments with extract had significant differences from the control treatment ($p<0.05$). In addition, the results of the sensory evaluation indicated that the highest and lowest rates of acceptance were in the doogh with natamycin and 0.3% khalvash, respectively. Likewise, an increase in the level of khalvash extracts resulted in a decrease in the acceptability level reaching its lowest level in the treatment of 0.3% ($p<0.05$). **Conclusion:** The use of khalvash extracts can be a suitable flavor and a fairly appropriate preservative in the doogh.

KEYWORDS

Doogh, herbal extracts, microbial properties, natamycin, preservative

INTRODUCTION

Animal husbandry and products are important to fulfill the universal demand for high-quality food. When it comes to animal products, taste and appearance have economic value. Doogh is an Iranian drink made from yogurt, water and salt. It is worth mentioning that the popularity of this fermented beverage arises from its organoleptic characteristics and its health benefits[1-4]. Doogh is the result of the lactic fermentation

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of milk and similar products are consumed in some countries in the Middle East and Central Asia[5-7]. Milk and dairy products are relatively adequate foods and are good sources of mineral nutrients. On the other hand, it should be emphasized that dairy products can be contaminated with dangerous environmental factors. Thus, the safety and hygiene of dairy products are very important[8]. The different climate (at the same time) in Iran makes the production of a wide range of dairy-fermented products. There are some herbs added to dairy-fermented products traditionally. For example, khalvash (*Mentha piperita*) is added to doogh in northern parts of Iran[9-11]. Incorporation of herbs in doogh may lead to more beneficial health impacts for the host as it has been done traditionally for years. One of the greatest tasks of the food industry is to keep the quality of food products[7,12,13].

A vast number of variations have changed the development of fermented dairy products and their quality. But the achievements are not adequate. This study aimed to evaluate the enrichment of doogh with the khalvash extract and investigate its physicochemical, microbial and sensory properties during storage at room temperature and in refrigerators.

MATERIALS AND METHODS

Study Area

Adopted protocol: The study started on 26th May, 2017 and ended on 26th June, 2017. This study was conducted in the laboratory of Mehrayin University, Bandar Anzali, Iran. Fourteen liter doogh and 18.9% khalvash extract used for this study. To prepare the khalvash extract, a fresh khalvash plant was obtained from Rasht Grand Bazaar. Then, it was dried in a suitable environment away from sunlight and powdered by a mill and passed through a sieve. The plant powder was mixed with a solvent (96% ethanol) at a ratio of 1:10 on a shaker (KL 2, Bühler Dual-Action Shaker, German) for 48 hrs and then passed through filter paper. The solvent was separated using a rotary (RE550, China) at a temperature of 45°C. Khalvash plant extract was used in doogh at levels of 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3. Natamycin was purchased from Iran Chemi Company and was used at 10 ppm in the control sample.

Raw milk for preparing doogh (milk with the characteristics): The pH = 6.68, acidity 15° Dornic, fat 3.5%, protein 3.2%, non-fat solids 8.8%, total solids 12.3% and microbial load 2.7×105 CFU mL^{-1} was standardized, followed by standardization of fat (1.8%) and pasteurization of doogh (90°C for 10 min), then cooling to 43-45°C and fermentation (2-3%) was done. Then, the greenhouse was kept at 42-45°C and cooled down to minus 10°C to stop the greenhouse at 110-100 Dornic acidity degrees, adding 50% water and less than 1% salt (0.7%) and mixing. Homogenization and pasteurization (72-76°C for 15 sec) were done as the final stages of production and then packaging and storage at 4°C temperature were done. Then natamycin at the level of 10 ppm to the control doogh sample and the extract of khalvash plant at the levels of 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 with the help of Tween 80 at the rate of 30% of the weight of the extract using A mixer was added to prepare doogh treatments.

After preparing the doogh samples, the samples were kept for 30 days, during this period every 15 days (days 1, 15 and 30) of control doogh containing natamycin and doogh samples containing 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3% of khalvash extract were sampled. Physicochemical characteristics (titratable acidity, pH, total solids, doogh measurement) and microbial test (mold and yeast) were evaluated.

Studied parameters: Then, physicochemical tests, pH measurement, total solids measurement, fat measurement, microbial test and sensory evaluation were performed based on the methods approved by Iran's National Standard. The samples were given to 15 randomly selected sensory assessors to determine the overall acceptability of each of them and they were asked to rate the samples on a scale of 1-5 according to the degree of interest (5 for "very pleasant" to 1 for "very unpleasant") (National Standard of Iran No. 695, 1387).

Statistical analysis: In this research, the effect of 8 treatments (6 levels of khalvash extract, one doogh sample containing natamycin and one doogh sample without additives) in three repetitions during storage on days 1, 15 and 30 on the physicochemical and microbial characteristics of doogh was investigated. To statistically analyze the data, the Analysis of Variance (ANOVA) method was used in the form of a completely random design and Duncan's Test at 5% level. Statistical analysis was done using SPSS statistical software program version 22.

RESULTS

Amount of pH and acidity

pH changes: The results of the statistical analysis showed that there is a statistically significant difference between the control group and natamycin treatments three times (days 1, 15 and 30) ($p<0.05$) as shown in Table 1. Also, the lowest pH was observed in the control treatment on the 30th day and natamycin treatment on the 1st day. On the other hand, the highest pH level was related to the treatment of 0.05% of khalvash on the 30th day.

The results of the statistical analysis related to the acidity factor (Table 2) showed that the highest level of acidity in the treatment with 0.25% of khalvash on the 30th day so that a statistically significant difference was observed between the said treatment and the treatment without the extract on the same day ($p<0.05$). Also, the amount of acidity in treatments of 0.5, 0.1 and 0.15% on the 15th day has the most statistically significant difference with the control treatment on the same day ($p<0.05$). In addition, no statistically significant difference was observed between the control treatments, different levels of khalvash extract and natamycin on the first day ($p<0.05$).

Table 1: Results of pH analysis of doogh treatments

Treatments	Day 1	Day 15	Day 30
1	4.24 ± 0.003^{Bac}	4.37 ± 0.002^{Fab}	4.20 ± 0.003^{Aac}
2	4.26 ± 0.003^{Ca}	4.29 ± 0.002^{Ha}	4.31 ± 0.003^{Fa}
3	4.26 ± 0.003^{Ca}	4.27 ± 0.002^{Ea}	4.28 ± 0.003^{Da}
4	4.28 ± 0.003^{Ea}	4.25 ± 0.002^{Ba}	4.27 ± 0.003^{Ca}
5	4.27 ± 0.003^{Da}	4.27 ± 0.002^{Da}	4.27 ± 0.002^{Ba}
6	4.27 ± 0.003^{Da}	4.25 ± 0.002^{Aa}	4.27 ± 0.003^{Ca}
7	4.26 ± 0.003^{Ca}	4.26 ± 0.002^{Ca}	4.27 ± 0.002^{Ba}
8	4.20 ± 0.003^{Aab}	4.26 ± 0.002^{Cbc}	4.28 ± 0.003^{Ebc}

Standard Deviation = ±, Different letters indicate a significant difference ($p<0.05$)

Table 2: Results of acidity analysis of doogh treatments

Treatments	Day 1	Day 15	Day 30
1	0.64 ± 0.003^{Aa}	0.83 ± 0.002^{Ab}	0.6 ± 0.002^{Aa}
2	0.79 ± 0.001^{Aa}	0.94 ± 0.003^{Db}	0.70 ± 0.002^{Ec}
3	0.80 ± 0.002^{Aa}	0.94 ± 0.003^{Db}	0.69 ± 0.002^{Cc}
4	0.78 ± 0.002^{Aa}	0.94 ± 0.003^{Db}	0.68 ± 0.002^{Cc}
5	0.79 ± 0.002^{Ab}	0.92 ± 0.003^{Cc}	0.67 ± 0.002^{Ba}
6	0.97 ± 0.002^{Ac}	0.90 ± 0.003^{Bb}	0.81 ± 0.002^{Ha}
7	0.83 ± 0.035^{Ab}	0.99 ± 0.003^{Ec}	0.72 ± 0.003^{Fa}
8	0.76 ± 0.002^{Ab}	0.90 ± 0.003^{Bc}	0.67 ± 0.002^{Ba}

Standard Deviation = ±, Different letters indicate a significant difference ($p<0.05$)

Table 3: Results of fat analysis of doogh treatments

Treatments	Day 1	Day 15	Day 30
1	1.25 ± 0.00^{Aa}	1.27 ± 0.00^{Aa}	1.39 ± 0.01^{Aa}
2	1.30 ± 0.01^{Aa}	1.32 ± 0.02^{Aa}	1.38 ± 0.01^{Aa}
3	1.69 ± 0.01^{Aa}	1.75 ± 0.08^{Aa}	1.73 ± 0.01^{Aa}
4	1.75 ± 0.04^{Aa}	1.72 ± 0.04^{Aa}	1.79 ± 0.07^{Aa}
5	1.90 ± 0.04^{Aa}	1.84 ± 0.03^{Aa}	1.95 ± 0.05^{Aa}
6	1.95 ± 0.01^{Aa}	1.85 ± 0.02^{Aa}	1.80 ± 0.02^{Aa}
7	1.79 ± 0.01^{Aa}	1.70 ± 0.01^{Aa}	1.81 ± 0.05^{Aa}
8	1.86 ± 0.03^{Aa}	1.80 ± 0.03^{Aa}	1.90 ± 0.03^{Aa}

Standard Deviation = ±, Different letters indicate a significant difference ($p<0.05$)

Table 4: Results of dry matter analysis of doogh treatments

Treatments	Day 1	Day 15	Day 30
1	91.90 ± 0.00^{Aa}	91.84 ± 0.02^{Aa}	91.69 ± 0.01^{Aa}
2	91.82 ± 0.01^{Aa}	91.75 ± 0.07^{Aa}	91.87 ± 0.03^{Aa}
3	91.78 ± 0.00^{Aa}	91.63 ± 0.08^{Aa}	91.64 ± 0.02^{Aa}
4	91.72 ± 0.07^{Aa}	91.79 ± 0.05^{Aa}	91.93 ± 0.02^{Aa}
5	91.95 ± 0.01^{Aa}	91.82 ± 0.03^{Aa}	91.79 ± 0.01^{Aa}
6	91.95 ± 0.02^{Aa}	91.91 ± 0.01^{Aa}	91.89 ± 0.04^{Aa}
7	91.44 ± 0.03^{Aa}	91.42 ± 0.01^{Aa}	91.51 ± 0.02^{Aa}
8	91.93 ± 0.03^{Aa}	91.89 ± 0.05^{Aa}	91.92 ± 0.01^{Aa}

Standard Deviation = ±, Different letters indicate a significant difference ($p<0.05$)

Table 5: Results of mold and yeast analysis of doogh treatments

Treatments	Day 1	Day 15	Day 30
1	$0.56\times10^{3}\pm0.00^{Fa}$	$2.92\times10^{3}\pm0.00^{Ib}$	$3.18\times10^{3}\pm0.00^{Hc}$
2	$0.40\times10^{3}\pm0.00^{Aa}$	$2.18\times10^{3}\pm0.00^{Hb}$	$2.41\times10^{3}\pm0.00^{Fb}$
3	$0.40\times10^{3}\pm0.00^{Aa}$	$2.09\times10^{3}\pm0.00^{Fb}$	$2.36\times10^{3}\pm0.07^{Ec}$
4	$0.52\times10^{3}\pm0.00^{Ea}$	$1.81\times10^{3}\pm0.00^{Eb}$	$2.30\times10^{3}\pm0.14^{Db}$
5	$0.50\times10^{3}\pm0.00^{Da}$	$2.05\times10^{3}\pm0.00^{Db}$	$2.30\times10^{3}\pm0.07^{Db}$
6	$0.56\times10^{3}\pm0.00^{Fa}$	$1.62\times10^{3}\pm0.00^{Cb}$	$1.72\times10^{3}\pm0.00^{Bb}$
7	$0.43\times10^{3}\pm0.00^{Ba}$	$1.45\times10^{3}\pm0.00^{Ab}$	$1.44\times10^{3}\pm0.05^{Ab}$
8	$0.47\times10^{3}\pm0.06^{Ca}$	$1.53\times10^{3}\pm0.42^{Ba}$	$2.05\times10^{3}\pm0.00^{Ca}$

Standard Deviation= ±, Different letters indicate a significant difference ($p<0.05$)

Table 6: Results of sensory evaluation of doogh treatment

Treatments	Score color and appearance	Fragrance score	Taste score	General acceptance
1	4.06 ± 1.01^{a}	3.4 ± 0.73^{a}	3.53 ± 0.63^{a}	3.64^{ae}
2	2.53 ± 1.18^{e}	3.13 ± 0.74^{a}	3.4 ± 0.50^{a}	3.64^{ae}
3	2.00 ± 0.84^{c}	3.33 ± 0.48^{a}	3.26 ± 0.79^{ab}	3.83^{c}
4	1.73 ± 0.79^{c}	2.86 ± 0.74^{a}	2.73 ± 0.79^{bc}	2.42^{b}
5	1.66 ± 0.48^{c}	3.00 ± 0.84^{a}	2.46 ± 0.83^{cd}	2.34^{b}
6	1.86 ± 0.74^{c}	2.8 ± 1.01^{a}	2.46 ± 0.99^{cde}	2.34^{b}
7	1.46 ± 0.51^{c}	2.53 ± 0.51^{a}	1.53 ± 0.51^{f}	1.83^{a}
8	4.0 ± 0.0^{b}	4.3 ± 0.73^{b}	3.53 ± 0.53^{a}	3.93^{f}

Standard Deviation = ±, Different letters indicate significant difference ($p<0.05$)

Number of fat changes

Changes in fat content: According to the results of the statistical analysis, no significant difference was observed between the control treatments, different levels of khalvash extract and natamycin at three different times ($p>0.05$) as shown in Table 3. The rate of change of dry matter the number of changes in dry matter. According to the results of the statistical analysis, no statistically significant difference was observed between the control treatments, different levels of khalvash extract and natamycin at three different times ($p>0.05$) as shown in Table 4.

Mold and yeast changes: The results of variance analysis obtained between different treatments on days 1, 15 and 30 can be seen in Table 5. The data obtained from the colony count of the control treatments and different levels of khalvash extract showed the strength of the extracts in preventing the growth of mold and yeast in comparison with the control treatment. In this way, the growth of mold and yeast in the control treatment was higher than in the treatments containing the extract. Also, the results of the statistical analysis showed that with time, the growth of mold and yeast decreased with the increase of different levels of khalvash extract. Thus, the lowest and the highest amount of mold growth was observed in the treatments of 0.3% khalvash extract and control on the 30th day, respectively. Also, a statistically significant difference was observed between the control treatments and 0.3% khalvash extract on the 30th day ($p<0.05$).

Sensory evaluation: The sensory evaluation results were shown in Table 6. The highest score of color and appearance was seen in the treatment without extract, while the lowest score of color was related to the

treatments with khalvash extract ($p<0.05$). The highest aroma score was observed in the natamycin treatment, but no significant difference was observed between the control treatments and other treatments with extracts ($p>0.05$). According to the results of the statistical analysis of the sensory evaluation, it showed that the acceptability level among the treatments with extracts was low. Whereas natamycin treatments without extract had higher acceptance than treatments with the extract.

DISCUSSION

All treatments showed a significant difference in terms of pH and acidity with the control sample ($p<0.05$). The addition of khalvash extract to doogh formulation increased acidity and decreased pH. The pH of doogh containing khalvash extract is closer to the pH of the national doogh standard and in comparisons during and between periods, the number of changes in the samples containing 0.05% of the extract from the previous days was not significantly different ($p<0.05$) and compared to the samples containing natamycin and no extract were better. As the pH increases towards the optimum pH, which is mostly neutral pH, the growth rate increases and as the pH decreases, the lag phase increases and as a result, the growth rate of microorganisms decreases. Zarali *et al*.[13] attributed the decrease in pH to the breakdown of the ester group. On the other hand, the growth of acid-resistant bacteria such as *Lactobacillus* may also be effective in this field. The researchers introduced the cause of the decrease in pH to *Lactobacillus bulgaricus*, which is the cause of acidification or over-acidification in yogurt. This bacterium works in the same way in the doogh products.

The researcher stated that during fermentation and storage, the process of reducing the acidity in the product will continue even until the pH is less than 3.5 and *Lactobacillus bulgaricus* is capable of over-acidification in the doogh product, which is consistent with the Biswas *et al*.[14].

Zadeh *et al*.[15] investigated the effect of mountain thyme plant essential oil on the physicochemical and sensory characteristics of doogh for 14 days. The essential oil of this plant was used at five different levels (500, 400, 300, 200, 100 and 50 ppm). The results of this study showed that there is a significant difference in acidity between 100 and 400 ppm treatments.

Also, the results of sensory analysis of doogh showed that dooghs containing different concentrations of thyme essential oil differ significantly in terms of taste, so higher concentrations had a negative effect on the taste of doogh and the best taste acceptance was observed at a concentration of 50 ppm.

In the study of Cava-Roda *et al*.[16], who investigated the antimicrobial effect of vanilla in milk, two pH ranges, 6 and 7, had no significant difference in MIC. Better dissolution of the extracts in the lipid phase of the cell membrane of the target bacteria at low pH improves the antimicrobial activity of the extract. In fact, the antimicrobial activity of extracts in foods with neutral pHs, such as milk and butter, is less than in acidic foods. At low pH, molecules with antimicrobial activity are more easily bound to the hydrophobic regions of the cell membrane and as a result, show a higher antimicrobial effect. In all the studies mentioned, the observed results are almost aligned with the results of this study. In the first times of storage, by increasing the amount of extract from the mint family and afterward increasing the substrate available for the growth of microorganisms, the metabolic activity of bacteria increases which causes a decrease in pH and also an increase in acidity in the samples containing the extract. In addition, the added mint family extract has an acidic pH, which can affect the pH of the samples containing the extract. But at the end of the storage period, the pH does not change much with the increase in the amount of mint extract. Microbial activity in the presence of khalvash extract has increased the delay phase in the cell growth graph and increased the delay time compared to the control sample. Also, the addition of essential oil or extract has significantly reduced the amount of cell mass production, because the energy required by the cell for repair or survival is more than cell reproduction. The reason for the increase in mold and yeast growth in doogh samples can be attributed to the synergistic effect of starter bacteria and acid

production by *Lactobacillus bulgaricus* bacteria and the increase in acidity during the storage period[17-19]. The results of this study showed that the growth of mold and yeast in the samples containing plant extracts was lower compared to the control sample and the extract of khalvash had a greater inhibitory effect on the growth of mold and yeast in doogh compared to the natamycin preservative and the sample without extract.

Therefore, based on the results of these studies, the essential oil of khalvash plant has anti-bacterial and anti-fungal properties. The results obtained from the measurement of the diameter of the lack of growth indicate that the essential oils of these two plants have a greater antibacterial effect compared to the natamycin and the essential oil of the khalvash plant also has a stronger antibacterial effect than the essential oil of the thyme plant. Abu-Shanab *et al.*[20] and his colleagues investigated the antimicrobial effect of khalvash extract against methicillin-resistant staphylococci and showed that both aqueous and alcoholic extracts of this plant are effective against this fungi[20].

According to the research results of Ghazghazi *et al.*[21], pennyroyal (*Mentha pulegium*) essential oil has antifungal activity against a wide range of microorganisms which is comparable to standard drugs. Based on the results of this research, the essential oil of khalavash has a stronger antifungal power than the natamycin, so from this point of view, the results are in line with previous studies.

According to these results, the essential oil of this plant and its products can be used in food as a beneficial food. On the other hand, considering the increasing limitations of using antimicrobial chemicals such as side effects and drug-resistance, there is a need to replace these substances with natural substances, including the essential oils of these plants and they can be useful in disease control.

There was a significant difference in the color and appearance score of the samples with khalavash extract compared to the control sample and the sample with natamycin preservative ($p<0.05$) as shown in Table 6. The best color and appearance were seen in the sample without khalavash extract. In terms of aroma and taste score, there was only a significant difference with samples containing natamycin and the sample containing natamycin had the best aroma and taste ($p<0.05$). While with the addition of khalavash extract, the taste score had a significant difference only with the samples containing 0.05% and above ($p<0.05$), but it did not have a significant difference with the sample containing natamycin ($p<0.05$), that is, with an increase the amount of khalavash extract worsened the taste score compared to the control sample.

According to the statistical results, all the sensory characteristics (smell, taste and color) had a statistically significant difference, in other words, the doogh containing the examined extract compared to the doogh without extract had a significant difference in terms of sensory characteristics ($p<0.05$) and the total was not accepted. Because no research has been done on this type of Iranian mint khalavash in Iran, no results were available for comparison. It should be mentioned that the essential oil or extracts of the mint family have a significant difference in sensory evaluation with the essential oil or extract of this type of mint, of course, the climatic conditions of growth are effective in these cases.

Therefore, the khalavash plant has a unique smell and taste that is completely distinguishable from the mint. Khalvash is warm at first, quite fragrant, almost gassy and bitter, which after a while creates a cool feeling. In terms of organoleptic characteristics, this extract is not accepted alone in doogh, but it can be used together with other flavorings. The use of other vegetable extracts along with the olive leaf extract can be tested. Reduction of the effects of plant extracts during pasteurization in the industry can be mentioned.

CONCLUSION

Khalavash plant extract was considered an additive in doogh production in this study. Although, the addition of khalavash extract decreased the percentage of sensory evaluation compared to the control sample and the sample containing natamycin, due to the high antioxidant and microbial quality of khalavash plant extract compared to chemical additives such as natamycin which is usually used in the production of beverages, this extract. The plant is rich in antimicrobial substances and antioxidants such as flavonoids and terpenoid compounds such as menthol, phenolthymol, terpinolene, phenol, etc. and with higher efficiency, the nutritional value of the product increases. Also, the presence of high amounts of therapeutic properties can be a good source for certain diseases.

SIGNIFICANCE STATEMENT

Food preservatives such as benzoic acid, sorbate, natamycin, etc. are widely used in the food industry. In some countries, including the United States, natamycin is widely used as a preservative in the dairy industry, including in cheese, yogurt, etc., due to the prohibition of its use in some countries, including Iran. Plants, including khalvash extract, can be considered as an alternative to natamycin, which, in addition to preventing mold, improves the physicochemical characteristics of these products.

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