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Effect of Mentha Piperita L. Powder on the Overall Acceptability, Coliforms, and Molds and Yeasts Counts of Borujerd Domestic Cheese

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Abstract. In this study, the antimicrobial effects of plant powder Mentha Piperita L. in at the levels of 0.1, 0.2 and 0.3 % on preventing the growth of mold, yeast and coliforms, as well as the, overall acceptability, pH and acidity of Borujerd domestic cheese samples during the cold storage were studied. The findings showed that with increasing the percentage of Mentha Piperita L. powder in cheese samples, the number of coliforms and mold and yeast, titratable acidity and overall acceptability significantly decreased ($p < 0.05$); and pH significantly increased ($p < 0.05$). Finally, the sample containing 0.2% Mentha Piperita L. had more utility in sensory properties than the others containing it.

Keywords: Mentha Piperita L., Borujerd domestic cheese, Coliform, Mold and yeast, Overall acceptability

1. INTRODUCTION

Natural food preservatives and flavorings have recently found a wide use and acceptability, even in some countries, consumers tendency to use foods containing preservatives or artificial flavors has shown a decreasing tendency. This is due to the recognition of the harmful effects of chemical compounds, flavors and preservatives, which are widely used in a variety of processed foods. Among the natural compounds that can serve as a preservative in food, plants and their derivatives have been paid much more attention than any other natural compounds and studies in relation to their antimicrobial effects and properties of plants giving a taste the foods in terms of food habits. Many studies have seen conducted in different countries and different cultures in this regard and various products of this type have been introduced to the market [1]. Among the dairy products, cheese has good quality protein that contains almost all the essential amino acids needed by the body [2]. Microorganisms play an important role in the processing, and determining the desirability of cheese flavor. However, in some circumstances, a human pathogen colony is formed during the curing cheese in the cheese samples, and causes diarrhea and other diseases [3]. Peppermint *Mentha piperita L.* plant with the scientific name of the *Lamiaceae* family herbs, due to by multiple drug effects, has attracted the attention of researchers. Peppermint herb herbaceous perennial with dark Peppermint aroma and a height of about 40-80 cm, opposite leaves, oval, pointed and downy stem, falling squares and, inflorescences and complex flowers at the end of the stem white or red-purple and capsule form fruits. This plant grows in most parts of Lorestan province. It is used in form of leaves, floral branches, plant oils, and sweat. Its chemical compounds contain essential oils, menthol, Menthone, Limonene (0.1-1%), flavonoids, Hyperitin, Mentozid, tannins, resins, Triterpène, *Tocopherol* (α and γ), carotenoids, phenolic acids, etc. Peppermint plant with hot and dry nature, in addition to being consumed as food herbs and spices, is used in traditional medicine as a

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energiser, stomach tonic, windbreaker, diuretic, diaphoretic, and antiseptic. It is also applied in diarrhea, nosebleeding, nervous headaches,, digestive pains, and migraines [4].

Antimicrobial activity and chemical composition of some essential oils have been studied. According to the results, the essence of peppermint oil has strong antimicrobial effect against *Staphylococcus aureus* and *Escherichia coli* [5]. Also the results of the study to evaluate the effect of some plant extracts against *Staphylococcus aureus* in feta showed that peppermint essential oil had the highest antimicrobial effect against *Staphylococcus aureus* than the other. Therefore, it can be used as preservative in food products [6]. In another study, the antimicrobial properties of the essential oil of *Mentha piperita L.* were evaluated against 21 pathogenic microorganisms and the results showed that it strictly prohibits the growth of pathogenic microorganisms; Menthol, is known for its antimicrobial properties [7].

Since the domestic Borujerd cheese is produced in small urban centers, and tribal areas, in these circumstances, the demand for sales is very important, so in most cases, due to the high volume of demand, the cheese ripening period is not completed, and it is sold fresh. For this reason, conditions in this study were considered similar to the actual conditions of production and sale of the product; this will be more helpful for consumers than producers. Traditionally and historically, after preparation of cheese, some plant powders are added to that mainly for improving the taste. Based on the antimicrobial properties of the plants, the main objective of this study was to evaluate the antimicrobial effect of peppermint powder on the cheese product, while considering the marketability of the product, evaluate its effect on the shelf life of the cheese (increase the shelf life for more than 2 weeks).

2. MATERIALS AND METHODS

2.1. Materials

Raw sheep milk from Golchehran village in Boroojerd, Rennet tablets from Iranian Industrial Enzymes Company (Iran), salts from Taban company (Iran), Peppermint powder from Agricultural Research Center of Khorramabad (Iran), Caustic soda, phenolphthalein, buffers 7 and 4, hydrochloric acid, Ringer tablets, culture media VRBD agar and YGC agar from Merck Company (Germany), sterile plates from Labtron Company (Iran), and methanol 70˚ and 96˚ from Nasr company (Iran).

2.2. Preparation Of Borujerd Domestic Cheese Samples

The steps of producing Borujerd domestic cheese samples is shown in Fig 1.

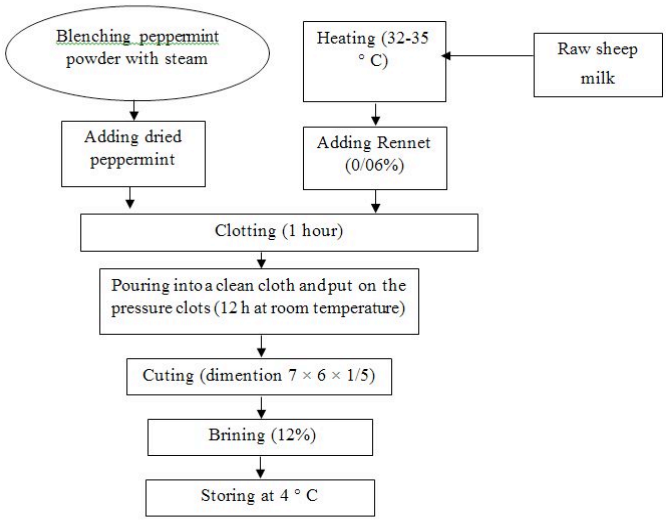

Figure 1. Flowchart of producing Borujerd domestic cheese samples containing Peppermint powder.

Four treatments were defined as follows:

C: The sample of Borujerd domestic cheese without Peppermint powder entitled control.

P1: The sample of Borujerd domestic cheese containing 0.1% Peppermint powder.

P2: The sample of Borujerd domestic cheese containing 0.2% Peppermint powder.

P3: The sample of Borujerd domestic cheese containing 0.3% Peppermint powder.

2.3. Analyses

2.3.1. Raw Milk

The raw milk parameters were determined by the following standards and methods:

pH with a pH meter in accordance with the Iranian National Standard No. 2852, titratable acidity with titration method in accordance with the Iranian National Standard No. 2852 [8], non-fat dry matter in accordance with the Iranian National Standard No. 637 [9], fat according to the Iranian National Standard No. 366 [10], and protein with Iranian National Standard No. 639 [11]..

2.3.2. The Final Product

During the cold storage (60 days at 7-day intervals), pH of the cheese samples was determined in accordance with the Iranian National Standard No. 2852 using a pH meter (Model Cyber Scan pH 510, England) [8], and their titratable acidity was measured in accordance with the Iranian National Standard No. 2852..

Mold and yeast colonies were counted with YGC agar[1] in accordance with the Iranian National Standard No. 10154 during the cold storage (60 days at 7-day intervals). Plates were

[1] Yeast Glucose Chloramphenicol -Agar

incubated under aerobic conditions at 25 ± 1˚C for 5 days; the colonies were counted after the end of incubation [10].

Coliform colonies were counted with VRBD agar[2] in accordance with the Iranian National Standard No. 1-5486 during the cold storage (60 days at 7-day intervals). Plates were incubated under aerobic conditions at 30˚C for 24 hours; the colonies after the end were counted of the incubation [13].

Sensory evaluation was carried out in accordance with the Iranian National Standard No. 695 by 20 trained panelists, based on a five-point Hedonic during the cold storage. In this paper, only the results of sensory evaluation have seen reported.

2.4. Statistical Analysis

A factorial experiment was used in a randomized complete block design (for sensory test) and completely randomized design (For pH, acidity and microbial tests). The experiment consisted of two factors that included Peppermint powder factor (at three levels, 4 combination treatments) and time factor (8 levels, days 0, 7, 14, 21, 28, 35, 42 and 49). For each treatment, 3 replicates were considered. To remove the variance of the difference between the evaluators (sensory test), each panelist was considered like a block. The statistical significance of the data was determined using Duncan test. P-value <0.05 was considered sufficient to reject the null hypothesis. Statistical analysis was performed by running the SAS 9.1 software. Krous Valis nonparametric test was used to analyze the data obtained from sensory tests.

3. RESULTS AND DISCUSSION

3.1. Changes İn Ph And Acidity During The Storage

The pH and acidity results of cheese samples containing Peppermint (Tables 1 and 2) showed that in the first day, pH of the control, P1, P2 and P3 samples was 6.14, 6.04, 6.24 and 6.37, respectively; and after 8 weeks, it declined to 5.39, 5.60, and 5.66 and 5.74, respectively. Therefore, pH of the samples containing Peppermint is higher than that of the control, and titratable acidity of the samples is lower than that of the control. Acidity of the control, P1, P2 and P3 samples increased from 0.21, 0.21, 0.21 and 0.19 (as percent of lactic acid) at the beginning of storage time to 0.32, 0.31, 0.31 and 0.28 (as percent of lactic acid) in the 49 th day, respectively. As can be seen, acidity increased during the storage time ($p < 0.05$).

Table 1. pH values of Borujerd domestic cheese samples during the cold storage *

day / treatment	0	4	14	21	28	35	42	49
C	6.14 ± 0.43^{abc}	6.01 ± 0.19^{abcdef}	5.81 ± 0.18^{bcdefgh}	5.52 ± 0.22^{ghijk}	5.27 ± 0.25^{k}	5.31 ± 0.23^{k}	5.35 ± 0.22^{jk}	5.39 ± 0.25^{ijk}
P1	6.04 ± 0.22^{abcd}	6.00 ± 0.20^{abcdef}	5.88 ± 0.16^{bcdefg}	5.58 ± 0.24^{ghijk}	5.37 ± 0.25^{ijk}	5.37 ± 0.25^{ijk}	5.46 ± 0.24^{hijk}	5.60 ± 0.20^{efghijk}
P2	6.24 ± 0.31^{a}	6.22 ± 0.27^{ab}	6.02 ± 0.27^{abcde}	5.67 ± 0.32^{defghijk}	5.44 ± 0.29^{hijk}	5.45 ± 0.29^{hijk}	5.52 ± 0.27^{ghijk}	5.66 ± 0.23^{defghijk}
P3	6.37 ± 0.24^{a}	6.37 ± 0.23^{a}	6.15 ± 0.28^{abc}	5.79 ± 0.24^{cdefghi}	5.47 ± 0.25^{ghijk}	5.50 ± 0.29^{ghijk}	5.60 ± 0.27^{fghijk}	5.74 ± 0.25^{cdefghij}

* Mean±Standard Deviation (SD) values with different superscript letters differ significantly ($P<0.05$).

Table 2. Titratable acidity values (as percent of lactic acid) of Borujerd domestic cheese samples during the cold storage*

[2] Violet Red Bile Dextrose - Agar

day / treatment	0	4	14	21	28	35	42	49
C	0.21±0.05^{ef}	0.22±0.05^{ef}	0.24±0.03^{bcdef}	0.32±0.04^{a}	0.35±0.03^{a}	0.35±0.03^{a}	0.34±0.04^{a}	0.32±0.04^{a}
P1	0.21±0.05^{ef}	0.22±0.05^{ef}	0.24±0.04^{cdef}	0.32±0.04^{a}	0.34±0.04^{a}	0.34±0.04^{a}	0.33±0.05^{a}	0.31±0.04^{ab}
P2	0.21±0.05^{ef}	0.21±0.05^{ef}	0.23±0.03^{def}	0.30±0.06^{abcd}	0.34±0.04^{a}	0.34±0.04^{a}	0.33±0.05^{a}	0.31±0.05^{abc}
P3	0.19±0.06^{f}	0.19±0.06^{f}	0.22±0.05^{ef}	0.28±0.04^{abcde}	0.33±0.04^{ab}	0.33±0.05^{a}	0.32±0.05^{a}	0.28±0.06^{abcde}

*Mean±Standard Deviation (SD) values with different superscript letters differ significantly ($P<0.05$).

Our finding showed that the pH value was the lowest in the fifth week, but it had some raise over the next weeks. The amount of acidity in the fifth week was higher than in the last week. Decrease in pH and increase in acidity during ripening time can be attributed to breaking down of lactose and production of lactic acid by lactic acid bacteria that proper adjustment of desired pH during the ripening time leads proteolysis on the right way. Acidity and pH of coagulum affect the syneresis, adjusting moisture, microbial flora, enzyme activity, texture and taste of cheese during ripening [14].

The high pH of the samples relative to the standard set of (5-5.2), in the National Standard No. 2852 can be attributed to the use of unpasteurized milk to produce cheese. This result corresponds to that of Mirzayi and Aligholinezhad study (2011) regarding the increase of pH of unpasteurized cheese at the end of the ripening period [14].

In the present study, the pH of samples containing different percentages of Peppermint powder was higher than that of the simple cheese, and the increase of plant powder added to the pH of cheese. Antimicrobial compounds such as Carvacrol, Eugenol, menthol be present in the plant may have a negative effect on the activity of lactic acid bacteria and other acid-producing bacteria in raw milk that increase the pH and the titratable acidity decreases compared to the control sample.

The results are in violation with Mahmoudi *et al*. (2010), who studied the effect of oregano on Iranian white cheese and reported that the pH of cheese did not change with the use of extracts of the oregano and increased concentration of oregano extract is ineffective on the lactic acid bacteria [15].

The current results are also inconsistent with the results of Ehsani *et al*. (2011); who examined the effect of different concentrations of the essential oils of anise and shallots on white cheese. They found that the pH of the cheese containing essential oil had no significant effect on milk pH comparing to the control subjects [16].

3.2. Changes İn Coliform Counts During The Storage

The results obtained from the coliform counts (Table 3) in cheese samples containing powder Peppermint revealed on the first day, the number of coliform counts was not different significant (log cfu/g) between the treatments but there was difference compared to control samples (C = 6.02, P1 = 5.95, P2 = 5.92 and P3 = 5.92). During the 60-day period, the total number of coliform decreased and there was a significant difference between the P1, P2, P3 and control samples. The most reduction in treatment P3 (containing 0.3% Peppermint powder) belongs to the fourteenth day, which is more different with the other treatments (4.41 log cfu / g) and in the seventh and eighth weeks, the number of coliform reached to the National Standards Limit No. 5486-1 (0.98 log cfu/g) ($p <0.05$).

Table 3. Changes in coliform counts (log cfu/g) of Borujerd domestic cheese samples during the cold storage*

day / treatment	0	4	14	21	28	35	42	49
C	6.02±0.12^{a}	5.98±0.12^{a}	5.89±0.13^{a}	5.82±0.10^{a}	5.78±0.13^{ab}	4.72±0.09^{cd}	3.81±0.09^{ef}	2.84±0.13^{gh}
P1	5.95±0.16^{a}	6.18±0.71^{a}	5.72±0.13^{ab}	4.99±0.56^{bcd}	4.43±0.45^{de}	3.82±0.49^{ef}	2.87±0.63^{g}	1.83±0.64^{hi}
P2	5.92±0.17^{a}	5.74±0.10^{ab}	5.51±0.22^{abc}	4.73±0.64^{cd}	4.45±0.47^{de}	3.15±0.65^{g}	1.57±0.99^{ij}	1.00±0.00^{j}
P3	5.92±0.15^{a}	5.59±0.18^{ab}	4.41±1.25^{de}	3.44±0.55^{f}	3.07±0.36^{g}	1.56±1.24^{ij}	1.00±0.00^{j}	0.98±0.03^{j}

*Mean±Standard Deviation (SD) values with different superscript letters differ significantly ($P<0.05$).

The importance of using essential oils is the existence of phenolic compounds in them, which explains the antimicrobial properties of essential oils, so that they influence on the cell membrane of bacteria, disrupt permeability, and lead to disruption of the cell membrane activity of the electron transfer admission, removal of nutrients, and synthesis of nucleic acids and ATPas [17]. Therefore, the higher is the amount of phenolic compounds in vegetable oil, the higher will be its antimicrobial properties. These include Carvacrol, Eugenol and *Thymol*. Carvacrol and *Thymol* are the most prominent phenolic compounds in different parts of the plants of Lamiaceae family (Peppermint, savory, oregano, etc.), but leaves, flowers and roots have different levels of these compound. In addition to the above-mentioned compounds, *Lamiaceae* family plants contain tannins, flavonoids, Saponins and bitter substances [18], which have proven antimicrobial properties, and have a negative effect on the activity of coliforms.

According to the result of this study, we can conclude that with the increase of the powder concentration, its effect on reducing the number of coliform would increase. The use of herbal powder at higher concentrations (0.3%) decreased the number of coliform per week 1.5-2 logarithm compared to the control sample. Antimicrobial compounds such as menthol, Carvacrol, *Thymol,* etc., which are found normally in herbs, have anti-microbial effects. The results of the present study correspond to the results of Alipur Eskandany and colleagues (2009) that confirm the effect of using essential oil of thyme in industrial soup in disappearance of *Bacilus. cereus* [19]. The most effective antimicrobial compounds reported in Peppermint include Carvone, limonene, menthol and Menthone [20], which reduce the total number of coliforms [20].

The current research results are in agreement with the results of Bonyadian and Karim research (2004) regarding the antimicrobial effect of volatile oils of herbs (Peppermint, tarragon, cumin, oregano and thyme) on E-coli bacteria in Iranian white cheese [21]. Hayaloglu and Fox (2008) have also reported the antimicrobial effects of herbal powders in Otlu[3] cheese, and their results are in according to our findings on the reduction of the food-born pathogens, particularly coliforms, with the use of powdered herbs (thyme, garlic, Peppermint, cumin and black pepper) [22].

3.3. Changes İn Molds And Yeasts Count During The Storage

The results obtained from the mold and yeast counts (Table 4) in the samples containing Peppermint powder showed that in the first day, the mold and yeast counts were the same in all treatments (1 logc fu/g); and during storage, number of molds and yeasts were increased; so that in the sixth week, they were increased by maximum and in the seventh and eighth weeks they showed a decreasing trend. The mold and yeast counts in different treatments had little difference in the sixth, seventh and eighth weeks (days 35, 42 and 49), and showed difference

[3] Traditional herbal cheese containing a variety of herbs powder produced in Turkey.

compared to the control sample. The lowest count of molds and yeasts (logcfu/g 5.84) belonged to P3 treatment (containing 0.3% Peppermint powder) in the 49th day ($p<0.05$).

Table 4. Changes in molds' and yeasts count (logcfu/g) of Borujerd domestic cheese samples during the cold storage*

day / treatment	0	4	14	21	28	35	42	49
C	1.00±0.00^{m}	1.91±0.11^{k}	3.89±0.02^{i}	4.92±0.04^{h}	6.20±0.03^{e}	7.13±0.02^{a}	7.08±0.04^{ab}	7.02±0.04^{abc}
P1	1.00±0.00^{m}	1.85±0.07^{kl}	3.80±0.02^{ij}	4.89±0.05^{h}	6.18±0.03^{e}	7.11±0.03^{a}	6.99±0.05^{bcd}	6.00±0.17^{f}
P2	1.00±0.00^{m}	1.89±0.10^{kl}	3.79±0.03^{ij}	4.88±0.04^{h}	6.17±0.06^{e}	7.10±0.04^{ab}	6.95±0.05^{cd}	5.95±0.17^{b}
P3	1.00±0.00^{m}	1.78±0.11^{l}	3.77±0.01^{j}	4.86±0.07^{h}	6.15±0.05^{e}	7.06±0.06^{abc}	6.90±0.05^{d}	5.84±0.09^{g}

*Mean±Standard Deviation (SD) values with different superscript letters differ significantly ($P<0.05$).

The number of molds and yeasts in the first two weeks was in the standard range (up to 100/ 1g) according to the National Standard No. 10154. The duration of the antimicrobial activity of plant powder corresponds with the finding of Bonyadian and Karim (2005), who studied and confirmed the antimicrobial effect of volatile oils (peppermint, pennyroyal, Tarḳan, thyme) on fungal population in Iranian white cheese [1];but after two weeks, the number of molds and yeasts increased beyond the standards mentioned. The results of this study are similar to those of Tarakci and Temiz (2009), who reported the number of molds and yeasts in Otlu plant cheese after ripening, to be 6.09 logcfu/g [23]. Hayaloglu and Fox (2008) also reported, the number of molds and yeasts in Otlu plant cheese as 2.3-5.3 logcfu/g that is consistent with our study [22]. Generally, the total counts of molds and yeasts in cheese samples (made from unpasteurized milk) are not listed in the standard range. Aromatic compounds in plants like ethanol and methanol caused the number of molds and yeasts in the cheese samples to be less than in the controls. This is in accordance whit the results of Sengul and colleagues (2006) regarding the effect of combination of ethanol and methanol herbs (Peppermint, oregano, garlic, pepper, etc.) in controlling the fungal growth, too [24]. Antifungal effects of yeasts and aromatic plants increase with the increasing rate of Peppermint powder; this result is consistent with the results of Bonyadian and Karim (2005) confirming the antimicrobial effect of plant volatile oils (peppermint, oregano, Thyme and Tarḳan) on the fungal population of Iranian white cheese [1].

3.4. Results Of Sensory Evaluation (Overall Acceptability) During The Storage

The results of sensory evaluation of the cheese samples containing powder showed that for flavor, color and overall acceptability (Table 5), treatment P2 (containing 0.2% peppermint powder), and for texture and odor, treatment P3 (containing 0.3% Peppermint powder) were accepted ($p<0/05$).

Table 5. Results of sensory evaluation (overall acceptability) of Borujerd domestic cheese samples during the cold storage*

day / treatment	0	4	14	21	28	35	42	49
P1	4.15±0.75^{ab}	4.05±0.51^{bc}	4.10±0.45^{ab}	3.80±0.52^{defg}	3.80±0.41^{defg}	3.75±0.44^{efgh}	3.55±0.76^{h}	3.70±0.57^{efgh}
P2	4.30±0.66^{a}	4.05±0.51^{bc}	4.00±0.32^{bcd}	3.90±0.31^{bcde}	3.75±0.44^{efgh}	3.70±0.57^{efgh}	3.80±0.41^{defg}	3.85±0.37^{cdef}
P3	3.60±0.50^{gh}	3.65±0.59^{fgh}	3.25±0.72^{ij}	3.10±0.79^{ijk}	3.05±0.69^{jk}	3.00±0.65^{k}	3.20±0.70^{ijk}	3.30±0.73^{i}

*Mean±Standard Deviation (SD) values with different superscript letters differ significantly ($P<0.05$).

In addition to the antimicrobial effects of aromatic plants on coliform, molds and yeasts, in this study, the plants maintained tissue, taste and smell of the cheese at desired and very

effective level so that even with high levels of molds and yeasts the tissue of cheese samples containing them was preserved. The cause of this phenomenon can be due to the effect of aromatic herbs in cheese on microbial flora, the degree of decompose of proteins and peptides (which are influenced by microbial flora), and the way of ripening of the cheese, in general, these results correspond to the finding of Tarakci and Temiz (2009) that used aromatic plants effects on the microbial flora in Otlu cheese and maintained its tissue [23].

4. CONCLUSİON

The research result revealed that increasing the percentage of Peppermint powder in Boroojerd domestic cheese samples increased pH level and decreased acidity. The maximum coliform count during the storage was observed in all samples containing Peppermint powder and control (in the first day), and the minimum number was in the samples containing 0.3% Peppermint powder (in forty-ninth day). By increasing the percentage of Peppermint powder, the coliform count in the cheese samples dropped during storage the cold. The lowest count of molds and yeasts was observed during the storage in all samples containing Peppermint powder and control (in the first day), and the highest count was observed in the control sample (in forty-ninth day). By increasing the percentage of Peppermint powder, the number of mold and yeast counts was reduced in cheese samples during the cold storage. In this study, the samples of cheese containing 0.2% Peppermint powder had the highest rating of overall acceptability; therefore, it was selected as the preferred treatment.

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