Production of Non-Carbonated Yogurt Drink (Dough) Enriched with Marjoram Extract and Evaluation of its Physicochemical and Microbial During Storage

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ABSTRACT

In this study, an experiment was conducted as factorial arrangement based on completely randomized design with three replications. Factors were: marjoram concentration (0, 1.5, 3 and 6%), storage condition (refrigerator (4°C) and ambient (25°C)) and storage duration (1, 15, 30 and 45 days). The results showed that samples stored in refrigerator temperature had minimal changes in their physicochemical properties. Dough samples with 1.5% extract in the fridge temperature showed the best acidity and pH in the fourth period. Samples stored at ambient temperature showed more changes than samples that were stored in refrigerator. The amount of phenolics in the samples increased with increasing of extract concentration. Total count of microorganisms with increasing of extract concentrations and decreased with decreasing temperature. In none of the samples mold and coliform was observed that showed high quality of pasteurization.

INTRODUCTION

Milk and its products have a major role in human nutrition and health in all stages of life [28]. Word “Dough” is taken from the Persian word “milk” and literally means a substance derived from milk. In the past, “Dough” refers to the production of yogurt remains after dilution with water and fat separation by using musk. But today industrial production is diluting standardized yoghurt with water. There are many benefits of using probiotics and prebiotic products in the production process of dough. It can increase the value of the product in terms of health [6]. Dough is a dairy beverage that is traditionally prepared in some countries, including Iran and Turkey. This useful product is a healthy drink that provides a quarter of the daily requirement of calcium and contains vitaminsB2, B6, B12 [29].

Marjoram (Origanum majorana) Lamiaceae Class, is widely distributed worldwide. This plant spread the North and North West regions and not found in the warmer areas of southern. Plants belonging to the genus Origanum rich aromatic compounds has been used for centuries as a spice. According to confirmed reports the Origanum genus in the Lamiaceae family contains chemical compounds such as phenolics and essential oils. For example, amount of essential oils in O. vulgare was reported nearly 0.5 to 1.5% and its main in gredients are carvacrol and thymol.

One of the main phenolic compounds, are flavonoids, that have been proven antioxidants properties. Marjoram has the highest amount of flavonoids. Flavonoids are 9 types that will be vary depending on the type of plants. Flavonoids have antibacterial, anti-inflammatory, antipyretic, anti-allergy properties. Flavonoid intake reduces the risk of cardiovascular disease. Therefore using marjoram in traditional medicine, is justified that it could be more because of their relatively high flavonoid compounds [19]. In many studies a direct correlation between total phenol and antioxidants activity of marjoram was observed.

Marjoram is expected to have the high radical scavenging Due to the high levels of total phenols. It is believed that a direct correlation between the amount of total phenol and Radical scavenging power of Pycrill diphenyl hydrazyl(DPPH) [22].

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Alma et al. studied the effect of antioxidant of essential oils in some Origanum (O. syriacum) and found that it was slightly less than ascorbic acid or BHT [3]. Ivanova et al. studied antioxidant activity and phenolics content in 21 extracts by measuring TEAC. O. vulgare have the highest phenolic content and antioxidant properties [10]. Phenolic compounds in marjoram are protocatechic acid and related glycosides, caffeic acid, and rosmarinic acid [15]. Collins and colleagues reported that the antioxidant properties of aqueous extract of Origanum is comparable to alpha-tocopherol, but is less effective than ascorbic acid [7]. This is due to the presence of thymol and carvacrol content is about 32% and 35% [4].

Dumb Meyer et al. studied effect of plant oils such as marjoram against C. albicans. After 7 days, the essential oil of marjoram, savory, mint and cinnamon had the highest inhibitory effect against the yeast (MIC = 500 ppm). Chromatographic analysis showed the strongest ingredient was carvacrol (MIC = 100 ppm), respectively [26]. Thymol, 4-terpinene, Cis-sabinene and Gamma-terpinene are an important phenolic compounds in the essential oils of Marjoram Vt. Shirazi, and have an important medicinal properties and antifungal activities [24].

This study aimed to produce a functional yoghurt drink by using Marjoram extract and evaluation of its physicochemical, microbial and sensory properties during storage time.

MATERIALS AND METHODS

Preparation of Origanum extract:

Marjoram (Origanum vulgare) was harvested in late June, after removing the redundant parts of the plant was dried in environmental conditions (ambient temperature) and then was split in mill (Model Kenwood CG 100). Powder samples (100 gr) mixed by 1000ml ethanol 99.6% (ratio 1: 10v/v) for 48h in a shaker (250 rpm) at room temperature. Extract was filtered with Whatman No. 1, under vacuum. Extract was concentrated in rotary evaporation at 38-40°C and then was dried at 45°C by vacuum oven to obtain constant weight. This extract was kept in impervious containers and refrigerated in 4°C during the time of trial [1]. A commercial Dough sample before adding any preservatives and essential oil were prepared from in Goldsash company of Sabzevar. Drink bottles (100 ml glass containers) were sterilized by autoclaving [17]. Adding extracts were conducted in concentrations 1.5, 3, and 6% and samples were stored at temperatures 25°C, 4°C. The following experiments were taken in order to control the quality of the dough at intervals of 15 days.

Physicochemical Tests:

The pH meter was used to measure the pH of the samples at 25°C. Titratable acidity was measured by titrating 5ml of sample with 0.1N NaOH using phenolphthalein as indicator [25].

The amount of phenolic compounds was measure by Folin ciocaltiu method. For this purpose, 1 ml of the sample was poured into a test tube and 1 ml ethanol 99.6% and 5 cc distilled water and 0.5 ml Fulin ciocaltiu indicator 50% were added then put samples in a dark place for 5 minutes and 1 ml sodium carbonate 5% was added and then placed on a shaker for 1 hr in the dark. Then by using a spectrophotometer absorbance was measured at a wavelength of 725 nm and the results were compared with gallic acid standard curve [30].

Microbial tests:

Coliform counts:

VRB media Culture is used to determine the presence of these microorganisms from the culture medium which was sterilized by boiling and didn’t need autoclaving. Then it was cooled to 40-45°C. For dilution, the liquid sample (1ml) was added in to a test tube containing 9 ml Ringer’s solution. 1 ml of the diluted sample was poured into the plate and medium culture in 40-45°C were added to the plate and was well mixed with pour plate method. The plates placed upside down to incubator for 24 h at 37°C. During the same period, red to purple colonies were counted and thenumber obtained by multiplying the inverse dilution [25].

Molds and yeasts count:

YGC agar medium was used for this purpose, which has been sterilized prior to use by autoclaving. After sterilization, the medium liquid is poured into a plate and refrigerated until firm. The prototype, which can be a liquid or diluted samples (dilution 0.1, 0.01, 0.001), 0.1 ml removed and was broadcast with L-shaped glass rod or pipette in the culture medium. Then incubated plates were placed in refrigerated incubator for 3 to 5 days at 25°C [25].

Total count of microorganisms:

For this purpose, samples can be diluted depending on the microbial load (The original dilution, dilution 0.1, 0.01, 0.001, 0.0001) diluted sample (1cc) was added to the plate. Then the sterilized medium (PCA) was added and well mixed with pour plate method. The plates were incubated for 48 hours at 37°C and...
placed upside down. During this time colonies were counted and according to the initial dilution, the total count was determined [25].

Statistical Analysis:

The obtained data were statistically analyzed using computerized SAS (ver 9.1). Effects of different treatments were analyzed by proc generalized linear model (GLM). Test of significance of the treatment difference was carried out on a basis of Duncan multiple-range test. The significant differences between treatments were compared with the critical difference at 5% and 1% levels of probability. Charts were plotted by excel software.

RESULTS AND DISCUSSIONS

Result of analysis of variance showed that pH and phenolic content was effected by marjoram extract concentration, storage condition and storage duration.

Table 1 shows the physicochemical characteristic of dough samples with enriched by marjoram extract. The highest pH value was observed in 1.5 % extract which had significant difference with other samples. The lowest pH value was measured in control sample which had no significant difference with 6% marjoram extract. This result in line with founding of Marshal et al (1997) that reported decreasing of pH during storage time. Mirchuly et al (2010) found that the production and storage of heated packed buttermilk (in PET and Pry pack containers) at 5, 25 and 33 °C, the pH decreases during storage temperature but this decrease in5 °C was lower [14]. Farahnudy (2002), according to tests carried out showed that between the chemical properties(pH)and yeast overgrowth is a significant correlation. As the yeast activity increased with increasing temperature and the decomposition of some compounds in dough, especially carbohydrates leads to production of organic acids and reduction in pH and sour taste in Dough [5].

As shown in table 1 dough with 1.5% marjoram extract had the lowest acidity. With increasing of Marjoram extract, phenolic content in dough was increased. There were significant difference among all treatment. Maximum phenolic content (0.59 gr/100ml) was observed in 6% and control sample had minimum phenolic content (0.55 gr/100ml). Gold et al (2010), reported the amount of water-soluble phenolics in Yogurt contains 10% extract of Palm Dates was reduced during the storage [8]. Zinodin and Baba (2009) also reported adding 10 % of water-soluble extracts of white varieties of Pitaya fruit improved features phenolic in yogurts [30]. Alma et al studied the antioxidant activity of Origanum(O. syriacum) essential oils. They found that presence of high concentrations of phenolic compounds such as carvacrol and thymol methyllether showed antioxidant activity [3].

Table 1: Effect of marjoram concentration on pH, acidity and phenolic content .

<table>
<thead>
<tr>
<th>pH</th>
<th>acidity</th>
<th>phenolic content</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.94±0.07 b</td>
<td>54.96±1.95 b</td>
</tr>
<tr>
<td>1.5</td>
<td>4.03±0.05 a</td>
<td>54.67±2.71 c</td>
</tr>
<tr>
<td>3</td>
<td>3.98±0.04 c</td>
<td>55.01±1.64 b</td>
</tr>
<tr>
<td>6</td>
<td>3.95±0.04 c</td>
<td>55.98±2.03 a</td>
</tr>
</tbody>
</table>

Means with the same letter in each column had not significance difference (Duncan α=0.05)

Table 2 shows storage condition effect on pH, acidity and phenolic content of dough during 45 days of storage. Storage of dough in refrigerator condition had the highest pH and phenolic content, but kipping in ambient condition had maximum acidity. Abboudi and Thompson (1996) with a 9-month retention of dates (Khelas or Kheniz variety) packed in polyethylene bags at 4-20 and -10 and 0 and 5 °C found that pH decreased during storage. But decreasing at -20 °C was lower than the others [2]. Tours and colleagues (2005) stated that the storage temperature and storage time of fruits and vegetables play an important role in the amount of phenolic compounds. During storage at 25 °C, the amount of phenolic compounds is reduced than to 7 and 15 °C [27]. Nematshahi et al(2014)studies showed that increasing cereal extract concentrations of 200 to 1600 ppm increased levels of polyphenol compounds in canola oil [18].

Table 2: Effect of storage condition on pH, acidity and phenolic content.

<table>
<thead>
<tr>
<th>pH</th>
<th>acidity</th>
<th>phenolic content (gr/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ambient</td>
<td>3.96±0.06 b</td>
<td>55.63±1.71 a</td>
</tr>
<tr>
<td>refrigerator</td>
<td>3.98±0.04 a</td>
<td>54.68±1.95 b</td>
</tr>
</tbody>
</table>

Means with the same letter in each column had not significance difference (Duncan α=0.05)

The initial pH values were 4.06 for first day of experiment pH value was decreased with increasing of storage duration from 4.06 to 3.87 at final storage time(45 days). There were significant difference among all treatment for pH values (table 3).Despite of pH , The highest acidity was measured at final storage time(58.179). There was a significant difference between treatments. As shown in table 3, increasing of storage time was
decrease phenolic component by 24.24 %. Maximum of phenolic content was measured at first day of experiment (0.66 gr/100ml) and minimum was observed at final storage time (0.50gr/100ml).

Table 3: Effect of storage time on pH, acidity and phenolic content.

<table>
<thead>
<tr>
<th>Storage time(day)</th>
<th>pH</th>
<th>Acidity (OD)</th>
<th>phenolic content (gr/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.06±0.007 a</td>
<td>52.36±0.72 d</td>
<td>0.66±0.12_a</td>
</tr>
<tr>
<td>15</td>
<td>4.02±0.01 b</td>
<td>54.05±0.53 c</td>
<td>0.59±0.05 b</td>
</tr>
<tr>
<td>30</td>
<td>3.95±0.03 c</td>
<td>56.04±0.37 b</td>
<td>0.55±0.04 c</td>
</tr>
<tr>
<td>45</td>
<td>3.87±0.04 d</td>
<td>58.17±1.05 a</td>
<td>0.50±0.004 d</td>
</tr>
</tbody>
</table>

Means with the same letter in each column had not significance difference (Duncan α=0.05)

Interaction effect of concentration and storage duration on phenolic component showed that in all concentration. The highest phenolic component obtained at first day which statistically similar with all concentration. Increasing of storage duration decreased linearly phenolic component in all concentration. 3 and 6% concentration had more decreasing in final day than other treatment. By reducing the percentage of extract and increasing the storage time, total amount of phenolic compounds was decreased (Figure 1).

![Fig. 1: Interaction of marjoram extract and storage time on phenolic compounds](image1)

Fig. 1: Interaction of marjoram extract and storage time on phenolic compounds

![Fig. 2: Interaction effect of storage condition and storage time on pH.](image2)

Fig. 2: Interaction effect of storage condition and storage time on pH.

With increasing of storage duration pH was decreased in ambient and refrigerator condition. The difference in pH was observed on 45days in the storage condition. This difference was increased with increasing storage time. The highest difference for pH was measured on 45 days which dough storage in ambient had the lowest pH (figure 2). In general, reducing the temperature of storage and increasing the storage time of samples, a total amount of phenolic compounds decreased. It can be concluded that increasing the storage time of yogurt samples produced the pH ranges decreased. So the highest level of pH was observed in dough samples stored at 4°C and the lowest level was in samples stored at 25°C. In general, probiotic bacteria including lactic acid
bacteria that produce low acid in the refrigerated. But traditional yogurt bacteria such as L. bulgaricus and S. thermophilus Unlike Probiotics are active during storage in refrigerator. Through fermentation of lactose and producing lactic acid, decreasing pH are significantly during storage [23]. By decreasing Storage temperature and increasing storage time in samples pH levels decreased. Marshall et al (1997) studied Changes in acidity and pH of yoghurt during storage period at 4 °C. During this time in all samples, increasing the acidity and pH reduction is seen. These changes were low due to the low ability of probiotic microorganisms for the production acidity in refrigerator [13].

Unlike pH, acidity was increased with increasing of storage duration in two storage condition. The highest acidity was observed at storage in 25°C and 45 days (figure 3).

![Graph showing acidity vs storage duration](image)

**Total count and yeast:**

Analysis of variance showed that concentration, temperature and time had significant effect on total count and yeast counts.

Table 4 shows total count and yeasts of different enriched dough samples. The result showed that the presence of total coliforms in the samples of dough treated with 6% had the lowest value. Increasing of concentration was decreased significantly total count during storage. There was no stable change in treatment. Mirchuly colleagues (2010) demonstrated based on experiments performed that the yeast grow that 33 °C per two packages (PET and Perry Pack), considerably more than other temperatures (5 and 25°C) [14]. Mortazavi et al (2000) showed that with increasing temperature and time, the growth of yeasts have been increased. This would correspond exactly with yeast physiological characteristics. Because optimum temperature for growth of most yeasts is in the range of 25-30°C and the maximum is 35-47°C. Most of the yeast growth enhanced in acidic environment in pH 4 to 4.5. Yeasts grow best under aerobic conditions, but a variety of fermentative yeasts are able to grow slowly in anaerobic environments. Many yeasts are easily grown in the presence of high concentrations of dissolved substances such as sugar and salt [16]. Yoghurt drink can be an ideal environment for the growth and activity of yeasts and probably the most effective natural methods to control their activities is keeping in freezing conditions, and temperatures dropped. The presence or absence of mold in samples should be noted that although the growth of mold, is slower than bacteria and yeast and if the conditions are favorable for all the microorganisms, usually molds, are notable to compete with yeasts and bacteria. But if the growth of mold begins, other steps of growth may perform too fast. On the other hand, molds need to grow aerobic conditions and a wide range of pH (2-8.5). Most molds grow in acidic conditions, and the optimum temperature for growth is in 25-30°C [16]. Farahmudy (2002), demonstrated that between the chemical properties (pH) and yeast over growth this a significant correlation. As the yeast activity increased with increasing temperature and the decomposition of some compounds in dough, especially of carbohydrates leads to production of organic acids and reduction in pH and sour taste in Dough (5). Zhang (2009) stated that in higher concentration of phenolic compounds, the scavenger of radicals increased because of increasing in hydroxyl amounts. The main mechanism of antimicrobial activity of plant extracts, are expressed cell wall degradation, damage to the cytoplasmic membrane, leakage of intracellular content of cells and eventually death of bacteria by phenolic compounds [31]. Bacterial growth was reduced by acidic conditions and with increasing temperature, effect of antibacterial properties of these compounds was due to the agar medium was used [20].

Salmakas et al (2008), investigated Thyme antibacterial activity against Listeria monocytogenes in meat that were kept in cold storage. In this study, concentrations of 0.3, 0.6 and 0.9% thyme oil extracts were tested. Concentration of 0.3% thyme was a weak antimicrobial activity but concentration of 0.6% antimicrobial activity was stronger and more effective [21]. Karim et al (2004) studied Antimicrobial effects of volatile oils of peppermint, tarragon, cumin, oregano and thyme in Escherichia coli studied in Iranian white cheese that, after 7
days at concentrations of 0.3 and 0.4 and 2%, respectively, causing a reduction of 2.5 times compared with controls the logarithm of the bacteria Escherichia coli [11]. Graf et al (2004) showed that the plant extract has inhibitory effects on fungi compared with bacteria. But vegetable oil is more anti-bacterial than fungicide [12]. Goel et al (1971) tested antimicrobial properties of yogurt, buttermilk, sour cream and cottage cheese. The results showed that total coliforms were reduced in sour cream but the rate of decline was less than the yoghurt. This is due to higher pH than we know these products. These researchers reported a pH range of our products listed below: 4.4 to 3.65 sour cream, buttermilk 4.9 to 4.1 is 4.7 to 4.18 [9].

According to the figure, it can be concluded that the maximum number of yeasts in the control yoghurt samples were at 25°C that showed significant differences with all treatments. Although the number of yeast extract concentrations at 25°C was higher than samples stored at 4°C, but there was no significant difference between treatments.

**Table 4**: Interaction of marjoram extract and storage time on yeast count.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>15</th>
<th>30</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>1.87±0.50</td>
<td>1.91±0.64</td>
<td>2.02±0.11</td>
<td>2.13±0.33</td>
</tr>
<tr>
<td>1.5%</td>
<td>1.83±0.25</td>
<td>1.47±0.71</td>
<td>1.29±0.003</td>
<td>1.26±0.71</td>
</tr>
<tr>
<td>3%</td>
<td>1.78±0.88</td>
<td>1.41±0.49</td>
<td>1.20±0.41</td>
<td>1.20±0.41</td>
</tr>
<tr>
<td>6%</td>
<td>1.73±0.39</td>
<td>1.35±0.21</td>
<td>1.13±0.03</td>
<td>1.11±0.39</td>
</tr>
</tbody>
</table>

Mean± standard deviation

**Table 5**: Interaction of marjoram extract and storage time on total count.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>15</th>
<th>30</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>2.42±0.78</td>
<td>2.43±0.13</td>
<td>2.45±0.10</td>
<td>2.42±0.32</td>
</tr>
<tr>
<td>1.5%</td>
<td>2.38±0.91</td>
<td>2.27±0.87</td>
<td>2.22±0.40</td>
<td>2.19±0.59</td>
</tr>
<tr>
<td>3%</td>
<td>2.36±0.64</td>
<td>2.22±0.53</td>
<td>2.17±0.31</td>
<td>2.16±0.55</td>
</tr>
<tr>
<td>6%</td>
<td>2.31±0.49</td>
<td>2.16±0.82</td>
<td>2.08±0.99</td>
<td>2.02±0.11</td>
</tr>
</tbody>
</table>

Mean± standard deviation

As shown in table 4 increasing of concentration Marjoram essential oil on dough significantly decreased yeast count in storage duration. There were no significant difference between 30 and 45 day for yeast count when dough enriched by marjoram essential oil with 3 or 6%.

**Conclusion:**

Storage temperature has a significant effect on the shelf life and physicochemical of non-carbonated yoghurt drink. It would be possible to use Ethanol extract of the marjoram to produce non-carbonated yoghurt drink. This will lead to an increase in the level of public health. Concentration of marjoram extract in dough is an important factor on the physicochemical properties of product. Samples contain 6% extract showed the highest phenolic content in 1st period and Samples contain 1.5% extract stored in environmental temperature showed the lowest phenolic content in 4th period.

According to the results of this study can be expressed that increasing the concentration of the extract in the samples formulations reduce the total count of bacteria and yeasts. Count of yeast declined at the second period and the third period onwards, the loss of the yeast is stable and there is no significant difference.

**REFERENCES**


