Effect of Oregano on the Growth of \textit{Lactobacillus acidophilus} and \textit{Bifidobacterium bifidum} in Probiotic Dairy Products

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\textbf{Abstract:} In the present study, the potential of producing foods containing probiotic bacteria with the bases of milk, yoghurt and oregano was evaluated. In order to determine the effects of different doses of oregano on the growth of two probiotic bacteria, \textit{Bifidobacterium bifidum} and \textit{Lactobacillus acidophilus} in milk and yoghurt produced in the first and second phases, respectively, 0.33 gram lyophilized bacteria were added to 1 liter low-fat sterilized milk, separately. The samples were then examined in terms of pH, acidity and microbial count during the incubation setting period and their respective permanence. In the samples with either \textit{Lactobacillus acidophilus} or \textit{Bifidobacterium bifidum}, it was observed that the increased concentration of oregano created a favorable taste of yoghurt of all the samples and the yoghurt containing 0.1% oregano were with the best tastes and the testifier sample containing 0% oregano was of greater permanence than the other samples. During 15 day period, the number of bacteria decreased and no significant difference between the testifier sample and the samples containing different concentrations of oregano was observed. Upon examination of the results, it was revealed that the increased concentration of oregano had a positive effect on the growth of the probiotic bacteria, \textit{Lactobacillus acidophilus} and \textit{Bifidobacterium bifidum} in probiotic milk and yoghurt. Also, the results showed that among the different concentrations of oregano, concentration of 0.2% compared to the testifier group had more effect on reducing cholesterol. In all different concentration of oregano triglyceride level was higher than the testifier group.

\textbf{Key words:} Oregano • Probiotic • \textit{Lactobacillus acidophilus} • \textit{Bifidobacterium bifidum} • Triglycerides • Cholesterol

\textbf{INTRODUCTION}

Probiotic microbes are live organisms that are used by eating and condition application in repellent number and cause creation of one or many healthy effects on host body. The ability of probiotics to withstand the normal acidic conditions of the gastric juices and the other microorganisms, allow them to be established in the intestinal tract [1].

\textit{Lactobacillus acidophilus} and \textit{Bifidobacterium bifidum} species are well known for their application in fermented milk products. Consumption of these bacteria is frequently related to the health promoting activities of some strains. Strains with beneficial characteristics are referred to as probiotic [2, 3].

Probiotics are beneficial microorganisms that protect the host against diseases and are defined as “live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance” [4].

Consumption of dairy product containing \textit{Lactobacillus} species can aid in the control of serum cholesterol in animal and humans.
Assimilation of cholesterol in the intestines by Lactobacillus species may reduce the amount of dietary cholesterol absorbed into the body. Because the ability of Lactobacillus species to assimilate cholesterol during growth varies by strain, the Lactobacillus strains for use as a dietary adjunct to potentially reduce serum cholesterol should be carefully selected [5].

Bifidobacteria are a focus of intensive international research for their essential role in fermented food especially for their ability to produce various antimicrobial compounds promoting their probiotic properties [6].

Recently design and product of probiotic productions with basic plant are considered for both rule of health nature of these food material groups (protein, fiber, vitamin, solute) and variation creation in product and consume [7]. The objective of this study was to create a technical method to produce and preserve a kind of fermented probiotic dairy product with acceptable aroma, while containing reasonable counts of probiotic bacteria.

**MATERIALS AND METHODS**

**Materials:** These included oregano plant extract, low-fat milk and yoghurt from supermarket, lyophilized *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (CHR Hansen Company, Denmark), serum cholesterol and triglyceride measuring kits (Cinnajen, Iran), high-fat diets of laboratory rats and MRS Agar and MRS Broth (Merck Company, Germany).

**Effect of Oregano on the Production of Probiotic *Bifidobacterium bifidum* Yoghurt at the Second Passage:** To produce *Bifidobacterium bifidum* yogurt in this stage, after providing 4 containers, 1 liter of low-fat sterilized probiotic milk (1% fat) from the control group at first passage and the (1.5%) starter of low-fat yogurt were added to each container. Different concentrations of oregano (0, 0.1, 0.2 and 0.3%) were added respectively to the containers and mixed properly so that oregano was uniformly dissolved. Afterwards, all the containers were placed in the incubator at 38°C. Approximately every 2 hours, the acidity and pH tests were done until acidity reached 90° Dornic. Then, the samples were taken out of the incubator and transferred to a refrigerator and stored at 2°C. The produced probiotic oregano yoghurt was evaluated every 7 days by counting the microbes using direct counting method [9-11] and after 10 days the yoghurt was evaluated for sensory properties, using questionnaires filled by 50 people. The respondents were asked to rate the factors of scent, taste and thickness on a scale ranging from very good, good, medium, to weak. The results were analyzed in a statistical descriptive test by SPSS version 17 software.

**Effect of Oregano on the Production of Probiotic *Lactobacillus acidophilus* Milk at the First Passage:** The procedure was carried out as described above and instead of *Bifidobacterium bifidum* the bacterium *Lactobacillus acidophilus* was added.

**Effect of Oregano on the Production of Probiotic *Lactobacillus acidophilus* Yoghurt at the Second Passage:** The procedure was carried out as described above and instead of *Bifidobacterium bifidum* the bacterium *Lactobacillus acidophilus* was added.

Having produced the above-mentioned products, we stored 1000 gram of each product in a disposable container in the refrigerator. Each sample was tested at days 1, 7, 14 and 21 for acidity, pH and sensory properties.

**Effect of Probiotic *Lactobacillus acidophilus* Milk Containing Oregano Extract on the Cholesterol and Triglycerides Levels of the Experimental Rats:** In order to evaluate the effect of probiotic *Lactobacillus acidophilus* milk with oregano extract on cholesterol and triglyceride levels in laboratory rats, initially the rats that had the same condition based on physiological and genetic structure and environmental conditions were
prepared. For this purpose, laboratory rats were reproduced in 45 days and then, the adult rats (45 days), cognate, equiponderant (200 gram) with same environmental and nutritional conditions (temperature, moisture, light, bed, water, feed and …) were obtained. Then, rats were divided into 6 groups of 6 pieces:

- Group 1: Control group with balanced ration.
- Group 2: Second control group with high fat ration.
- Group 3: Testifier group fed with *Lactobacillus acidophilus* milk which had no oregano extract.
- Group 4: Testifier group fed with probiotic *Lactobacillus acidophilus* milk with 0.1% oregano extract.
- Group 5: Testifier group fed with probiotic *Lactobacillus acidophilus* milk with 0.2% oregano extract.
- Group 6: Testifier group fed with probiotic *Lactobacillus acidophilus* milk with 0.3% oregano extract.

In the beginning of division, blood samples were taken from group 1 as initial control group and sent to the laboratory and the first group was removed. Then the 5 remaining group were fed for 14 days with high-fat ration prepared from pellet and animal fat with the rate of 3 to 1, after 2 weeks, blood samples were taken from second control group and sent to the laboratory and second group was removed. The high-fat ration was stopped and group 3 (testifier) was fed with balanced ration for 7 days, group 4 was fed with probiotic *Lactobacillus acidophilus* milk with 0.1% oregano extract for 7 days, group 5 was fed with probiotic *Lactobacillus acidophilus* milk with 0.2% oregano extract for 7 days and group 6 was fed with probiotic *Lactobacillus acidophilus* milk with 0.3% oregano extract for 7 days.

During this period, milk provided to every rat was 30 ml daily and after these 2 weeks, blood samples were taken from the 4 remaining groups and sent to the laboratory.

### Effect of Probiotic *Bifidobacterium bifidum* and Oregano Extract on Cholesterol and Triglyceride Levels of the Experimental Rats:

All the same procedures were followed as mentioned above with the difference of using *Bifidobacterium bifidum* instead of *Lactobacillus acidophilus*.

### Measurement of Serum Cholesterol and Triglycerides:

Blood samples were centrifuged at 3500 rpm for 5 minutes and serum was separated. Serum cholesterol and triglycerides of the experimental rats were calculated using the cholesterol and triglyceride measuring kits (Cinnajen, Iran) according to the manufacturer instructions.

### RESULTS

Table 1 shows the acidity degrees of oregano milk and yoghurt *Lactobacillus acidophilus*, during storage time in the refrigerator and table 2 shows the growth rates of microbes in oregano *Lactobacillus acidophilus* milk and yoghurt at the storage time. Table 3 shows the acidity degrees of oregano *Bifidobacterium bifidum* milk and yoghurt and table 4

<table>
<thead>
<tr>
<th>Oregano milk</th>
<th>1 day</th>
<th>7 day</th>
<th>14 day</th>
<th>21 day</th>
<th>Oregano yoghurt</th>
<th>1 day</th>
<th>7 day</th>
<th>14 day</th>
<th>21 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>45</td>
<td>51</td>
<td>78</td>
<td>98</td>
<td>0%</td>
<td>92</td>
<td>97</td>
<td>108</td>
<td>-</td>
</tr>
<tr>
<td>0.1%</td>
<td>45</td>
<td>52</td>
<td>68</td>
<td>105</td>
<td>0.1%</td>
<td>96</td>
<td>110</td>
<td>118</td>
<td>-</td>
</tr>
<tr>
<td>0.2%</td>
<td>46</td>
<td>54</td>
<td>62</td>
<td>89</td>
<td>0.2%</td>
<td>103</td>
<td>113</td>
<td>125</td>
<td>-</td>
</tr>
<tr>
<td>0.3%</td>
<td>49</td>
<td>51</td>
<td>54</td>
<td>83</td>
<td>0.3%</td>
<td>103</td>
<td>115</td>
<td>120</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oregano milk</th>
<th>first week</th>
<th>second week</th>
<th>Third week</th>
<th>Oregano yoghurt</th>
<th>first week</th>
<th>second week</th>
<th>Third week</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>7.25×10³⁰</td>
<td>5.25×10³⁰</td>
<td>4.5×10³⁰</td>
<td>0%</td>
<td>9.25×10³⁰</td>
<td>3.75×10³⁰</td>
<td>3×10³⁰</td>
</tr>
<tr>
<td>0.1%</td>
<td>13×10³⁰</td>
<td>11×10³⁰</td>
<td>6.5×10³⁰</td>
<td>0.1%</td>
<td>11.25×10³⁰</td>
<td>4.25×10³⁰</td>
<td>3×10³⁰</td>
</tr>
<tr>
<td>0.2%</td>
<td>10.75×10³⁰</td>
<td>4.5×10³⁰</td>
<td>3.25×10³⁰</td>
<td>0.2%</td>
<td>12.25×10³⁰</td>
<td>4.25×10³⁰</td>
<td>3.5×10³⁰</td>
</tr>
<tr>
<td>0.3%</td>
<td>5×10³⁰</td>
<td>3.25×10³⁰</td>
<td>2×10³⁰</td>
<td>0.3%</td>
<td>15×10³⁰</td>
<td>6.75×10³⁰</td>
<td>3.75×10³⁰</td>
</tr>
</tbody>
</table>
Table 3: The acidity level based on Dornic degree in the oregano *Bifidobacterium bifidum* milk and yoghurt within 21-day storage in the refrigerator

<table>
<thead>
<tr>
<th>Acidity level in Dornic degree</th>
<th>Oregano milk 1 day</th>
<th>7 day</th>
<th>14 day</th>
<th>21 day</th>
<th>Oregano yoghurt 1 day</th>
<th>7 day</th>
<th>14 day</th>
<th>17 day</th>
<th>21 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>44</td>
<td>51</td>
<td>55</td>
<td>59</td>
<td>0%</td>
<td>90</td>
<td>95</td>
<td>135</td>
<td>-</td>
</tr>
<tr>
<td>0.1%</td>
<td>42</td>
<td>53</td>
<td>53</td>
<td>58</td>
<td>0.1%</td>
<td>98</td>
<td>110</td>
<td>133</td>
<td>-</td>
</tr>
<tr>
<td>0.2%</td>
<td>43</td>
<td>46</td>
<td>48</td>
<td>53</td>
<td>0.2%</td>
<td>99</td>
<td>110</td>
<td>117</td>
<td>-</td>
</tr>
<tr>
<td>0.3%</td>
<td>44</td>
<td>48</td>
<td>49</td>
<td>54</td>
<td>0.3%</td>
<td>93</td>
<td>112</td>
<td>114</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4: Growth of microbes in the oregano *Bifidobacterium bifidum* milk and yoghurt

<table>
<thead>
<tr>
<th>Oregano milk</th>
<th>first week</th>
<th>second week</th>
<th>Third week</th>
<th>Oregano yoghurt</th>
<th>first week</th>
<th>second week</th>
<th>Third week</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>16×10⁶</td>
<td>6.75×10⁶</td>
<td>5.75×10⁶</td>
<td>0%</td>
<td>13.75×10⁶</td>
<td>4×10⁹</td>
<td>3.75×10⁹</td>
</tr>
<tr>
<td>0.1%</td>
<td>19×10⁶</td>
<td>11×10⁶</td>
<td>12.6×10⁶</td>
<td>0.1%</td>
<td>9.75×10⁹</td>
<td>5.25×10⁹</td>
<td>3×10⁹</td>
</tr>
<tr>
<td>0.2%</td>
<td>22.5×10⁶</td>
<td>9.75×10⁶</td>
<td>4.75×10⁶</td>
<td>0.2%</td>
<td>8.75×10⁹</td>
<td>6.75×10⁹</td>
<td>3.5×10⁹</td>
</tr>
<tr>
<td>0.3%</td>
<td>18.65×10⁶</td>
<td>12.25×10⁶</td>
<td>5.5×10⁹</td>
<td>0.3%</td>
<td>5.5×10⁹</td>
<td>6×10⁹</td>
<td>3.25×10⁹</td>
</tr>
</tbody>
</table>

Table 5: Effect of probiotic *Lactobacillus acidophilus* and *Bifidobacterium bifidum* milk with varying doses of oregano on triglyceride and cholesterol levels

<table>
<thead>
<tr>
<th>Lactobacillus acidophilus</th>
<th><em>Bifidobacterium bifidum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>Control group1</td>
<td>139</td>
</tr>
<tr>
<td>Control group2</td>
<td>303</td>
</tr>
<tr>
<td>Control group3</td>
<td>257</td>
</tr>
<tr>
<td>Non oregano</td>
<td>277</td>
</tr>
<tr>
<td>0.1%</td>
<td>230</td>
</tr>
<tr>
<td>0.2%</td>
<td>170</td>
</tr>
<tr>
<td>0.3%</td>
<td>209</td>
</tr>
</tbody>
</table>

shows the growth rates of microbes in oregano *Bifidobacterium bifidum*. Table 5 shows effect of probiotic *Lactobacillus acidophilus* and *Bifidobacterium bifidum* milk with varying doses of oregano on triglyceride and cholesterol levels.

**DISCUSSION**

In recent years, the probiotic bacteria, as the food additives, have been introduced into numerous foods, of which the dairy products especially yoghurt which has played an important role in carrying these bacteria (such as *Bifidobacterium bifidum* and *Lactobacillus acidophilus*). Eating regularly the sufficient amounts of living cells “the minimum treatment” is required if the consumer is to benefit from the probiotic products. The daily recommended amount of the yoghurt containing 10⁶ CFU.ml⁻¹ probiotic bacteria is 100 gram. It is also very important to investigate the survival of these microorganisms within the interval between storage in the refrigerator and consumption [12].

Media with milk base or whey are bed-making media wholly; milk is a suit medium for growing microbes. As it has nutritive substances and high pelage and pH near to neutral and is easily digestable [7].

In the present study, the effects of oregano on the growth of the bacteria *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in probiotic milk and yoghurt were investigated.

The acidity, pH and survival of the bacteria in the oregano probiotic milk and yoghurt were evaluated at 2 hours intervals till reaching 42° Dornic acidity degrees for milk and 90° Dornic degree for yoghurt in the incubator at 38°c and also within 27-day period of storage in the refrigerator.

Probiotic milk 0.3% oregano with *Lactobacillus acidophilus* turned to 42° Dornic acidity more quickly than other samples and then samples with 0.1 and 0.2% oregano and finally control sample.

Shelf life of this product was measured 21 days in refrigerator and during this time, acidity of 0.3% milk of oregano was lesser than other samples.
According to direct microbial counting, highest counted number in first week was by 0.1% probiotic milk oregano sample with *Lactobacillus acidophilus*, followed by 0.2%, control and 0.3% and in the second and third weeks 0.1% samples showed the highest bacterial count, followed by control, 0.2 and 0.3% samples respectively.

Probiotic yoghurt of 0.3% oregano sample with *Lactobacillus acidophilus* turned to acidity at 90° Dornic more quickly than other samples and then samples 0.2, 0.1% and finally control sample and then they were put in refrigerator with 2°C temperature.

Shelf life of this product in the refrigerator was determined 15 days and during this time, acidity of yoghurt 0.3% oregano was lesser than other samples. Although benefit of probiotic products is their medical effect, their feeling effect is very important too. Among probiotic products, fermentation products are universally favorable like probiotic yoghurt and this is attributed to their seldom feeling effect. Probiotic yoghurt is the most important probiotic product [7].

Regarding yoghurt samples containing oregano of different densities with *Lactobacillus acidophilus* there were meaningful differences among color, density and taste of products (p >0.05). Increasing in density of oregano lead to favor, taste, essence and color of yoghurt but also lead to decreased density of yoghurt as control yoghurt that included 0% oregano, had more density compared to other samples. Yoghurt samples included *Lactobacillus acidophilus* bacteria and different densities of oregano differed greatly in essence and smell.

Direct microbial counting in 3 weeks showed that high quantities were present in 0.3, 0.2, 0.1% and control, respectively and this indicated that bacteria were growing as the density of oregano was increased.

Samples of probiotic milk with *Bifidobacterium bifidum* turned to 42° Dornic acidity and after that they were put in refrigerator at 2°C temperature.

Products’ shelf life in refrigerator was 21 days and during this time, acidity in milk with 0.2% of oregano was lesser than other samples.

According to direct microbial counting, The counted quantity at 0.1% oregano density from first day to 3° weak was more than other densities. Counted batteries in all densities of oregano and control sample were decreased in 3° weak compared to first day, although, the logarithmic rate was 10. Deterrent and fatality effect of low pH on *Bifidobacterium bifidum* is more than *Lactobacillus acidophilus* so, during shelf-life of product and increasing fermentation process that lead to decrease pH, is one of the main reason of decreasing *Bifidobacterium bifidum* growing

Probiotic yoghurt 0.2 and 0.3% oregano samples including *Bifidobacterium bifidum* turned to 90° Dornic acidity more quickly than other samples and then sample with 0.1% oregano and finally the control sample and then they were put in refrigerator at 2°C temperature.

Shelf-life of this product in refrigerator was more than 15 days and during this time, acidity of yoghurt in other samples was 114-135°Dornic and acidity was increased during shelf-life because of fermentation process.

The questionnaire results showed that there was much difference between yoghurt samples including *Bifidobacterium bifidum* and different densities of oregano in color and taste of products. Yoghurt sample with *Bifidobacterium bifidum* and different densities of oregano were not different in essence, smell and density.

The addition of oregano to the probiotic *Lactobacillus acidophilus* milk resulted in decreasing serum cholesterol in laboratory rats which was especially detected with 0.2% density.

Addition of oregano to the probiotic *Bifidobacterium bifidum* milk at 0.2% resulted in decreased cholesterol level compared to control group. Increasing density of oregano in probiotic *Bifidobacterium bifidum* milk had inverse relation to serum triglyceride level in laboratory rats. As, most of the decrease of triglyceride resulted from the probiotic *Bifidobacterium bifidum* milk with 0.1% density of oregano and this level of decreasing was the same as initial control group.

Probiotic products produce oily acids with short sequence and stop cholesterol syntheses in liver and move plasma cholesterol to liver [7].

In a study of the effects of soya powder on the growth of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, in probiotic products, it was demonstrated that the shelf life for the acidity reaching the desired level during incubation decreases for the milk with both bacteria and combined soya and malt, compared to the milk with only soya. As for the yoghurt with both bacteria, the same results were yielded and incubation time for the yoghurt with malt and soya was decreased [9-10].

The effect of honey on the growth of the above-mentioned bacteria introduced simultaneously into dairy products and drinks was investigated and the results indicated that the yoghurt with only *Lactobacillus acidophilus* tasted sourer than the yoghurt with both bacteria. The products containing *Bifidobacterium bifidum*, compared to those with *Lactobacillus acidophilus*, were with slower growth rate and also tasted less sour and were of longer permanence. They were not
of favorable taste when honey concentration was increased and the control was of the best taste among all the samples [11].

In another study addressing the effect of spearmint on the bacterial growth, it was demonstrated that the increased spearmint concentration promoted the growth of the bacteria in probiotic milk and yoghurt [13].

In another study addressing the effect of juice on the bacterial growth, it was demonstrated that the increased juice product promoted the growth of the bacteria in probiotic orange and apple [1].

As a final conclusion we can say, the samples were more lasting using Lactobacillus acidophilus bacteria than Bifidobacterium bifidum.

REFERENCES