ORIGINAL ARTICLE

Physicochemical and Organoleptic Properties of Lighvan Cheese Fortified with Protulaca Oleracea Seed Oil

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KEYWORDS
Omega 3, 6 and 9 fatty acids
Lighvan cheese
Protulaca Oleracea seed oil
Fortification

ABSTRACT: Cheese has high nutritional value in human health although is naturally poor in essential fatty acids. Essential fatty acids revealed crucial roles in nutritional diet and have been suggested as disease prevention agent. Protulaca Oleracea (purslane) has considerable amounts of omega 3, 6 and 9 fatty acids as well as magnesium, potassium and vitamin C. The aim of this study is production and characterization of Lighvan cheese fortified with Protulaca Oleracea seed oil. Results indicated that increasing of Protulaca Oleracea seed oil caused significant increased omega 3, 6 and 9 concentration in cheese (P<0.05). Protulaca Oleracea seed oil fortification showed no significant effects on physicochemical properties of Lighvan cheese (P>0.05). Lighvan cheese containing 2.5% Protulaca Oleracea (purslane) seed oil showed the highest sensory attributes.

INTRODUCTION

Cheese is the dairy product, considered as important food as a point of nutritional value. Cheese has been produced by traditional procedures since long time ago in Iran. Lighvan cheese, the semi-hard cheese, is the most popular traditional cheese made from raw sheep’s milk in East Azerbaijan Province of Iran. Lighvan cheese is characterized by high taste acceptability and considerable amounts of proteins especially casein which is beneficial for indigestion disease [1] but is naturally poor in essential fatty acids. A large body of scientific reports suggests that high essential fatty acids dietary intake associated with health and reductions in cardiovascular diseases [2].

Protulaca oleracea is an herb, known as purslane,
which has considerable amount of polyunsaturated fatty acids. Purslane has been considered as rich sources of antioxidants, Vitamin A, B, C and E, beta-carotene and essential amino acids as well as minerals such as potassium, calcium, magnesium and iron [3, 4]. Fatty acid composition of purslane contains palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 n9c), linoleic acid (C18:2 n6c) and α-linolenic acid (C18:3 n3)[5]. α-Linolenic acid is an ω3 fatty acid that is essential in the human diet as a precursor for the synthesis of longer chain fatty acids and the prostaglandin group of mammalian hormones. Oil seeds such as brassica, flax and soya are main sources of linoleic acid and particularly purslane seed contains considerable amounts of linoleic acids [6]. There are several reports in the literature corresponding to the health effects of omega 3 fatty acids on cholesterol reduction, arthritis treatment, mental depression therapy, burns healing and prevention of cancer cells growth [7-9]. Studies on omega 3 fortification of dairy products using fish oil have been found in the literature. Ye et al. fortified processed cheese with omega 3 of fish oil and reported oxidation and the “fishy” flavor of fish oil limit the level of fortification. In addition, they concluded that encapsulation is a suitable way to reduce the above-mentioned problem [10]. However, the major problem with this kind of fortification is unacceptable sensory properties especially in low fat dairy products as Kolanowski and Weißbrodt confirmed [11].

The objectives of this study are i) to formulate of Lighvan cheese with purslane oil extract and ii) to study the effects of purslane fortification on characteristics of Lighvan cheese.

MATERIALES AND METHODS

Materials
Sheep milk was provided from Almalo village, Sahandabad County, East Azerbaijan Province, Iran. Composition of sheep milk was 7.1% fat, 5.7% protein and 18.2% total solids that were measured using Milkoscan (model 134 A/B N, Foss Electric, Denmark) [12]. Renin was purchased from Mitoy Company, Japan and salt was purchased from Pars Kaveh Company, Iran. Purslane seeds provided from Mashhad traditional market and oil was extracted by cold press method.

Cheese preparation
About 24 kg of sheep milk were hygienically filtered at 30 °C and then divided into four portions in steel containers. Purslane seed oil was mixed with sheep milk at three ratios of 1:5, 2:5 and 3:5 using Blender (MJ-176NR, National, Japan). Then rennet was added to sheep milk in order to curd formation after one hour. The curds were pressed in textile filter in order to separate whey. The curds were then cut longitudinally and transversally and pressed again to remove residue whey. The curds were moulded and placed in 15% salt brine after 3 hours. Finally, the moulded curds salted and stored in 11% salt brine at 8±2 °C for 3 months for ripening.

Physicochemical analysis of cheese
Regarding the cheese samples, their water contents were analyzed in triplicate by heating to a constant weight using a moisture analyzer (Sartorius Ltd., Epsom, UK) and fat according to the Gerber method [13]. For pH measurement, the grated cheese samples (10 g) mixed with equal quantities of distilled water (10 mL) and the pH of dispersion was measured using pH-meter [14]. Titratable acidity of cheese samples was expressed as lactic acid percentage. Cheese samples were analyzed for salt content by Volhard [13]. The nitrogen content (protein) of cheese samples was determined using Kjeldahl method [15].

Fatty acid composition
Two grams of cheese fat samples (fortified and control) were mixed with 0.4 mg of esterification detector and shaked in 110-120 °C for 3 h. The separated upper phase was used for further analysis. Fatty acid composition of purslane oil and fortified cheese analyzed using gas
chromatography. YL Model 6100 GC equipped with flame ionization detector was used. Characterization of capillary column (TR-CN100) was with 0.2-micrometer internal diameter, 0.25 micrometer thin coating and 30-meter length at 80 to 200 °C temperature. The GC was operated with helium carrier gas with 99.99% purity [16].

**Sensory properties**

The sensory quality of cheese sample was evaluated by a 15-member panelist group who were skilled with quality attributes of food products. Panelists scored for sensory characteristics including color, odor, texture, internal and external appearance using a five point hedonic scale (1; very bad to 5; excellent) [17].

**Statistical Analysis**

The means of treatments were subjected to one-way analysis of variance (ANOVA) at 95% confidence level using SPSS 16 software. Sensory results were analyzed using nonparametric Fridman test at 0.05 significant levels. All analysis was performed at three replications.

**RESULTS AND DISCUSSION**

**Effects of purslane seed oil on physicochemical properties**

The results of physicochemical analysis were shown in Table 1-3. The results indicated that the efficiency of cheese making (kg cheese/kg milk) increased as purslane oil concentration increased. The efficiency of cheese making depends on milk type, fat content, total solid (milk density), milk temperature (temperature of inoculation), the amount of rennet, rennet coagulation capacity, removed whey content, molding pressure and salting.

### Table 1. Cheese making efficiency

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>Cheese + 1.5% PO</th>
<th>Cheese + 2.5% PO</th>
<th>Cheese + 3.5% PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency</td>
<td>34.83%</td>
<td>36.83%</td>
<td>37.33%</td>
<td>39.33%</td>
</tr>
</tbody>
</table>

1: purslane oil

There is no significant difference ($P>0.05$) between pH and acidity of samples due to purslane oil addition however, the significant reduction in pH and acidity after aging process was observed ($P<0.05$) which can be attributed to lactose fermentation and lactic acid production by bacteria (Table 2).

### Table 2. pH and acidity of cheese samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>pH</th>
<th>Acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 90</td>
</tr>
<tr>
<td>Control</td>
<td>5.8±0.11</td>
<td>4.8±0.13</td>
</tr>
<tr>
<td>Cheese + 1.5% PO</td>
<td>5.7±0.12</td>
<td>4.8±0.17</td>
</tr>
<tr>
<td>Cheese + 2.5% PO</td>
<td>5.6±0.15</td>
<td>4.7±0.17</td>
</tr>
<tr>
<td>Cheese + 3.5% PO</td>
<td>5.6±0.09</td>
<td>4.7±0.11</td>
</tr>
</tbody>
</table>

Values are recorded as mean ± standard deviation

Means followed by different superscripts in each column are significantly different ($P<0.05$)

The results of moisture, fat, salt and protein contents of cheese samples were shown in Table 3. The results revealed that the highest fat content related to cheese containing 3.5% purslane oil. The more purslane oil
concentration caused significant more fat content ($P<0.05$) although the aging step did not affect fat content of cheeses samples significantly ($P>0.05$) [18]. Salt concentration in brine and consequent osmotic pressure caused penetration of salt into cheese and resulted in equilibrium, which led to moisture loss during aging [19]. Results showed that moisture content of fortified cheese with purslane oil decreased before aging as purslane oil increased, however there is no significant difference between moisture content of cheese containing 1.5 and 2.5% purslane oil ($P>0.05$). In fact, increasing of fat content resulted in more fat in dry matter and subsequent less moisture content, which led to reduced lipolysis [18]. Moisture content did not substituted as much as reduced fat in low fat cheese [20]. Salt content of samples decreased as purslane oil increased which can explained by preventing role of purslane oil from penetration of salt into cheese structure. In fact, fat globules fasten capillary structure and extend penetration duration so cheese contained more fat content needs more time for salt diffusion into cheese [21]. Salt content of cheese increased during aging because of cheese curds storing in salt brine [19]. Protein content of cheese decreased significantly ($P<0.05$) during aging due to proteolysis. Proteolysis leads to increased solubility and water absorption of proteins due to releasing of polar groups such as amines and carboxylic groups of amino acids and peptides. Therefore, the more proteolysis causes the more soluble content, the more water absorption and subsequent the less total solid [3].

### Table 3. Chemical properties of cheese samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Moisture</th>
<th>Salt</th>
<th>Fat</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 90</td>
<td>Day 7</td>
<td>Day 90</td>
</tr>
<tr>
<td>Control</td>
<td>60±0.32a</td>
<td>59±0.25b</td>
<td>4.17±0.037a</td>
<td>17.1±0.66a</td>
</tr>
<tr>
<td>Cheese + 1.5% PO</td>
<td>58.6±0.99b</td>
<td>59.75±0.45a</td>
<td>3.99±0.066a</td>
<td>20.03±0.37b</td>
</tr>
<tr>
<td>Cheese + 2.5% PO</td>
<td>58.02±0.19bc</td>
<td>59.58±0.34a</td>
<td>3.81±0.026a</td>
<td>4.52±0.03a</td>
</tr>
<tr>
<td>Cheese + 3.5% PO</td>
<td>57±0.63c</td>
<td>59.19±0.64ab</td>
<td>3.13±0.032c</td>
<td>4.38±0.01ab</td>
</tr>
</tbody>
</table>

Values are recorded as mean ± standard deviation
Means followed by different superscripts in each column are significantly different ($P<0.05$)

### Gas chromatography

The results of gas chromatography were shown in Table 4. The considerable amounts of omega fatty acids especially omega-3 fatty acids in purslane oil was observed and the omega-6 to omega-3 ration calculated as 1.5 which is an ideal ratio.

### Table 3. GC results of main purslane oil fatty acids

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Name</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>Palmitic acid</td>
<td>16.62</td>
</tr>
<tr>
<td>C18:0</td>
<td>Stearic acid</td>
<td>4.80</td>
</tr>
<tr>
<td>C18:1n9c</td>
<td>Oleic acid</td>
<td>20.35</td>
</tr>
<tr>
<td>C18:2n6c</td>
<td>Linoleic acid</td>
<td>33.11</td>
</tr>
<tr>
<td>C18:3n3</td>
<td>Alpha - linolenic acid</td>
<td>21.93</td>
</tr>
</tbody>
</table>
The results indicated that purslane oil addition caused significant increase in omega 3, 6 and 9 ($P<0.05$). Ninthly gram of purslane oil which contained 21.93, 33.11 and 20.53 % of omega 3, 6 and 9, respectively added to 6 kg of milk in which cheese contained 1.5% purslane oil and resulted in increasing of cheese making efficiency from 34.83 to 36.83% as well as increasing of omega essential fatty acids from 0.29 to 3.56, 0.8 to 5.37 and 6.57 to 9.79% for omega 3, 6 and 9 respectively.

### Table 5. Omega fatty acids of cheese samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>C18:3n3 Day 7</th>
<th>C18:3n3 Day 90</th>
<th>C18:2n6c Day 7</th>
<th>C18:2n6c Day 90</th>
<th>C18:1n9c Day 7</th>
<th>C18:1n9c Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.29±0.021</td>
<td>0.67±0.04</td>
<td>0.8±0.05</td>
<td>0.26±0.06</td>
<td>6.75±0.88</td>
<td>9.19±0.49</td>
</tr>
<tr>
<td>Cheese + 1.5% PO</td>
<td>3.56±0.16</td>
<td>3.15±0.06</td>
<td>5.73±0.63</td>
<td>3.28±0.12</td>
<td>9.97±0.54</td>
<td>10.99±0.94</td>
</tr>
<tr>
<td>Cheese + 2.5% PO</td>
<td>4.44±0.14</td>
<td>4.43±0.03</td>
<td>6.03±0.42</td>
<td>4.04±0.28</td>
<td>13.45±0.47</td>
<td>13.93±0.20</td>
</tr>
<tr>
<td>Cheese + 3.5% PO</td>
<td>5.62±0.37</td>
<td>5.64±0.07</td>
<td>8.38±0.36</td>
<td>6.86±0.15</td>
<td>17.53±0.78</td>
<td>17.79±0.54</td>
</tr>
</tbody>
</table>

Values are recorded as mean ± standard deviation
Means followed by different superscripts in each column are significantly different ($P<0.05$)

**Sensory properties**

Sensory assessment of cheese fortified with purslane oil is presented in Figure1. The panelists marked the lowest scores of flavor, aroma and appearance (color) for cheese containing 3.5% purslane oil and the cheese contained 3.5% purslane oil obtained the lowest total acceptability score. Porous structure is texture characteristic of Lighvan cheese. Different aroma is the other characteristic of Lighvan cheese, which is related to sheep milk and microorganisms activity. It was expected that purslane oil covered the special aroma of Lighvan cheese because of distinct odor of purslane oil. The panelist distinguish particular aroma of purslane oil only at 3.5% concentration level. The individual flavor is another characteristic of Lighvan cheese, which is correlated to sheep milk and bacteria activity. Purslane oil affected flavor of Lighvan cheese especially at 3.5% concentration level. The fortified cheese contained 3.5% purslane oil seemed darker than the other treatments.

![Figure 1. Sensory properties of cheese samples](image-url)
CONCLUSION

This study exhibited the possibility of purslane oil as omega essential fatty acid source in traditional Lighvan cheese formulation without undesirable altering of physicochemical and organoleptic properties. Purslane seed oil could enrich omega 3, 6 and 9 fatty acids content of cheese considerably. Increasing of purslane oil in formulation lead to increased omega fatty acid content although cheese contained 3.5% purslane oil did not obtain acceptable sensory characteristics and 2.5% purslane oil concentration showed better organoleptic properties.

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REFERENCES
