Influence of Plant Based Coagulant (Enzyme Extracts from Albizia and Sunflower Seeds) on Quality of Domiati Cheese.

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ABSTRACT

The effects of protein extract of albizia (Abizia lebbeck) and sunflower (Helianthus annuus) seeds on milk-clotting activity, lipolysis, proteolysis, textural characteristics and flavor development in Domiati cheese over 60 days picked during 60 days were studied. Changes in the chemical composition of experimental Domiati cheese samples seemed to be not affected by plant based coagulant (albizia and sunflower seeds). On the other hand, cheese was within legal requirements for Domiati cheese in Egypt. Lipolysis and proteolysis of pickled cheese were higher when coagulated with protein extract of albizia and sunflower seeds, compare with control, while hardness, adhesiveness, gumminess and chewiness were lower in cheese made using plant based coagulant than control. A significant variation in cohesiveness and springiness were found among the plant based coagulant used in Domiati cheese making. The cheese made using protein extract of albizia (A. lebbeck) received high scores in flavour acceptability and texture, compared with that made by mixing protein extract of sunflower (H. annuus) and control.

Keywords: Domiati cheese, Albizia, sunflower, Texture profile analysis

INTRODUCTION

Domiati cheese is the most common and popular in Egypt. It is a different compare with other pickled types by the fact that a high percentage of salt (Up to 15%) is put directly to the cheese –milk (Abou-Donia, 2007), rather than at the process end to the cheese curd. Domiati cheese was consumed fresh or after pickling period for several months (Ayad 2009). Domiati cheese has been made from buffalo’s milk, cow’s milk or a mixture of both. It is made by coagulating the milk with calf rennet.

Chymosin is a aspartic protease found in calf rennet and used for enzymatic coagulation of milk in cheese-making. The essential cleavage occurs at Phel05-Met106 bond of k-casein, resulting form the cheese curd (Jolles, et al., 1963).

Enzymatic coagulation may be achieved by a several types of proteolytic enzymes from different sources, such as microbial species (Cryphonectria parasitica, R. pusillus and Rhizomucor miehei) or plant coagulants. The use of animal rennet may be restricted for several reason, such as vegetarian or consumer interest regarding genetically modified food (e.g., France, Germany and Netherlands forbid the application of recombinant calf rennet in food industries). Moreover, the infection of animal with bovine spongiform encephalopathy has decreased both supply and demand for animal rennet (Roseiro et al., 2003).

Plant coagulant has been identified from Calotropis procera (Sanni et al., 1999), Ananas comosus (Cattaneo et al., 1994), Ficus bengalensis, Opuntia phylloclades, Cersea triangularis, Urophorbia caducifolia, F. elastica, E. hista (Umar, et al., 1990), Lactuca sativa (Lo, et al., 2002). Unfortunately, almost of these plant coagulants have been reported to be unsuitable because of production of extremely bitter cheeses. However the aqueous extracts and protein extracts of Cyanara cardunculus, Helianthus annuus and Abizia lebbeck have been found to possess proteolytic enzymes which clotted milk readily, without developing any desirable flavour or bitterness in cheese after pickled period (3 months) (Otani et al., 1991; Cordeiro et al., 1994; Park et al., 2000; Sidrach et al., 2005).

The objective of the present work was to study the potential ability of protein extracts from Helianthus annuus and Abizia lebbeck seeds as calf rennet replacer for production of Domiati cheese.

MATERIALS AND METHODS

Preparation of crude enzyme extract

Ten grams of albizia seeds and sunflower seeds were crushed, followed by soaking the mill seeds in distilled water (100 ml) containing NaCl (1%) and sodium azide (0.02%) for 24 hours at 5°C with agitation. The mixtures were then filtered to give crude aqueous extracts. The precipitation of proteins from crude aqueous extracts were performed by using ammonium sulfate at 40% saturation, the mixture was kept for 45 min at 4°C. The mixture was centrifuged (16,000xg at 4°C for 15 min), and the pellets were removed and added gradually to the supernatant up to 60% saturation in the case of Helianthus annuus or 70% in the case of Abizia lebbeck and were kept for 45 min at 4°C, followed by centrifugation (15,000x g at 4°C for 10 min). The supernatants were discarded and the pellets were dissolved in 20 ml of distilled water, the mixture was put in dialysis tubing at 4°C for 48h to remove salts and finally dried to give the protein extracts of H. annuus and A. lebbeck (Egitto et al., 2007).

Cheese making procedure:

Domiati cheese was made as described by Abou-Donia (2008), the milk was heated to 50°C and salted using sodium chloride to give final concentration of 12%. Each quantity of milk was divided into three parts. A suitable amount of commercial rennet and 20mg ml - 1 of H. annuus and A. lebbeck protein extracts were added to coagulate milk cheese within 2-3h. The cheese curds were placed in the wooden frames lined with cheese cloth. After 3 to 4 h, and cheese curds were pressed using weights. The cheese was then cut into blocks of about 10 x 10 x10 cm. The cheese blocks were transferred into cans and filled with brine (12% salt). The cans were stored at room temperature (20 to 30°C) for 60 d.
Cheese Composition analysis

Total protein was determined by the Kjeldahl method (AOAC, 2000) and content of fat by Gerber method (AOAC, 2000), the moisture content was measured according to AOAC, (2005), pH meter (CG710, West Germany) was used to measure pH values of cheese samples according to Ling, (1963).

Microbiological analysis

Ten grams of each cheese sample were mixed for 5 min with 90 ml of sterilized 2% sodium citrate solution and serially diluted using sterilized saline (0.85% NaCl). Appropriate dilutions of sodium citrate solution of cheese samples were plated on tryptone soya agar (TSA) at 35°C for 48 h, MacConkey agar for enumerating coliform bacteria at 37°C for 48 h, Potato dextrose agar for enumerating the molds and yeasts at 20°C for 5 d, and staphylococcus medium 110 for enumeration of staphylococci at 37°C for 48 h (Difco's Manual, 1985).

Evaluation of proteolysis and lipolysis

The water –soluble extract (WSE) was determined according to Kuchroo and Fox (1982), and were estimated in water soluble extract by using the Cd-ninhydrin method (Foldertsma and Fox, 1992). Free fatty acids were estimated by the method of Deeth et al., (1975).

Evaluation of textural properties of cheese

Determination of Textural characteristics of cheese samples were carried out by texture analyzer (TA 1000, Lab Pro), USA. Samples of cheese were divided into 50 mm3. A two-bite penetration test was implemented with the TA 60 degree cone, Perpex probe for Domiati cheese operated at a crosshead speed 50 mm/sec. Hardness, Adhesiveness, cohesiveness, springiness and gumminess and chewiness were estimated in triplicate as reported by Szczesniak et al., (1963) and Bourne, (1978).

Sensory Assessment

Evaluation of sensory properties of cheese samples was carried out at the Dairy Department, Mansoura University by 10 panelists, including staff members, consumers and cheese producers. Each one of panelists was given 3 cheese blocks (7 x 3 x 3 cm) per sample. Cheese samples were placed in identical plastic sample cups identified by a random three digit number. The coded cheese samples were randomly presented to panelists. The Panelists were asked to award the cheese a total grade out of 100, to assess whether each sample was closed to Domiati cheese and to give additional comments. Cheeses were evaluated at the end of pickled period and the following scale was used: 0 – 30 = unacceptable; 31-60=poor; 61-85=acceptable; 86-100=good.

Statistical Analysis

Data were statistically analyzed using SAS software package (SAS institute, 2004) was used ANOVA.

RESULTS AND DISCUSSION

Chemical composition of Domiati cheese

The total solid in all cheeses decreased significantly (P < 0.05) over pickling (Fig.1). The total solid increased over the first month of pickling (Fig.1). Treating cheese with different coagulants in the present study, had no significant influence on the total solid of Domiati cheese (Fig.1). The average content of total solid of fresh Domiati cheeses is in agreement with that reported by Awad et al. (2001).

The contents of protein and fat in cheeses were found to be dependent on the total solid content in cheeses over pickling (Fig. 2 & Fig.3). There was no significant difference (P < 0.05) of protein and fat content in all cheese treatments (Fig. 2 & Fig.3). The salt in moisture content increased insignificantly (P < 0.05) during the period of pickling (Fig. 4). Similar results were found by Awad et al. (2001).

The overall aged Domiati chemical composition was consistent with the typical composition of Domiati cheese (Abd El-salam and ALichanidis, 2004) and maintaining the legal limit for Domiati cheese (Egyptian Standards, 2000).

![Figure 1](image1.png)

**Figure 1.** The moisture content of Domiati cheese made with different coagulant types. Values are means of at least three separate determinations, and error bars represent ±SD.

![Figure 2](image2.png)

**Figure 2.** The effect of coagulant types on protein content of Domiati cheese. Values are means of at least three separate determinations, and error bars represent ±SE.
Figure 3. The influence of coagulant types on fat content of Domiati cheese. Values are means of at least three separate determinations, and error bars represent ±SE.

Figure 4. The effect of coagulant types on salt content of Domiati cheese. Values are means of at least three separate determinations, and error bars represent ±SE.

Yield percentage of Domiati cheese
The impact of different coagulant types on yield percentage of Domiati cheese were presented in Figure 5. The yield (%) in all cheeses decreased significantly \((P < 0.05)\) during pickling (Fig. 5). The treatments used in this study had no significant \((P > 0.05)\) influence on the yield percentage of Domiati cheese.

pH values of cheese over Pickling
The pH values of cheese samples measured during pickling are listed in Table 1. The pH values decreased gradually during pickling period (table1). The pH values of fresh Domiati cheese made using albizia seed protein extract was the lowest compare with control and sunflower seed protein. This might be attributed the albizia seed protein extract had greater protein degradation ability by activation of non starter lactic acid bacteria.

Lipolysis of Domiati cheese
The lipolysis occurred in picketed Domiati cheese was determined in terms of total free fatty acids (FFA) (Table1). There was significant increasing \((P < 0.05)\) in FFA associated with progressing period of pickling (Table 1). Cheese made by protein extract of Albizia seed showed higher FFA values over pickling than cheese made by rennet, followed by using protein extract of sunflower seed as rennet replacer, compare with control. High FFA values in cheeses containing protein extract of sunflower seed and albizia seed might be attributed to the activation of microflora in cheeses to the release lipases and intracellular esterases.

Proteolysis of Domiati cheese
Free amino acids release \((mM leucine equivalents)\) in WSE of cheese samples at different stages of pickling are presented in Table 1. The values of free amino acid (FAA) were the highest in treatment made using protein extract of A. lebbeck, compare with other treatments throughout the period pickling. This finding is imputed to several factors such as low pH values, high content of moisture, high content of viable bacteria, and high residual proteolytic enzymes. FAA were raised significantly \((P < 0.05)\) as storage pickling progressed in all treatments. These results are consistent with Egito, et al., (2007), who reported that protein extract of Albizia and sunflower seeds could be a potentially animal rennet replacer, the protein extract of albizia seed was higher than protein extract of sunflower, the both of protein extracts were exhibited good milk-clotting and caseinolytic ability required for ripening of cheese.

Assessment of microbial profile of Domiati cheese
The total viable bacterial count in cheese samples throughout the period of pickling is shown in (Fig. 6). There was no significant difference \((P > 0.05)\) in total viable bacterial count of cheese samples. A gradual decrease in total viable bacterial count was detected over pickling in all cheese samples, resulting in a bout a 2 log reduction after 2 months. The present results were similar to those reported by El-Koussy et al., (1976); Ahmad et al., (1978); Abou-Donia, (1981) and Abd-El-Khalek et al., (2008), who investigated a decrease in the total viable counts over pickling of Domiati cheese. Staphylococci, coliform bacteria, yeasts and molds were not detected in any samples of Domiati cheese over pickling period. The results were in agreement with those found by Awad et al., (2010).
Texture Profile analysis of experimental Domiati cheese

Texture profile analysis parameters of experimental cheese samples were determined (Table 2). Texture properties of cheese samples were significantly (P<0.05) affected by using substitution of calf rennet by protein extract of A. lebbeck and H. annauus seeds. Hardness, Adhesiveness, gumminess and chewiness were significantly lower (P<0.05) in cheese samples made by using protein extract of A. lebbeck compared with other treatments (Table 2), followed by cheese made using protein extract of H. annauus. However, the cohesiveness and springiness were significantly higher in cheese coagulated with protein extract of A. lebbeck seed. A gradual decline in cohesiveness, springiness, gumminess and chewiness were detected during the progress of pickling period in all cheeses, in contrast to hardness and adhesiveness were significantly higher in all samples of cheese over pickling (Table 2). This result was in perfect harmony with those presented by Koca and Metin (2004); Volikakis et al., (2004); Korish and Abd Elhamid (2012). The lower values of hardness, gumminess, chewiness and adhesiveness in cheese coagulated with protein extract of H. annauus and A. lebbeck seeds might be related to high ability of these protein extracts for hydrolysis of casein particles compare with chymosin (Egito et al., 2007). The matrix of cheese is formed by interconnected casein particles and the solubilization of colloidal calcium phosphate (CCP) and hydrolysis of these molecules will decrease hardness (De Jong, 1976; Creamer and Olson, 1982; Creamer et al., 1982).

Table 1. The effect of coagulant types on pH, free amino acid (FAA) and Free fatty acids.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH 1 d</th>
<th>pH 30 d</th>
<th>pH 60 d</th>
<th>FAA 1 d</th>
<th>FAA 30 d</th>
<th>FAA 60 d</th>
<th>FFA 30 d</th>
<th>FFA 60 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.49±</td>
<td>5.63±</td>
<td>4.79±</td>
<td>0.02±</td>
<td>0.09±</td>
<td>0.22±</td>
<td>0.51±</td>
<td>1.12±</td>
</tr>
<tr>
<td>(Chymosin)</td>
<td>0.08</td>
<td>0.18</td>
<td>0.04</td>
<td>0.004</td>
<td>0.007</td>
<td>0.035</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>Protein extract of H. annauus</td>
<td>6.26±</td>
<td>5.34±</td>
<td>4.45±</td>
<td>0.033±</td>
<td>0.197±</td>
<td>0.283±</td>
<td>0.63±</td>
<td>1.25±</td>
</tr>
<tr>
<td>Protein extract of A. lebbeck</td>
<td>6.08±</td>
<td>5.12±</td>
<td>4.24±</td>
<td>0.045±</td>
<td>0.273±</td>
<td>0.363±</td>
<td>0.67±</td>
<td>1.38±</td>
</tr>
<tr>
<td>Data are means ±S.E., N=3; mean values in the same column with different letters in the superscript are indicated significantly different (p&lt;0.05).</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Table 2. The influence of pickling period on hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pickling period</th>
<th>Hardness (Newton)</th>
<th>Adhesiveness (Jole)</th>
<th>Springiness (Millimeter)</th>
<th>Cohesiveness (Dimensionless)</th>
<th>Gumminess (Newton)</th>
<th>Chewiness (Jole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1 day</td>
<td>7.38±0.17</td>
<td>37.44±0.26</td>
<td>0.75±0.06</td>
<td>0.40±0.04</td>
<td>2.92±0.26</td>
<td>2.23±0.12</td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>8.67±0.04</td>
<td>38.55±0.58</td>
<td>0.55±0.07</td>
<td>0.25±0.03</td>
<td>2.42±0.25</td>
<td>1.56±0.18</td>
</tr>
<tr>
<td></td>
<td>60 days</td>
<td>9.33±0.27</td>
<td>40.32±0.56</td>
<td>0.42±0.08</td>
<td>0.24±0.05</td>
<td>2.20±0.51</td>
<td>1.03±0.31</td>
</tr>
<tr>
<td>Mean</td>
<td>7.30±0.04</td>
<td>38.77±0.39</td>
<td>0.57±0.03</td>
<td>0.31±0.03</td>
<td>2.51±0.14</td>
<td>1.61±0.06</td>
<td></td>
</tr>
<tr>
<td>protein extract of H. annauus</td>
<td>1 day</td>
<td>6.20±0.04</td>
<td>34.80±0.37</td>
<td>0.75±0.03</td>
<td>0.43±0.03</td>
<td>2.72±0.41</td>
<td>2.03±0.30</td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>7.21±0.32</td>
<td>35.93±0.24</td>
<td>0.58±0.02</td>
<td>0.33±0.04</td>
<td>2.35±0.16</td>
<td>1.36±0.10</td>
</tr>
<tr>
<td></td>
<td>60 days</td>
<td>8.49±0.24</td>
<td>38.05±0.34</td>
<td>0.43±0.05</td>
<td>0.25±0.03</td>
<td>2.16±0.17</td>
<td>0.96±0.19</td>
</tr>
<tr>
<td>Mean</td>
<td>7.30±0.13</td>
<td>36.26±0.39</td>
<td>0.59±0.03</td>
<td>0.34±0.03</td>
<td>2.41±0.14</td>
<td>1.45±0.03</td>
<td></td>
</tr>
<tr>
<td>protein extract of A. lebbeck</td>
<td>1 day</td>
<td>5.73±0.15</td>
<td>33.69±0.25</td>
<td>0.77±0.03</td>
<td>0.45±0.03</td>
<td>2.57±0.11</td>
<td>1.93±0.12</td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>6.44±0.18</td>
<td>34.66±0.33</td>
<td>0.65±0.04</td>
<td>0.34±0.05</td>
<td>2.19±0.28</td>
<td>1.81±0.11</td>
</tr>
<tr>
<td></td>
<td>60 days</td>
<td>7.06±0.14</td>
<td>36.13±0.14</td>
<td>0.45±0.04</td>
<td>0.31±0.03</td>
<td>2.17±0.21</td>
<td>0.94±0.25</td>
</tr>
<tr>
<td>Mean</td>
<td>6.41±0.13</td>
<td>34.83±0.24</td>
<td>0.62±0.03</td>
<td>0.37±0.03</td>
<td>2.31±0.14</td>
<td>1.35±0.03</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ±S.E., N=3; mean values in the same column with different letters in the superscript are indicated significantly different (p<0.05).
acids, is answerable for the characteristic cheese flavour (Kanawjia et al., 1995). Panelists found differences (P< 0.05) in body and texture between cheese made using protein extract of A. lebbeck and control. The making of Domiati cheese using protein extract A. lebbeck seed replaced the highest grades in acceptability of body and texture.

Figure 6. Sensory evaluation of Domiati cheese using different coagulant types. Values are means of at least three separate determinations, and error bars represent ±SE.

CONCLUSION
It is deduced from the present study that protein extract of A. lebbeck and H. annauus seeds yielded Domiati cheeses with higher proteolysis and lipolysis, higher flavour intensity, lower pH, as well as increased acceptability of body and texture of resultant cheese compared with control. Further work is in progress to create to possible use protein extract of A. lebbeck and H. annauus seeds for production soft cheese as rennet replacer.

REFERENCES


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