PRODUCING OF PROBIOTICS MONTEREY CHEESE AND STUDY ITS CHEMICAL COMPOSITION

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ABSTRACT
Chemical composition of three manufactured Monterey cheese (Control Monterey cheese (C), therapeutic Monterey cheese with the single probiotic strain (A) and therapeutic Monterey cheese with mixture probiotic strains (B)) by adding probiotics bacteria was studied at 0, 14, 28 and 42 days of ripening period. Moisture content decreased in all manufactured Monterey cheese (C, A and B) during the ripening period, protein and fat content increased after 42 days of ripening period and reached 23.41, 23.78 and 23.63% respectively for protein whilst 31.20, 31.03 and 30.43% for fat. Salt and ash content in manufactured monterey cheese by using a mixture of probiotics strains was higher than both manufactured monterey cheese with single probiotic strain and control product (without probiotics)

Key Words: Probiotics, Monterey cheese, Chemical composition.

INTRODUCTION
Tended attention went to fortify food with vital supplements by using therapeutic bacterial strains with a healthy effect in order to improve consumer health and immunity. It has been shown evidently the close link between food and health not only on the development of the disease levels, but also skipped it to combat the disease itself, so the studies tended to produce many manufactured foods especially dairy products due to increasing its consumption as a result because they contain vital supplements, which represent the appropriate means to deliver these supplements to the consumer, the therapeutic cheese represents a favorable environment for keeping vital supplements since this product is a suitable carrier of the bacteria and the most important advantage that these products are therapeutic when they contain no less than $6^{10}$ CFU/g to perform a therapeutic role by improving the balance of intestinal flora, and getting back the real balance of intestinal flora requires the presence of 80-85% of the vital supplements and for this reason, recently the concept of therapeutic food became common (Vetvicka and Vetvickova, 2016). Castro et al., (2015) defined functional foods; those foods that contain some health-promoting components that go beyond the traditional nutriaants; one way in which foods can be modified to become functional is by adding probiotics, in addition to the viability of probiotics in cheese, the incorporation of probiotic bacteria should not affect the sensory characteristics (flavor, texture, and appearance) of conventional (non-probiotic) cheeses. Although several studies have shown that probiotic cultures didn’t considerably affect the Sensory quality of cheese, it is thought that their addition might contribute to different flavors and texture characteristics (Karimi et al., 2012 a, b).

The ingestion of cheese supplemented with probiotic bacteria has been associated with a variety of benefits to human health, such as improvements in the immune system, improvements in oral and intestinal health in the elderly and reinforcement of intestinal immunity (Lollo et al., 2012; Albenzio et al., 2013a, b). The study aims to use probiotics (Lactobacillus acidophilus and Bifidobacterium longum) bacteria in manufacturing of Monterey cheese to study the chemical changes of manufactured cheese and comparing it with traditional Monterey cheese.

MATERIALS AND METHODS
Milk: Cow milk was obtained from an agricultural research station (College of Agriculture/University of Basra) and was used in cheese manufacturing.
Rennet: Microbial rennet (Rhizomucor pusillus) was obtained from Meito Sangyo Co LTD, Japan was used in cheese manufacturing.
Starters: Lactococcus lactis ssp. lactis and Lactococcus lactis ssp. cremoris from Chr-Hansen (Denmark), Lactobacillus acidophilus probiotic bacteria for the single starter and Bifidobacterium longum and Lactobacillus acidophilus for the mix-ure starter from CVS/Pharmacy (Japan).
Monterey Cheese: Traditional Monterey cheese manufactured according to Kosikowski (1970). For probiotic Monterey cheese with single probiotic strain was manufactured by adding 10% of Lb. acidophilus 10$^{10}$ CFU/g after adding the starter and following the same steps of the manufacturing procedure. While therapeutic Monterey cheese with mixture probiotic strains was manufactured by adding 10% of (Lb. acidophilus and Bif. Longum) 10$^{15}$ cfu/g after adding the starter and following the same steps of manufacturing procedure. The ripening time was 6 weeks at 16°C and 85% humidity.
Chemical tests for cheese: Moisture content was determined according to Egan et al., (1988), total protein content was determined according to Uaboi-Egbenni et al., (2010), the total ash calculated by multiplying nitrogen value by the factor 6.38. Soluble nitrogen was determined according to Tavaaria et al., (2003). Fat percentage was determined by using the Gerber method according to Egan et al., (1988). Salt was determined according to Newlander & Altherton (1964). Ash was determined according to Osborne & Voogt (1978).

RESULTS AND DISCUSSION

Chemical composition of Monterey cheese:

Moisture content: Table 1 illustrates the moisture content of Monterey cheese produced from cow milk and inoculated with 1% of cheese starter (Lactococcus lactis ssp. lactis and Lactococcus lactis ssp. cremoris), (control treatment C), Monterey cheese inoculated with 1% of cheese starter and 10% Lb. acidophilus (treatment A), Monterey cheese inoculated with 1% of cheese starter and 10% mixture of Lb. acidophilus and Bif. longum (treatment B). The changing in moisture content of the manufactured cheeses was studied during ripening period of 0, 14, 28 and 42 days.

<table>
<thead>
<tr>
<th>Ripening Period</th>
<th>Samples</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
<td>40.99</td>
<td>40.12</td>
<td>39.47</td>
<td>39.11</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>41.23</td>
<td>40.20</td>
<td>39.41</td>
<td>39.00</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>41.78</td>
<td>40.60</td>
<td>39.71</td>
<td>38.96</td>
</tr>
</tbody>
</table>

Results showed that moisture content of Monterey cheese (C) at zero time was 40.99% and decreased to 39.11% after 42 days of ripening, while the moisture content of Monterey cheese samples (A and B) at zero time was 41.23%, 41.78% and decreased at the end of ripening period to 39.00%, 38.96% respectively.

The results revealed that there were statistically significant differences at (P<0.01) for the moisture content of the three manufactured pieces of cheese by using different bacterial strains along with the progress of ripening period.

Results show that Monterey cheese (C) had the highest moisture content followed by (A) and (B) samples, respectively. The increasing of moisture content’s losing in sample (B) comparing with the two other samples was due to the high content of microorganisms which have the main role in increasing the acidity and decreasing pH and then to reduce the moisture.

In addition, the moisture content is inversely proportional to the period of cheese ripening, knowing that the temperature of ripening used (16°C) and relative humidity 85% and that hinder the evaporation of water rapidly from ripened cheese as well as decreasing the evaporation of moisture, which is attributed to the wax, as manufactured cheese samples remained within semi-hard cheeses specifications, which including Monterey cheese.

These differences in the moisture content of the manufactured cheeses during ripening periods may be attributed to the heterogeneity of pressing operations of the different treatments as well as for evaporation during ripening.

The results were consistent with Sabikhi et al., (2014) when they manufactured Edam cheese by using probiotic strain Bif. bifidum as they had noticed a reduction in the moisture content, they attributed that for the evaporation process during ripening periods.

Study results were close to Dantas et al., (2016) when they manufactured soft (Minas Frescal) cheese without and with the addition of probiotic strain Lactobacillus casei Zhang, they found decrease in moisture content at the end of ripening period, and attributed the reason of this decrease to the low pH and high titratable acidity due to increasing the activity of probiotic bacteria and consumption of the remaining lactose.

The decline in moisture content’s percentage result in significant changes at (p<0.01) in the other solid components of the manufactured cheeses along with progress of ripening periods and this has led to a gradual increase in the percentages of protein, fat, salt and ash.

Protein content: Table 2 illustrates protein content of Monterey cheese samples C, A and B, which was 23.33, 23.13 and 22.89 % respectively at zero time for the three samples and this ratio increased after 42 days of ripening to reach 23.78, 23.64 and 23.41% respectively. It is noticed increasing protein content of sample B during 42 days of ripening followed by samples A and C respectively. Statistical analysis results of protein content of the three cheese samples showed that there were no significant differences at (p<0.01). The reason was due to decreasing of moisture content, which led to increase protein ratio, these results show that the addition of probiotics had no significant effect on the protein content and this is an indication of the potential use of these probiotic strains in manufacturing of Monterey cheese without any effect on their characteristics.

<table>
<thead>
<tr>
<th>Ripening Period</th>
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<th>0</th>
<th>14</th>
<th>28</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
<td>23.33</td>
<td>23.51</td>
<td>23.65</td>
<td>23.78</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>23.13</td>
<td>23.38</td>
<td>23.57</td>
<td>23.64</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>22.89</td>
<td>23.15</td>
<td>23.35</td>
<td>23.41</td>
</tr>
</tbody>
</table>

The results agreed with de Oliveira et al., (2012) who found that protein content increased during the stages of ripening of five semi-dry cheese (coelho) samples using probiotic strains (Lc. Lactis, Lc. cremoris, Lb. acidophilus LA-5, Lb. paracasei, Bif. bifidum BB-12) to decreasing moisture content percentage along with the progress of ripening periods.
Soluble Protein Content: Table 3 shows soluble protein content of Monterey cheese samples C, A and B, results showed an increase in the soluble protein content of the three manufactured cheese samples with progress of ripening periods, it was at zero time 0.241, 0.245 and 0.248% for cheese samples C, A and B respectively and after 42 days of ripening reached 0.780, 0.853 and 0.875% respectively, it was noticed from the results superiority of sample B followed by the sample A then sample C and for statistical analysis of the three cheese samples for the content of soluble protein, it was observed significant differences at (p<0.01), the reason was due to the ability of added probiotics to secrete protease enzymes, which show the influence in the proteolysis of the cheese, especially that the used probiotic strains of Bifidobacterium and Lactobacilli produce enzymes that hydrolyze protein into amino acids and peptides.

Table 3: Soluble protein content % of three Monterey cheese manufactured samples.

<table>
<thead>
<tr>
<th>Ripening Period Days</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.241</td>
<td>0.503</td>
<td>0.759</td>
<td>0.780</td>
</tr>
<tr>
<td>A</td>
<td>0.245</td>
<td>0.592</td>
<td>0.801</td>
<td>0.853</td>
</tr>
<tr>
<td>B</td>
<td>0.248</td>
<td>0.621</td>
<td>0.843</td>
<td>0.875</td>
</tr>
</tbody>
</table>

These results agreed with Bergamini et al., (2006) during study the effect of adding two probiotic strains (Lb. acidophilus and Lb. paracasei) on the proteolysis of semi-dry cheese, as results showed that the bacteria Lb. acidophilus has a clear impact on the secondary decomposition of protein by increasing the low-molecular weight compounds such as peptides and free amino acids, while Lb. paracasei showed little effect on proteolysis during the ripening period could be attributed to probiotic strains used in manufacturing and the type of cheese.

De Oliveira et al., (2012) also pointed out the reason for the high rate of proteolysis of ripened therapeutic cheese attributed to enzymes already existing in milk as well as the use of mixed cultures of probiotic strains that increase the level of proteolysis and thus leads to increase the production of peptides and free amino acids.

Result agreed with Sabikhi et al., (2014) who manufactured Edam cheese by using therapeutic strains of Bif.bifidum, results showed a significant increase in soluble protein percentage with the progress of ripening periods of 90 days, the reason has been attributed to enhanced probiotic strains used and their ability to decompose by protease enzymes and production of peptides, especially that alpha-casein decomposing more than beta-casein at the end of ripening period.

The results of the study are consistent with what Hong-Xin et al., (2015) have found during manufacturing a typical cheddar cheese without and with the use of probiotic strains Lb. casei LC2W and mixture starter of Lc. lactis, Lc. cremoris and St. thermophilus, they attributed the reason of increasing soluble protein percentage for both samples to the difference of strains in its proteolysis ability and illustrated that Lactobacilli strains produce proteases that proteolyze protein along with the progress of ripening periods.

The results agreed with the findings of Santillo and Albenzio (2015) regarding proteolysis when they studied the effect of adding probiotic strains (Bifidobacterium ssp. and Lactobacillus) when they manufactured three samples of ripened cheese, mixed probiotics overcome on the single probiotic and the control cheese regarding increasing of degradation degree and they attributed the reason to rennet enzymes and probiotic strains used.

Study results agreed with Dantas et al., (2016) when they manufactured two samples of Minas Frescal soft cheese without and with the use of probiotic strain (Lactobacillus casei Zhang), they attributed the increase of soluble protein due to the activity of the added strain.

Fat content: Table 4 shows fat content % of Monterey cheese samples C, A and B which was 30.35 and 30.14 and 29.54% respectively at zero time, and this content increased after 42 days of ripening to 31.20 and 31.03 and 30.45% respectively, it is noticed increasing of fat content of sample B ahead of the other two samples C and A but there were no significant differences at (p<0.01). The reason is due to the variation in moisture content of the manufactured cheese or may be attributed to the lost fat with whey during manufacturing processes or ripening periods.

This result agreed with the result of Dabevska-Kostoska et al., (2015) who found an increase in fat content percentage for two samples of probiotic brined cheese by using Lb. casei strain and they attributed that to decreasing moisture content and increasing of solid components percentages.

Table 4: Fat content % of three Monterey cheese manufactured samples.

<table>
<thead>
<tr>
<th>Ripening Period Days</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>30.35</td>
<td>30.78</td>
<td>31.07</td>
<td>31.20</td>
</tr>
<tr>
<td>A</td>
<td>30.14</td>
<td>30.57</td>
<td>30.87</td>
<td>31.03</td>
</tr>
<tr>
<td>B</td>
<td>29.54</td>
<td>29.96</td>
<td>30.27</td>
<td>30.45</td>
</tr>
</tbody>
</table>

These results agreed with the results of Dantas et al., (2016) when manufactured two samples of Minas Frescal soft cheese without and with the addition of probiotic strain Lactobacillus casei Zhang, they found an increase in the fat content of the two samples at the end of the storage period, they referred the reason of
this increase to decreasing of moisture content and increasing of total solids’ percentage.

Salt content: Table 5 illustrates salt content % of Monterey cheese samples C, A and B, which was 1.30, 1.32 and 1.35% respectively at zero time and reached at the end of ripening period after 42 days to 1.45, 1.53 and 1.55% respectively. Sample B was the highest in salt content comparing with samples A and C, and there were no significant differences for the three samples of cheese at a probability level of (p<0.01), and the reason attributed to decreasing the moisture content and increasing total solid percentage.

Table 5: Salt content % of three Monterey cheese manufactured samples.

<table>
<thead>
<tr>
<th>Ripening Period Samples</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.35</td>
<td>1.39</td>
<td>1.44</td>
<td>1.45</td>
</tr>
<tr>
<td>A</td>
<td>1.32</td>
<td>1.37</td>
<td>1.45</td>
<td>1.53</td>
</tr>
<tr>
<td>B</td>
<td>1.30</td>
<td>1.36</td>
<td>1.43</td>
<td>1.55</td>
</tr>
</tbody>
</table>

This result agreed with Dabevska-Kostoska et al., (2015) who found an increase in salt percentage of two Brined cheese by using Lb. casei strains and they attributed the reason to decreasing moisture content and increasing total solid percentage.

Ash Content: Table 6 shows ash content percent-age of Monterey cheese samples C, A and B, which was 3.60, 3.68 and 3.77% respectively at zero time and increased after 42 days to reach 4.27, 4.20 and 4.16% respectively. It is noticed increasing ash percentage of sample B comparing with samples A and C, but when conducting statistical analysis, there were no significant differences at probability level of (p<0.01), and attributed the reason of increasing ash content to decreasing moisture content along with the progress of ripening and increasing of total solid percentage.

Table 6: Ash content % of three Monterey cheese manufactured samples.

<table>
<thead>
<tr>
<th>Ripening Period Samples</th>
<th>0</th>
<th>14</th>
<th>28</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>3.77</td>
<td>3.95</td>
<td>4.10</td>
<td>4.16</td>
</tr>
<tr>
<td>A</td>
<td>3.68</td>
<td>3.90</td>
<td>4.11</td>
<td>4.20</td>
</tr>
<tr>
<td>B</td>
<td>3.60</td>
<td>3.85</td>
<td>4.09</td>
<td>4.27</td>
</tr>
</tbody>
</table>

Abd El-Gawad et al., (2011) assured that the reason of the continuing decreasing in moisture of waxed cheeses refers to the evaporation during the ripening period, which affects the equilibrium of other components, including ash.

Conclusion
The results of this study indicated that the addition of probiotics did not negatively influence the ripening process of Monterey cheese and its chemical composition. Therefore, Lb. acidophilus alone or gathering with Bif. longum is a suitable adjunct that could complement the normal Monterey cheese starter to enhance the probiotic attributes of the cheese. Monterey cheese can be an effect-ive vehicle for the delivery of probiotic Lb. acidophilus and Bif. longum to the consumers with the same characteristics of Monterey cheese.

REFERENCES


