Influence of probiotic on microbiological quality of kariesh cheese

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Abstract: Thirty random milk samples were collected from dairy farms at Sharkia Governorate; each sample was divided into two parts. The first part was used as control while the second one was inoculated with active culture of two probiotic strains (Lactobacillus casei (ATCC- 334) and Lactobacillus plantarum (ATCC-8014)) in ratio 2% of milk. Both parts were manufactured into Kariesh cheese followed by microbiological analysis. The results indicated that out of examined control karish cheese samples 30(100%) were showed bacterial growth, the minimum was 1.0×10⁶ cfu/gm, the maximum was 8.8×10¹⁰ cfu/gm and the mean value was 7.86×10⁸±3.05×10⁶ cfu/gm. While fresh kariesh cheese with probiotics showed that count was ranged from 9.0×10⁶ to 3.0×10⁷ with a mean value of 6.42×10⁶±1.29×10⁶. There was significance difference between two groups (P<0.05). All examined control kariesh cheese samples were contaminated with Coliforms while only 24(80%) of examined kariesh samples with probiotics were harbor Coliforms and the count were ranged from 2.0×10³ and 2.0×10⁵ to 1.9×10⁵ and 1.2×10⁵ with a mean value of 4.66×10³±9.3×10² and 1.47×10⁴±4.6×10³ for control samples and samples with probiotics respectively Staphylococcus aureus count in control and kariesh samples with probiotics. All control kariesh cheese samples were positive for Staphylococcus aureus, the count was ranged from 1.0×10⁴ to 2.9×10⁵ and the mean value was 4.2×10⁵±1.2×10⁵. Out of examined kariesh cheese samples with probiotics 21(70%) were contaminated with Staphylococcus aureus. The high level of contamination was 8.0×10⁵, the low level was 2.0×10⁴ and the mean value was 9.4×10⁴±3.7×10⁴. There was significance difference between two groups (P<0.05). 20% of examined control cheese samples contained Coagulase positive Staphylococcus aureus while none of kariesh cheese with probiotics was contaminated by such organism. Yersinia enterocolitica failed to be detected in neither control samples nor karish samples with probiotics. The obtained results indicated that kariesh cheese is of inferior quality and hazardous food, as it is considered as source of food borne illness and addition of probiotics leads to significance decrease in total bacterial and staphylococcus counts also coagulase positive Staphylococcus aureus failed to be detected in kariesh samples with probiotics. So there is a great need for rising up, developing and spreading the use of probiotics where karish cheese is made as a natural and gentle way to preserve food, with a view to improving the quality, hygiene and safety of food.

Keywords: Probiotics; kariesh cheese; microorganisms

1. Introduction
Kariesh cheese is one of the most popular local types of fresh soft cheese of the Egyptian cities. The increasing demand for it by Egyptian consumers is mainly attributed to its high protein content and low price. Kariesh cheese is made from defatted or skims cow or buffalo milk, or a mixture of both. Cow or buffalo are milked directly into special earthenware pots, which are kept undisturbed in a suitable place to allow the fat to rise to the surface forming a cream layer and the partly skimmed milk sours and clots. Then the cream layer is removed, and the curd is poured onto a mat which is tied and hung with its contents, to allow the whey drain until the desired texture of the cheese is obtained. Finally, the cheese is cut into suitable pieces, and salted then it is ready to be consumed as fresh cheese. The shelf-life of the fresh cheese is between one and two weeks (Abou-Donia, 1984. and Aldo, et al., 2013). It is generally made of raw skimmed buffaloes’ or cow’s milk which is often of poor bacteriological quality owing to the high microbial load present in raw milk and the objectionable condition under which it was produced (Salwa et al., 2012). Kariesh cheese contains amount of sugar, some water, soluble vitamins and most of calcium and phosphorus. The quality and composition of kariesh cheese may vary considerably due to such factors as the quality and composition of the clotted skimmed milk, the method of manufacture, the time required to complete the whey drain, the quality of salt added and the method of handling finished cheese (Aldo et al., 2013).

A recent trend in cheese manufacture is production of natural flavoured cheese made in short time with highly nutritive value and good microbiological quality as for human consumption. (Hosny et al., 2011) Kariesh cheese is sold uncovered and without container where the risk of contamination...
is high so it is considered as a good medium for the growth of different types of spoilage and pathogenic microorganisms.(Dawood et al., 2006).

Probiotic may reduce the incidence of disease or decrease the severity of disease outbreaks. Probiotics are defined as live microorganisms that administered in adequate amount produce a health benefits to the host. (Reid et al., 2003). The mechanism used include the production of inhibitory substances against pathogens, competition for essential nutrients and adhesion sites, the supply of essential nutrients and enzymes and the modulation of interaction with the environment and the development of beneficial immune response. (Gomez and Balcazar, 2008). Several visions have been proposed to improve kariesh cheese including therapeutic or probiotic effects of kariesh cheese by using cultures containing Bifidobacterium sp. (Abd-Elhamid, 2012). Recently there has been significant commercial interest in using lactic acid bacteria and propionibacteria as natural food preservative to enhance food safety and stability as anti microbial systems possessed by these bacteria offer potential food effective natural preservation methods. (Effat, 2000). Lactobacilli have the longest history as biotherapeutic agents (probiotics) and are still the most common ingredients among those intended for consumption by farm animals. This choice of probiotic bacteria seems appropriate because the normal gastrointestinal microbiota of these animals is particularly rich in lactobacilli (Tannock, 1997).

2. Material and Methods

Materials:
1- Milk samples
Thirty random milk samples were collected from dairy farms at Sharkia Governorate; each sample was divided into two parts.

2-Probiotic strains
Two probiotic strains Lactobacillus casei (ATCC-334) and Lactobacillus plantarum (ATCC-8014).

Methods:
1- Culture preparation:
The two probiotic strains Lactobacillus casei (ATCC- 334) and Lactobacillus plantarum (ATCC- 8014) were grown in MRS broth (biovita) at 30°C for 48hours in anaerobic condition (Zambou et al., 2004)

2- Kariesh cheese making
The first part was used as control while the second one was inoculated with active culture of two probiotic strains in ratio 2% of milk. Both parts were manufactured into Kariesh cheese according to method adopted by Fahmi, (1960).

3- Cheese analysis
25 gram cheese was homogenized for one minute with 225ml sodium citrate (2%). Total plate count on plate count agar (Oxoid), Total Coliform on violet red bile agar (Oxoid), Staphylococcus aureus on Baird Parker medium (Oxoid) and Yersinia enterocolitica on CIN medium (Oxoid). Methods of microbiological analysis were conducted according to APHA, (1992).

3. Results and Discussion
Concerning to total colony count listed in table (1) out of examined control kariesh cheese samples 30(100%) were showed bacterial growth, the minimum was $1.0×10^6$ cfu/gm, the maximum was $8.8×10^{10}$ cfu/gm and the mean value was $7.86×10^6±3.05×10^9$ cfu/gm. While fresh kariesh cheese with probiotics showed that count was ranged from $9.0×10^5$ to $3.0×10^7$ with a mean value of $6.42×10^6±1.29×10^6$. There was significance difference between two groups ($P<0.05$). These results were in agreement with that reported by Abou Dawood et al. (2005) and Hosny et al. (2011).

Table (2) showed that all examined control kariesh cheese samples were contaminated with Coliforms while only 24(80%) of examined kariesh samples with probiotics were harbor Coliforms and the count were ranged from $2.0×10^3$ and $2.0×10^2$ to $1.9×10^8$ and $1.2×10^5$ with a mean value of $4.66×10^4±9.3×10^3$ and $1.47×10^4±4.6×10^5$ for control samples and samples with probiotics respectively. Slightly higher counts were reported by Hosny et al. (2011) while Ahmed, (1988) recorded nearly similar counts. There was no difference between two groups ($P>0.05$). E. coli can become acid resistant and survive acid extremes similar to the human gastric barrier (Benjamin and Datta, 1995). Sindt, et al. (2002) concluded that E. coli and total coliforms provides inclusive information that encompasses several pathogenic strains of bacteria and is an appropriate model to determine reduction strategies for controlling acid-resistant, pathogenic, enteric bacteria.

Table (3) summarized Staphylococcus aureus count in control and kariesh samples with probiotics. All control kariesh cheese samples were positive for Staphylococcus aureus, the count was ranged from $1.0×10^5$ to $2.9×10^7$ and the mean value was $4.2×10^5±1.2×10^6$. Out of examined kariesh cheese samples with probiotics 21(70%) were contaminated with Staphylococcus aureus, The high level of contamination was $8.0×10^5$, the low level was $2.0×10^3$ and the mean value was $9.4×10^4±3.7×10^4$. There was significance difference between two group ($P<0.05$).

These findings were nearly similar to those reported by Abou Dawood et al. (2005) while Kaldes, (1997) and Hosny et al. (2011) reported lower counts.
Table (1): Total colony count of kariesh cheese samples (n=30).

<table>
<thead>
<tr>
<th>Positive samples</th>
<th>Total colony count</th>
<th>Mean ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>%</td>
<td>Minimum</td>
</tr>
<tr>
<td>Control group</td>
<td>30</td>
<td>100%</td>
</tr>
<tr>
<td>Fresh kariesh cheese with probiotics</td>
<td>30</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table (2): Coliforms count of Kariesh cheese samples (n=30).

<table>
<thead>
<tr>
<th>Positive samples</th>
<th>Coliforms count</th>
<th>Mean ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>%</td>
<td>Minimum</td>
</tr>
<tr>
<td>Control group</td>
<td>30</td>
<td>100%</td>
</tr>
<tr>
<td>Fresh kariesh cheese with probiotics</td>
<td>24</td>
<td>80%</td>
</tr>
</tbody>
</table>

Table (3): Staphylococcus aureus count of kariesh cheese samples (n=30).

<table>
<thead>
<tr>
<th>Positive samples</th>
<th>Staphylococcus aureus count</th>
<th>Mean ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>%</td>
<td>Minimum</td>
</tr>
<tr>
<td>Control group</td>
<td>30</td>
<td>100%</td>
</tr>
<tr>
<td>Fresh kariesh cheese with probiotics</td>
<td>21</td>
<td>70%</td>
</tr>
</tbody>
</table>

Table (4): Incidence of coagulase positive Staphylococcus aureus in examined kariesh cheese samples (n=30).

<table>
<thead>
<tr>
<th>Coagulase positive Staph. aureus</th>
<th>Positive samples</th>
<th>Mean ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>6.0</td>
<td>20.0%</td>
</tr>
<tr>
<td>Fresh kariesh cheese with probiotics</td>
<td>0.0</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

The results presented in table (4) stated that 20% of examined control cheese samples contained Coagulase positive *Staphylococcus aureus* while none of kariesh cheese with probiotics was contaminated by such organism. *Yersinia enterocolitica* failed to be detected in neither control samples nor Kariesh samples with probiotics. El-Gamal et al. (2013) failed to detect Yersinia enterocolitica from examined cheese and yoghurt samples. The Egyptian standards for kariesh cheese did not mention an account on the acceptable level of total bacterial count but indicated that *Coliforms* are less than 10cfu/gm and absence of coagulase positive *Staphylococcus aureus*. *Lactobacillus* spp. showed a broad inhibitory spectrum against *Staphylococcus aureus* and *E. coli* and the inhibitory substance of certain lactobacilli were bacteriocin and lactic acid. (Ryan et al., 2008 and Amin et al., 2009). The obtained results indicated that kariesh cheese is of inferior quality and hazardous food, as it is considered as source of food borne illness and addition of probiotics leads to significance decrease in total bacterial and staphylococcus counts also coagulase positive *Staphylococcus aureus* failed to be detected in kariesh samples with probiotics. So there is a great need for rising up, developing and spreading the use of probiotics where kariesh cheese is made as a natural and gentle way to preserve food, with a view to improving the quality, hygiene and safety of food.

References: