Detection about fungal contamination in products milk in local and manufacturing yogurt and cheese in Al-Diwaniya city

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**ABSTRACT**

This study included random examination of milk products such as yogurt and cheese in both types the local and manufacturing milk product, to detect the fungal contamination in these products. Yogurt was a well source of essential nutrients such as protein, essential minerals (Ca, P, Mg, Zn, K) and vitamins such as B1, B9, B2 B12, B3. Yogurt increase the body product of cytokine, activity of phagocytic cell, antibodies, T-cell and natural killer cell, also it induced immune system against other diseases such as cancer, gastrointestinal and allergic syndrome. Caseins are the main protein in cheese; which exist in the form of aggregates after combination with colloidal calcium phosphate common knowledge as micelles of casein. Caseins in cheese are nutritionally rich due to the high supply of essential amino acids, phosphate and calcium. Samples taken from market of al-Diwaniya city about ten samples for each type, these samples cultured on SDA by use dilution method and after incubated found several types of fungi were appeared, such as Candida albicans the general average for it cell in 1 ml about $2.7 \times 10^2$ (cell/ml) in samples of local yogurt, $7.6 \times 10^1$ (cell/ml) in samples of manufacturing yogurt, in cheese; about $1.8 \times 10^1$ (cell/ml) in samples of local cheese and $1.3 \times 10^0$ in manufacturing cheese. The fungi also appeared in samples such as Penicillium notatum, Aspergillus flavus, Fusarium oxysporium, Trichoderma harzianum, the higher frequency rate was recorded in samples of local yogurt about 13.7%, 25.4%, 19.6%, 11.3% respectively.

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**INTRODUCTION**

Milk and dairy products are a permanent part of our everyday life can be affected by fungal spoilage in a variety of ways. Yoghurt is produced by fermenting of lactic acid of milk by addition of primary culture containing *Streptococcus thermophilus* and *Lactobacillus Bulgaricus* (Tamime, 2002). Yogurt was a well source of essential nutrients such as portion, Ca, P, Mg, Zn, K and vitamins B1, B9, B2, B12, B3 (Barr et al., 2000). Studies appeared that yogurt amortize, increase the body product of cytokine, activity of phagocytic cell, antibodies, T-cell and natural killer cell, also yogurt induced immune system against other diseases such as cancer, gastrointestinal and allergic syndrome (Meydani and Ha, 2000; Isolauri et al., 2002). The other product cheese presented a good origin for major nutrients, such as proteins, lipids, minerals and vitamins, it was a good source for peptides and amino acids which play antimicrobial and anti-carcinogenic roles, cheese
was made from milk of several animals such as cows, buffaloes, sheep, and it was significant food component in useful feed of American, Asian and European (Ash and Wilby, 2010).

Among the milk proteins, caseins are the main protein in cheese; which exist in the form of aggregates after combination with colloidal calcium phosphate common knowledge as micelles of casein. Caseins in cheese are nutritionally rich due to the high supply of essential amino acids, phosphate and calcium (Farrell et al., 2004).

Lactoferrin is the multifunctional glycoprotein existed in the cheese, these proteins have an important roles include host defense against a variety of microorganism, iron homeostasis, anticancer, antiviral and anti-inflammatory activity (Elbarbary et al., 2010). Cheese can produce BPs not only during ripening process but also during the digestion of cheese in gastrointestinal tract (Parrot et al., 2003).

These BPs have a favorable effect on the human health and their activity is based on their composition of amino acid and ability to reach the target site (Ángel Gómez-Ruiz et al., 2002). Milk Fat Globule Membrane MFGM is found in significant quantities in various cheese which is rich in proteins, glycoproteins, fats such as sphingolipids, glycerophospholipids, and enzymes (Lopez, 2010). Salt plays a very important role in the process of making cheese, it had a preservatives function in cheese by preventing the increase of spoilage bacteria (Adams and Moss, 2007).

Mycotoxins can define a group of toxins produced by fungi or yeast as secondary metabolites, fungi and yeast developed in places with little water availability and unsuitable for growth of bacteria (Jouany et al., 2001). The bad methods of preparation and storage of animal feedstuffs, such as grain and silage, can increase mycotoxin contamination (Bryden, 2012). When animal eat contaminated feedstuffs, mycotoxins were metabolized, biotransformed, and transferred to animal products such as milk or meat, so becoming a hazard to health of human (Bruerton, 2001).

The worry form mycotoxin contamination in dairy products began in the 1960 when reported the first cases of containerization by aflatoxin M1(AFM1) and aflatoxin B1, by eat contaminated feedstuff (Creppy, 2002).

The main objective of the current study is to know the extent of fungal contamination of dairy products and to compare the amount of fungi contaminated between L products with hand-made dairy products with those packaged in special laboratories.

MATERIALS AND METHODS

Samples of cheese and yogurt were collected from the market of Al-Diwaniya city from random places, about 10 samples for each other. The cultivation media is sabouraud dextrose agar (SDA). The method of culture was; scrapping of cheese surface and 1gm of scrapping cheese was taken for isolation the contaminated fungi and solved in 9.0 ml sterile water and mixed well then make serial dilutions from 10-1 to 10-5. One ml of each last three dilution (10-3,10- 4 and 10-5) was put into petri dish then poured melted SDA medium and mixed well then left to solidify. Then these petri dishes were incubated in incubator at 25°C for 5-7 days. After incubated several colonies growth on medium (American Public Health Association, 2017).

Identification of fungi was depended on the visible notice of fungal colony such as morphological characteristics of colonies, the vegetative and reproduction strictness observed using a light microscope with oil objective glass of 100x. the following taxonomic keys were used (Gandomi et al., 2009; Balajee et al., 2007).

RESULTS AND DISCUSSION

After the end of incubation period of the dishes for milk product as cheese and yogurt, result showed they included types of innate serious such as Candida albicans, in Table 1 results showed the general average of cell by ml was about; 2.7×10^4 in local yogurt, 7.6×10^4 in manufacturing yogurt, 1.8×10^4 in local cheese and 1.3×10^4 in manufacturing cheese. Candida albicans identified by the characteristics of the dishes and under the microscope, smooth colonies on the agar surface, the whole colonies consist of blastospore, colonies of candida contained 6% hyphae and pseudohyphae, and the blastopores is a diploid. Imperfect yeast capable at yeast to hyphae transition induced by environmental factors in various cheeses (Stelzner, 1990; Scherer and Magee, 1990). Yeasts are nonfilamentous, unicellular, circular to oval shape and are generally facultative anaerobe (Pal, 2007). Most causes of yogurt spoilage was yeast in first (Suriyarachchi and Fleet, 1981). In yogurt samples many species of fungi appear showmen in Table 2, such as Penicilliumnotatum, Aspergillus flavus, Fusarium oxysporum and Trichoderma harzianum that showed highest frequency rate in local yogurt as about 13.7%, 25.4%, 19.6%, 11.7% respectively.

Since been diagnosed Aspergillus flavus by several features such as conidial heads radiate or vary loosely columnar, color of colonies brownish coni-
### Table 1: General average for enumeration *Candida albicans* in yogurt and cheese

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Number of sample</th>
<th>Yeast (cell/ml)</th>
<th>General average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>The minimum rate</td>
<td>The maximum rate</td>
</tr>
<tr>
<td>Local yogurt</td>
<td>10</td>
<td>$2.1 \times 10^2$</td>
<td>$3.4 \times 10^2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$1.2 \times 10^2$</td>
<td>$1.4 \times 10^2$</td>
</tr>
<tr>
<td>Manufacturing yogurt</td>
<td>10</td>
<td>$1.6 \times 10$</td>
<td>$2 \times 10$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$1.2 \times 10$</td>
<td>$3 \times 10$</td>
</tr>
<tr>
<td>Local cheese</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manufacturing cheese</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: The frequency rate of fungi in yogurt sample

<table>
<thead>
<tr>
<th>Fungi types</th>
<th>Frequency rate in local yogurt</th>
<th>Frequency rate in manufacturing yogurt</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Penicillium notatum</em></td>
<td>13.7</td>
<td>9</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>25.4</td>
<td>3.5</td>
</tr>
<tr>
<td><em>Fusarium oxysporium</em></td>
<td>19.6</td>
<td>6.8</td>
</tr>
<tr>
<td><em>Trichoderma harzianum</em></td>
<td>11.7</td>
<td>4.5</td>
</tr>
</tbody>
</table>

### Table 3: The frequency rate of fungi in cheese sample

<table>
<thead>
<tr>
<th>Types of fungi</th>
<th>Frequency rate in local cheese %</th>
<th>Frequency rate in manufacturing cheese %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Penicillium notatum</em></td>
<td>19.2</td>
<td>10.7</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>10.8</td>
<td>3.5</td>
</tr>
<tr>
<td><em>Fusarium oxysporium</em></td>
<td>8.6</td>
<td>2.3</td>
</tr>
<tr>
<td><em>Trichoderma harzianum</em></td>
<td>4.3</td>
<td>4.1</td>
</tr>
</tbody>
</table>

dia smooth to roughened, conidiophores arising primarily from the substrate (*Kwon-Chung et al.*, 1998). Dangerous of *Aspergillus flavus* become from produced four types of aflatoxins B1, B2, G1 and G2, the aflatoxin B1 usually found at highest concentration in food and feed contaminated with fungi (*Sweeney*, 1998).

These results agree with *Tsai et al.* (1988) that fungi found in the surface of yogurt causes change the colour and taste of it such as *Aspergillus sp, Mucorsp, Penicilliumsp*; and agree with *De et al.* (2014) they isolated fungi Aspergillus sp, Mucor sp and Penicilliumsp from the yogurt.

*Fusarium oxysporium* its features were macroconidia spherical to elongated, slightly, curved unicellular, hyaline, swollen at the base and pointed at the tip usually septate (*Brayford*, 1996).

In cheese samples the frequency rate of fungi was different Table 3 as about 19.2%, 10.8%, 8.6% and 4.3% for fungi *Penicillium notatum, Aspergillus flavus, Fusarium oxysporium* and *Trichoderma harzianum*. Respectively in local cheese while in manufacturing cheese about 10.7%, 3.5%, 2.3% and 4.1% respectively.

These results agree with *Kure et al.* (2001) that they isolated *Trichoderma harzianum* from cheese (*Sambrook*, 1989).

*Penicillium notatum* was recognized by it features, its colonies on SDA surface were flat, filamentous, and texture was velvety, wooly and cottony in texture on SDA medium. The colonies initially white and become blue green, gray green, olive gray, yellow or pinkish in time. Visualized as globose to elongated sausage-shaped cells that multiply by fission (*Nicoletti et al.*, 2008). Many authors isolated *Penicilliumsp* from cheese such as *Kure et al.* (2001).

### CONCLUSION

From the results above become clear that the local milk products carried high ratio of yeasts and fungi comparative with manufacturing milk products which carried lower ratio, this signifies the bad steps in manufacturing and storage of local products. So it have to control the Safety conditions seri-
ously in local factories.

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Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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