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Full Length Research Paper

Occurrence and identification of yeast species isolated from Egyptian Karish cheese

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This study aims at identifying the diversity and abundance of yeast associated with Egyptian Karish cheese, employing comparison between conventional laboratory techniques and API20 kits techniques in yeast identification. A total of one hundred samples (fifty each) of Egyptian raw and pasteurized Karish cheese milk were randomly collected from farmers and markets in Cairo and Giza Districts. The occurrence of yeast in raw and pasteurized Karish cheese milk were 100 and 38% with a mean value of 7 ± 1.1 l and 1 ± 0.31 $\log_{10}$ cfu g^{-1}, respectively. Yeast strains isolated from both raw and pasteurized karish cheese samples were identified and characterized using both conventional methods and API 20 C AUX as a commercial identification system. The most prevalent isolates belonged to *Trichosporon cutaneum* (25%), *Candida catenulata* (23%), *Yarrowia lipolytica* (13%), *Debaryomyces hansenii* (13%), *Kluyveromyces lactis* (6%), *Geotrichum candidum* (7%), *Candida zeylanoides* (5%), *Candida lambica* (3%), *Candida albicans* (2%), *Cryptococcus formans* (1%), *Rhodotorula glabrata* (1%) and *Saccharomyces cerevisiae* (1%). There was no significant difference (P 0.05) between the conventional method and the API20 kits test. However, the results of this study reveal that API 20 kits are simple, highly useful and are commercially available kits that considerably shorten the time required for the identification of yeast in cheese.

Key words: Yeast identification, Karish cheese, API 20 kits.

INTRODUCTION

Karish cheese is one of the most popular local types of fresh soft cheese in Egypt. The increasing demand by Egyptian consumers is mainly attributed to its high protein content and low price (Osman et al., 2010). Karish cheese is traditionally made from skim cow or buffalo's milk which is extracted directly into special earthenware pots known as (shalia) and kept undisturbed in a suitable place to allow the fat to rise to the surface forming a cream layer. 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 drainage of the whey. This process of squeezing takes two or three days until the desired texture of the cheese is obtained. Finally, the cheese is cut into suitable pieces and salted cheese is left for a few hours in the mat till whey no longer drains out, then it is ready to be consumed as fresh soft cheese (Ojokoh, 1998).

This traditional method affords many opportunities for microbial contamination. It is generally made from raw milk often of poor bacteriological quality and produced under unsatisfactory conditions. Also, this product is sold uncovered without a container, thus the risk of contamination is very high. Therefore, it can be considered as a good medium for the growth of different types of spoilage and pathogenic microorganisms (Yousef, 2007; Dawood et al., 2009).

It is widely recognized that yeasts can be an important component of the microflora of many cheese varieties because of the low pH, low moisture content, high salt concentration and refrigerated storage of these products (Devoyod, 2008). Nevertheless, yeasts play a dual role depending on the cheese. In fact, in some cheese types they make a positive contribution to the development of flavor and texture during the stage of maturation, while in other varieties, yeasts can be regarded as spoilage organisms. Yeast spoilage is recognized as a problem primarily in fermented milk and cheese (Brocklehurst and Lund, 1985; Fleet, 1990).

The sources of these yeast infections are located along the whole chain of production from the farm to the final

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product. The main defects caused by spoilage yeasts are fruity, bitter or yeasty off -flavors, gas production, discolorration changes and texture. Assessment of cheese spoilage by yeasts is complicated by subjective judgments on whether yeast activity during maturation is detrimental or beneficial to product quality. The main mechanisms by which yeast growth influences the final quality of cheese are: fermentation of lactose, utilization of lactic acid and lipolytic and proteolytic activities (Tudor and Board, 2010). Particularly, over-ripening during maturation could be interpreted as spoilage. In fact, continued lactose fermentation could lead to increased acidity, gassiness and fruity flavors, while continued hydrolysis of protein and fat could contribute to bitter and rancid flavors as well as a softening of product texture. However, the use of yeasts in dairy industry could determine potential advantages including production of flavor components and acceleration of ripening by means of its lipolytic and proteolytic properties, fermentation of lactose, assimilation of lactate and positive interactions with the primary starter cultures (Rohm et al., 2010).

Yeasts in some cheese types can periodically cause both economic and public health problems. Yeasts themselves are not commonly the cause of defects in cheese unless they ferment lactose. In this case, they can grow rapidly and produce a characteristic yeasty or fruity flavor and obvious gas (Dennis and Buhagiar, 2007; Dillon and Board, 2008; Daryaei et al., 2010). There are numerous references concerning the significance of the presence of yeasts in dairy products, where they may contribute positively to the characteristic taste and flavor development during the stage of maturation or, on the contrary, may lead to product spoilage (Ebrahim, 2008; Mahmoud, 2009).

In Egypt, the information about the involvement of Karish cheese in human illness and economic losses are unknown. Therefore, this study was designed to cover the following items: (1) Enumeration of the yeast popula-tions in raw and pasteurized Karish cheese milk samples, and (2) Isolation and identification of the yeast species, using both conventional method and commercial identification system API 20 Aux test.

MATERIALS AND METHODS

Collection of samples

One hundred (fifty each) raw and pasteurized Karish cheese milk samples were obtained from retail farmers and supermarkets in Cairo and Giza governorates. Each cheese sample was represented by one whole cheese (500 g). All samples were transported to the laboratory under refrigeration and analyzed on arrival for both sensory and chemical examination as well as isolation and identification of yeast.

Sensory examination

Karish cheese samples were scored using a score card for flavor (50 points), body and texture (35 points), and appearance and color (15 points). The scores were averaged by five well trained panelists of stuff members from the Food hygiene department, Faculty of veterinary medicine, Cairo University, according to the methodologies of Nelson and Trout (1981).

Chemical analysis

All Karish cheese samples were chemically examined for pH using a pH meter (model SA 720) . Moisture and salt content were applied according to AOAC (2003).

Isolation of yeast according to the method of Van der Walt and Yarrow (2009)

Ten grams of the product were taken from the inner part of the cheese, diluted in 90 ml of sterile solution of 2% (w/v) sodium citrate (Sigma, St. Louis, MO, USA) and homogenized in a Stomacher (PBI, Milan Italy) for 30 s. For all samples, ten fold serial dilutions were prepared in a sterile solution of 2% (w/v) sodium citrate and the numbers of yeasts were determined by surface plating on yeast potato dextrose agar (PDA) (Microbiol, Cagliari, Italy) with chloramphenicol (0.01%) (Microbiol), after incubation at 25°C for 5 days. All samples were prepared and analyzed in duplicate. Yeast colonies were sorted on the basis of their morphology (smoothness of surface, regularity of border, consistency, color, etc.), streaked to single colonies on yeast potato dextrose agar media (1% yeast extract, 2% dextrose, 2% peptone and 1.5% agar), incubated for 5 days at 25°C and checked for purity. Counts for each individual type of colony were made in order to estimate the relative occurrence of the various yeasts present in the samples. Yeast species counts were calculated as number of colony forming units per gram of sample and were reported as $\log_{10}$ cfu g^{-1}.

Identification of the isolated strains

The isolates were identified using the conventional tests and were checked using the API 20 Aux kits (bioMerieux, Rome, Italy).

API 20 C method according to Dolan and Woodward (2007)

As recommended by the manufacturer, each isolate was subcultured prior to testing, to ensure viability and purity. Yeast inoculum suspensions were prepared from 48 h cultures grown on sabouraud dextrose agar plates at 30°C. Yeast cells were suspended in 2 ml of RapID Yeast Plus Inoculation Fluid to achieve a turbidity which completely obliterate the black lines of the inoculation card supplied with the kits. Each yeast suspension was dispensed into a RapID Yeast Plus panel, and the panels were then incubated for 4 h at 30°C. Immediately after the incubation time, RapID Yeast Plus Reagents A and B were added to the designated cavities and color reactions were evaluated by following the manufacturer's directions. A six-digit microcode was derived and compared to the codes in the RapID Yeast Plus Code Compendium for the identification of the isolate. All microcodes were also sent to the manufacturer for confirmation. Molten (50°C) API basal medium ampoules were inoculated with yeast colonies, and the suspension was standardized to a density below 1+ (lines can be clearly distinguished) on a Wickerham card. Each cupule was inoculated, and the trays were incubated for 72 h at 30°C. Cupules showing turbidity significantly heavier than that of the negative control cupule (0 cupule) were considered positive. Identification was made by generating a microcode and using the API 20C Analytical Profile

Table 1. Statistical analytical results of raw and pasteurized Karish cheese milk samples based on sensory examination (50 samples each).

Cheese samples	Organoleptic scores			
	Flavo (50)	Body and texture (35)	Appearance and color (15)	Total scores (100)
Raw milk	49±0.01	34±0.08	15±0.0.06	98
Pasteurized milk	44±0.01	34±0.04	14±0.05	92

Table 2. The mean chemical composition of the examined Karish cheese samples.

Cheese samples	Moisture (%)	Salt (%)	pH
Raw milk	77.4 ±0.049	1.28±0.03	4.16±0.8
Pasteurized milk	60.0±0.08	1.27±0.03	4.20±0.01

Index or Voice Response System (for profiles not found in the index).

Conventional method according to Kurtzman and Fell (2000)

Tubes were read after 24 and 48 h and again after 10 days for evidence of gas production, which indicated fermentation of the carbohydrate substrate.

Analysis of data according to Ott (2009)

The results are presented as mean and standard errors. The analysis of variance (ANOVA) test was conducted to test the possible significance (P 0.05) among mean values of sensory, chemical and yeast count using Fishers Least Significance Difference (LSD).

RESULTS AND DISCUSSION

The data illustrated in Table 1 show the total score of raw Karish cheese milk in comparison with pasteurized milk samples. There was a significant difference (P>0.05) between raw and pasteurized Karish cheese samples. Practically, similar findings were reported by Yousef et al. (2001), Hamam (2005) and El -Batawy (2009). The flavor of raw Karish cheese milk had the highest total score compared to pasteurized cheese samples. This may be due to the natural flora initially present in raw milk which participates in flavor production (Brocklehurst and Lund, 1985).

As shown in Table 2, the mean chemical composition of raw and pasteurized milk cheese samples had a mean moisture content of 77.4 ± 0.04, while the pasteurized milk samples had 60.0 ± 0.08. There was no significant difference (P>0.05) between raw and pasteurized samples. The moisture content of pasteurized cheese samples was lower than raw cheese samples. This may be attributed to the effect of heat treatment on the capacity of the cheese protein to hold water (Shabatai, 2010). However, almost similar findings were reported by Abd El- Salam et al. (2003) and Ghosh et al. (2006). Higher results were recorded by Kanka et al. (2007), Schaffer et al. (2008) and El-Batawy (2009). The mean salt content and pH value in both raw and pasteurized Karish cheese samples were 1.28±0.03 and 1.27±0.03, and 4.16±0.8 and 4.20±0.01, respectively. There was no significant difference (P>0.05) between pH and salt content in both raw and pasteurized cheese samples. Nearly similar findings were obtained by Abd El-Salam et al. (2003), Lalaguna (2008), and Omer and Elshirbiny (2003).

The composition of most cheese falls within certain compositional ranges. Moisture and salt content are considered the most important compositional factors (Fox and McSweeney, 2009*)*. The higher moisture means more potential for off flavors, which result in many soluble breakdown products of acids, sugars, proteins and lipids (Ceylan et al., 2003; El-Sharoud et al., 2009*)*. There was no regulation concerning the addition of sodium chloride to this product and the amount of salt added depended on the cheese markers themselves. The apparent variation among the chemical examinations of examined raw and pasteurized cheese samples was due to the fact that this type of cheese relied upon individual dairies. Thus, there is no standard production process and thus there is variation in the composition and properties of the milk processed (Turkoglu et al., 2007).

Data depicted in Table 3 revealed that the highest total yeast count was found in raw Karish cheese milk samples (100%), followed by 38% in pasteurized Karish cheese milk samples with a mean log of 7.89±1.1 and 1.00±0.31, respectively. There was a significant difference (P>0.05) between raw and heat treated Karish cheese milk samples, although nearly similar findings were reported by Ahmed et al. (2008), Devoyod (2008), Salwa et al. (2008) and Qing et al. (2010). Higher results were obtained by Kaldes et al. (2006), while lower results were obtained by Brocklehurst and Lund (1985), Elkohly (2001) and Said et al. (2009). The Egyptian Standards (2005) specify that the total yeast count does not exceed 10 cfu g^{-1} detected in the cheese. The International Commission on Microbiological Specifications for Foods

Table 3. Statistical analysis based on total yeast count of the examined Karish cheese samples (50 samples each).

Cheese samples	Total no.	No. of positive sample	Percentage	Mean log.
Raw milk	50	50	100	$7.89^{a} \pm 1.1$
Pasteurized milk	50	19	38	$1.00^{b} \pm 0.31$

Table 4. Incidence of the isolated yeast strains using conventional method and APi 20 C Aux.

Isolated yeast species	API20 Aux	Conventional method
	Percentage of correctly identified strains	Percentage of correctly identified strains
Trichosporon cutaneoum	25	25
Candida catenulata	23	23
Yarrowia lipolytica	13	13
Debaryomyces hansenii	13	13
Kluyveromyces lactis	6	6
Geotricum candidum	7	6
Candida zeylanoides	5	5
Candida lambica	3	3
Candida albicans	2	2
Cryptococcus formans	1	1
Saccharomyces cerevisiae	1	1
Rhodotorula glabrata	1	1

(2005) has classified cheese as a high risk potential hazard. A high yeast count often indicates neglected hygienic measures during production and handling, contamination of raw material, unsatisfactory sanitation, or unsuitable time and temperature during storage and/or production. It may also refer to the suitable pH of cheese for yeast growth as well their wide distribution in the environment (Aponte et al., 2010).

From the obtained results, it is obvious that most of the examined raw milk of Karish cheese samples failed to conform to the Egyptian standard (2005) as they exceeded the accepted level. The Egyptian standards for Karish cheese have proposed a limit for the total yeast count to be less than 10 g^{-1}. The high incidence in the examined samples may be attributed to poor sanitation during preparation and or storage of the product. There are numerous sources of yeast contamination. These include the use of contaminated milk, the observed dirty premises and utensils used as observed during sample collection, the use of bare hands in preparing the products (personal communication with the handlers), equipment, through persons taking part in manufacturing and handling of the product, improperly cleaned servers and debris falling into uncovered raw karish cheese milk. The high total yeast counts have resulted from inadequate processing (Aly et al., 2010) . Yeast spoilage constitutes a major economic loss in the cheese industry through developing undesirable changes, such as slimness, red color and yeasty flavor (Sarais et al., 2009). This may be due to their capacity to produce lipolytic and proteolytic enzymes (Fleet and Mian, 2009; Tornadijo et al., 2010).

A comparison of a number of correctly identified organisms by both methods is presented in Table 4. The API 20 C system correctly identified about 100% of the isolates compared to 99% by the conventional method. The API system identified all of the members of the yeast genera. The conventional method correctly identified all organisms except for four isolates. The identification of isolated yeast species revealed the presence of *Trichosporon cutaneum* (25%), *Candida catenulate* (23%), *Yarrowia lipolytica* (13%), *Debaryomyces hansenii* (13%), *Kluyveromyces lactis* (6%), *Geotrichum candidum* (7%), *Candida zeylanoides* (5%), *Candida lambica* (3%), *Candida albicans* (2%), *Cryptococcus formans* (1%), *Rhodotorula glabrata* (1%) and *Saccharomyces cervisiae* (1%).

It was reported that *Trichosporon* spp. caused formation of a surface film on the cottage cheese leading to spoilage (Nichol and Harden, 2006; Welthagen and Viljoen, 2009). Presence of this species in high concentrations could indicate poor hygiene and ineffective cleaning procedures and show the need for improved sanitization procedures (Seiler and Busse, 2009; Viljoen et al., 2010). *Y. lipolytica* resulted in a browning spoilage of cheese (Vorbeck and Cone, 2009; Westall and Filtenborg, 2010), while *C. zeylanoides* was isolated from feta cheese, but it was not possible to determine whether spoilage was associated with this species or not (Eklund et al., 2005; Diriye et al., 2007; Rohm et al., 2010).

The source of the isolation of T*. cutaneum* varies considerably, although many of them are of human and animal origin (Kreger-van Rij, 2009) . The presence of this species in high concentration could indicate poor hygiene and ineffective cleaning procedures, and show the need for improved sanitization procedures. *Y. lipolytica and C. zeylanoides* have also been isolated from spoiled cottage cheese. *Y. lipolytica* in high concentration resulted in an unwanted texture of feta-cheese due to the degradation of fat through production of lipolytic and proteolytic enzymes (Westall and Filtenborg, 2010).

Candida spp. are the most common cause of fungal infection in immune compromised persons known as candidiasis. Candidiasis is caused by infection of species within the genus *Candida,* predominantly with *C. albicans. Candida* species are ubiquitous fungi that represent the most common fungal pathogens that affect humans. Oropharyngeal colonization is found in 30 to 55% of healthy young adults, and *Candida* species may be detected in 40 to 65% of normal fecal florae. *C. albicans* can infect all areas of the skin as well as the mucous membranes. Infections by *C. albicans*, especially the ones found in the mucous membranes, are contagious (Hidalgo, 2011).

At present, the identification of yeast is generally performed by biochemical methods. However, many research findings have demonstrated that this method is not only complicated and labor intensive, but also time-consuming. This study confirmed the results on the identification of yeasts by biochemical and API 20 kit methods and found that there was no significant difference (P>0.05) between the conventional method and API 20. Therefore, it may be a useful tool for the biochemical identification of yeast isolates. They have almost the same sensitivity, but API 20 is easier, faster and rapid. However, almost similar findings were reported by Donald et al. (2009). On the other hand, Kitch and Badawi (2008) found significant differences between the conventional method and API 20 kits test. The data obtained in this study revealed that 'API 20 kits' was highly simple, useful, commercially available and considerably shortened the time required for identification of yeast in cheese. Conclusively, there is a need for continuous monitoring of Egyptian Karish cheese by educating producers, distributors and retailers on good sanitary practices during processing and sale of the product and the possible danger of contaminated Karish cheese.

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