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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Yeasts and filamentous fungi associated with some dairy products in Egypt

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Summary This work aimed to identify and evaluate the diversity of yeasts and filamentous fungi that contaminate some dairy products (fresh buffalo milk, plain yoghurt, butter and Kareisk cheese) in Assiut city, Egypt. The identification was based on phenotypic characteristics, and genotypically in case of yeasts. The pHs of all dairy products lie within the acidic range, but yoghurt registered the highest value. A total of 41 genera and 89 species + 3 varieties were recovered from all dairy products investigated on DRBC (26 genera representing 59 species + 1 variety), DG18 (32 genera, 56 species + 2 varieties) and MY50G (31 genera, 64 species + 2 varieties). From these, filamentous fungi were represented by 21 genera and 59 species + 2 varieties, and yeasts by 20 genera and 30 species + 1 variety. In all products except yoghurt, the xerophilic media (DG18 and MY50G) supported more diversity of fungal species than the general medium (DRBC). Reversely, DRBC supported more propagules originated from all products than DG18 and DRBC. On the other hand, the highest numbers of propagules were recovered from Kareish cheese and butter on MY50G, while the lowest numbers were recorded from yoghurt and raw milk on MY50G. Moreover, yeasts constituted the greatest part of propagules from the four products on the three isolation media except the raw milk on MY50G. From yeasts, *Candida*, *Cyberlindnera*, *Debaryomyces*, *Galactomyces*, *Kazachstania*, *Kluyveromyces*, *Myerozyma*, *Pichia*, *Rhodotorula*, *Trichosporon*; and from filamentous fungi, only *Aspergillus*, *Cladosporium*, *Mucor* and *Penicillium* were found contaminating all dairy products. Possible medical problems related to the presence of these fungi or to their metabolic products are discussed.

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Introduction

Yeasts play diverse roles in affecting the quality and safety of food products. They have been traditionally used for the preparation of bread, beer, and wine. Some yeasts have been also shown to contribute to the development of desirable flavor during cheese ripening, and are thus considered for use as starter cultures [1]. Because of their antagonistic action toward fungi, certain yeasts have been developed as biocontrol agents of food spoilage fungi, and others are being considered as probiotic organisms [2]. However, several yeasts metabolize organic acids in fermented foods causing an increase in the pH and thus allowing the growth of spoilage and pathogenic bacteria [3]. Some yeasts may further pose threats to food safety given their association with opportunistic infections and other adverse conditions in humans [2]. It is therefore relevant to identify yeasts associated with different foods and establish relationships between the characteristics of examined foods and the diversity of their yeast biota [4].

Milk is an extremely nutritious food. It is an aqueous colloidal suspension of proteins, fat and carbohydrates that contains numerous vitamins and minerals such as calcium, phosphorus, sodium, potassium and magnesium [5]. Milk proteins are ideal in that they are complete and have high essential amino acids composition [6]. Preservation of milk by fermentation aims at converting it into more stable nutritious and desirable products such as yoghurt, cheese and butter milk [7]. Fermented foods are popularly accepted for their flavour, better keeping quality, and the fact that fermentation creates variety among foods [8]. Although milk and its various derivatives are vital human foods, it provides an excellent medium for the growth of a wide range of microorganisms [6]. Worldwide cow's milk is the most commonly used but milk from water buffalo, goats, sheep, camels and yaks is also used in various parts of the world [9]. The microbiological quality of milk and dairy products is influenced by the initial microbiota of raw milk, the processing conditions, and post heat treatment contamination [10].

Of milk products, yoghurt is the most popular type of fermented milk in Egypt. Due to its nutritive value, yoghurt is recommended for sick and convalescent people. It inhibits the bacterial flora of intestine, which may lead to constipation, autointoxication and colitis, as well as, it helps in the absorption of calcium and phosphorus [11]. Kariesh cheese (defatted cheese) is also one of the most popular local types of fresh soft cheese in Egypt due to its high protein content and low price [12]. The traditional method of production under unsatisfactory conditions affords many opportunities for microbial contamination. Also, this product is sold uncovered without a container, thus the risk of contamination by different types of spoilage and pathogenic microorganisms is very high [13,14]. Fungi including yeasts can be an important component of the microbiota of many cheese varieties because of the low pH, low moisture content, high salt concentration and refrigerated storage of these products [15].

Butter is a dairy product of high nutritive value made by churning fresh or fermented cream or milk. Butter is a water-in-oil emulsion resulting from an inversion of the cream, an oil-in-water emulsion; the milk proteins are the emulsifiers. Salt, flavorings and preservatives are sometimes added to butter. Butter remains solid when refrigerated, but softens to a spreadable consistency at room temperature [16]. It could, if contaminated, constitute a public health hazard besides economic losses throughout its deterioration [17].

The presence of yeast and filamentous fungi in dairy products are objectionable, as they grow at a wide range of temperature and pH values, resulting in spoilage of the product [18]. Their counts are used as index of storability and sanitary quality of the products. Such fungi might cause gas and off flavor in cheese and rancidity or other flavor defects in butter due to their proteolytic activity [19]. It was also stated that yeast species mainly of the genera *Candida*, *Debaryomyces*, *Galactomyces*, *Fellomyces*, *Mycoderma*, *Pichia*, *Saccharomyces* and *Rhodotorula* by lactose assimilation decrease the quality of dairy products and some representatives of the genus *Rhodotorula* cause staining and give bitter taste to these products [20–23].

The aim of this work was to identify and evaluate the diversity of yeasts and filamentous fungi that contaminate some dairy products collected from farmers' homes (butter and Kareisk cheese) or supermarkets (fresh buffalo milk and plain yoghurt) in Assiut city. The identification was based on phenotypic characteristics, and genotypically in case of yeasts.

Materials and methods

Collection of samples

A total of 20 samples were collected from 4 types of dairy products namely, raw milk, plain yoghurt, kareish cheese, and butter (5 samples each) from different places in Assuit, during the period from November 2012 to April 2013. Raw (fresh, non-boiled) milk samples and yoghurt samples (without any preservatives and additives) were collected from different dairy products markets. Kareish cheese and butter samples were collected from the farmers' homes and put directly each into a clean plastic bag. All samples were brought into the laboratory and kept at 5 °C till pH determination and fungal analysis.

Determination of pH

To determine the pH in dairy product samples, a pH meter (Orior Research Model GOHL Digital Ionalyzer) was used. The electrode was immersed directly in the raw milk and yoghurt. Emulsions were made by warming butter samples in a water bath and by squeezing of Kareish cheese samples.

Isolation of fungi

The dilution-plate method [24] was used for enumeration of different fungal species as employed in this laboratory by Moubasher and his collaborators as follows:

- one millilitre of raw milk or yoghurt samples taken, while one gram of squeezed cheese were placed in sterile 250 ml Erlenmeyer flasks containing each 99 ml of sterile distilled water and the final dilution was (1:100). The flasks were then shaken on a mechanical orbital shaker for 30 minutes. In case of butter one gram was put in a sterile 250 ml Erlenmeyer flask containing 99 ml of sterile

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