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MYCOLOGICAL QUALITY OF LABAN RAYEB SOLD IN ASSIUT CITY (With 3 Tables)

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التقييم الميكولوجي للبن الرايب المباع في مدينة أسيوط

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تم جمع خمسين عينة من اللبن الرايب (٢٥ عينة من لبن الفرز المتخمر و ٢٥ عينة من اللبن الرايب المبستر) من بيوت الفلاحين والسوبر ماركت المختلفة بمحافظة أسيوط وذلك لفحصها ميكولوجيا لمعرفة مدى تلوثها بالفطريات والخمائر وكذلك المفرز منها لانزيم البروتيز لماله من تأثير على طعم ورائحة المنتج النهائي. وقد تم العزل على مستنبت Malt extract والتحصين عند درجة ٢٥° م لمدة تتراوح من ٥-٧ أيام ولقد تبين من الفحص أن عينات اللبن الفرز المتخمر أكثر تلوثا من عينات الرايب المبستر حيث كان عدد العينات الموجبة على التوالي (١٨ عينة) ٧٢% ، (١٢) ٤٨% وكذلك العدد الكلي للفطريات في المللي الواحد ١٠ × ٩٨ ، ١٠ × ٦٥ ، مستعمرة على التوالي كما وجد أيضا أن العينات ملوثة بالخمائر بنسبة ٣٦% ، ٢٤% من العدد الكلي للفطريات المعزولة على التوالي. كذلك تم التعرف على العديد من الفطريات على المستنبت المستخدم وكان أكثرها انتشارا هي الأنواع التي تنتمي إلى أجناس الكلاوسوريوم والينسيليم والاسبرجلس والالترناريا بالنسبة للبن الفرز المتخمر، أما بالنسبة للبن الرايب المبستر فكانت أجناس الكلاوسوريوم والاسبرجلس ثم الجبريلا فيوجيكوري والينسيليم والالترناريا. هذا وقد تم اختبار ٧٣ عترة تنتمي إلى ١٣ جنس لمعرفة مقدرتها على افراز انزيم البروتيز وقد وجد أن ٦٢ عترة تمكنت من افرازه بدرجات متفاوتة وتمثلت أعلى نسبة افراز (٣٠ عترة) في فطر الاسبرجلس نيجر وفلاس وتيرس وسيدوى بينما كانت ١٩ عترة متوسطة الافراز تمثلت في الفيوزيريوم أوكسى سبوريم والاسبرجلس نيجر والكلاوسوريوم والينسيليم بينما كانت ١٣ عترة ضعيفة الافراز. وقد تمت مناقشة الأهمية الصحية والاقتصادية للفطريات.

SUMMARY

The mycological quality of raw and pasteurized Rayeb was investigated in fifty samples gathered randomly from different farmers' houses and supper markets at Assiut City using the dilution plate method on malt

extract agar which incubated at 25°C. The obtained results indicated that raw Rayeb was more polluted than the pasteurized one (72% and 48 % respectively), the average counts of fungi were 98×10^3 and 65×10^3 colonies/ml on the two types of substrates, respectively. Eleven species belonging to 20 genera were identified. The most prevalent fungi in raw Rayeb were *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria* species. While in case of pasteurized Rayeb, *Cladosporium*, *Aspergillus*, *Gibberella fujikuroi* and *Alternaria* species were the most common. Concerning 73 isolates examined for protease production about 30 exhibited strong protease production. The more prevalent isolates among them were related to *A.niger*, *A.flavus*, *A.terrus* and *A.sydowii* while 19 isolates were moderately producers including *Fuserium oxysporum*, *Aspergillus niger*, *Cladosporium* and *penicillum* species, where as 13 isolates were weakly producers. The public health significance of the isolated fungi were discussed.

Key words: *Mycological quality, Laban Rayeb.*

INTRODUCTION

It is believed that fermented milks may have originated in middle east as early as 1300 before century (BC) as means of preserving milk. Then they spread through central and Eastern Europe and their common link is based on lactic acid fermentation by single or a mixture of organisms. Laban Rayeb is one of the important fermented milks consumed by different ages in Egypt and throughout the world and characterized by high nutritive value, maintenance of normal intestinal microflora, its nutritional enhancement and to its great effects in reduction of both serum cholesterol levels and carcinogenic activity (Varnam and Sutherland, 1994).

Mold contamination not only causes deterioration of food and feeds but also can adversely affect the health of humans and animals since they are capable of producing toxic metabolites known as mycotoxins causing cases of food poisoning, liver cancer in human (Mossel, 1982 and Foster, *et al.* 1983). Under adversity of moisture, pH and temperature conditions economic losses and various degrees of food decomposition were taken place, they can cause lipolytic and proteolytic spoilage including discoloration, poor appearance, rancidity and off flavour of food (Besancon *et al.*, 1992 and Jakobsen and Narvhus, 1996).

Molds get entry to raw Rayeb either before, during and or post processing while presence of molds in the pasteurized Rayeb indicates the storage in the time of pasteurization process or may be due to the post pasteurization contamination.

Proteases are one of the most important groups of enzymes constituting two thirds of the total industrial enzymes marketed (Gerhart 1990 and Singh *et al.*, 1990). They play an important role in the production of fermented foods and in the dairy industry for clotting of milk (Singh *et al.*, 1994). Also the flavour of the final milk product is mainly due to the proteolytic activity (Lopez-Diaz *et al.*, 1996). In addition, protease from microorganisms have especially drawn much attention for their use in biotechnology.

Fungi influence the biochemical characters and flavour of the product and its appearance is commercially be undesirable and often result in down grading of the product (Chapman and Sharpe 1990; Roudot-Algaron *et al.*, 1993; Bouton and Grappin 1995 and Beuvier *et al.*, 1997).

So there is an increasing demand by the dairy industry for high quality products free from microorganisms (Desmases *et al.*, 1997). The objective of this study was to characterize the fungal contamination of raw and pasteurized Rayeb during the stage of consumption also screening of the isolated strains for production of protease enzyme was determined.

MATERIAL and METHODS

1- Collecting of samples:

Fifty random samples of raw and pasteurized Laban Rayeb (25 for each) at the stage of consumption were collected from different farmer's houses and supermarkets in Assiut City.

Raw Rayeb transferred to the laboratory in sterile bottles and all the samples were kept at 4°C until fungal analysis.

2- Preparation of samples:

Eleven ml from the product were transferred into a sterile flask containing 99 ml of sterile peptone water according to APHA, 1992.

3- Estimation of the fungi counts:

The dilution plate method (Johnson and Curl, 1972) was applied by using malt extract agar (Speck, 1976) with antibiotic for characterization of the mycoflora contaminated the product. Three plates for each sample were used and incubated at 25°C for 5-7 days and the developing fungi were counted and identified according to

Cooney and Emerson (1964); Raper and Fennell (1965); Ellis (1971); Booth (1977) and Pitt (1979).

3- Screening of the isolates for proteolytic activity:

Seventy three isolates representing thirteen species were tested for their ability to produce protease enzyme using the procedure of El-Gendy, 1966.

RESULTS

The obtained results of the examined samples were summarised in Tables 1-3.

Table 1: Analytical results of total mold counts in the examined Egyptian Laban Rayeb samples.

Samples	Positive samples	
	No	%
Raw Rayeb	18/25	72
Pasteurized Rayeb	12/25	48

Table 2:Total count (Tc)/ml in all samples and percentage frequency (F%) of fungal genera and species recovered from raw and pasteurized Laban Rayeb on malt agar at 25°C.

Genera and Species	Tc	Raw Rayeb		Tc	Pasteurized Rayeb	
		No. +ve samples	F%		No. +ve samples	F%
<i>Acrimonium strictum</i>	2	1	4	4	3	12
<i>Alternaria</i>	3	3	12	1	1	4
<i>A.alternata</i>	2	2	8	-	-	-
<i>A.tenuissima</i>	1	1	4	1	1	4
<i>Aspergillus</i>	2	2	8	14	5	20
<i>A.flavus</i>	-	-	-	1	1	4
<i>A.niger</i>	-	-	-	3	2	8
<i>A.parasiticus</i>	1	1	4	-	-	-
<i>A.sydowii</i>	-	-	-	10	1	4
<i>A.terreus</i>	1	1	4	-	-	-
<i>Cladosporium</i>	71	6	24	33	9	36
<i>C.cladosporioides</i>	34	6	24	25	9	36
<i>C.herbarum</i>	19	3	12	-	-	-
<i>C.sphaerospermum</i>	18	1	4	8	3	12
<i>Cunninghamella elegans</i>	1	1	4	-	-	-
<i>Epicoccum purpurascens</i>	-	-	-	1	2	8
<i>Fusarium oxysporum</i>	-	-	-	6	2	8
<i>Gibberella fujikuroi</i>	-	-	-	-	-	-
<i>Penicillium</i>	14	5	20	3	3	12
<i>P.aurantiogriseum</i>	-	-	-	1	2	8
<i>P.corylophilum</i>	2	1	4	-	-	-
<i>P.duclauxii</i>	12	2	8	1	1	4
<i>P.oxalicum</i>	-	-	-	1	1	4
<i>Rhizopus stolonifer</i>	2	1	4	1	1	4
<i>Sterilemy celia</i>	1	1	4	2	2	8
Total counts	98			65		
Number of genera = 11	8			8		
Number of species = 20	13			13		
Yeasts	1087	36		180	24	

Table 3: Screening of fungal isolates for protease production

Species	NIT	NIP	Degree of production		
			High	Moderate	Weak
<i>Acremonium strictum</i>	3	1	-	1	-
<i>Alternaria alternata</i>	5	4	2	-	2
<i>Aspergillus flavus</i>	11	10	9	1	-
<i>A. niger</i>	13	13	10	2	1
<i>A. sydowii</i>	6	5	3	1	1
<i>A. terreus</i>	5	5	4	1	-
<i>Cladosporium cladosporioides</i>	4	4	-	2	2
<i>C. herbarum</i>	3	2	-	1	1
<i>C. sphaerospermum</i>	6	4	-	2	2
<i>Fusarium oxysporum</i>	8	6	2	3	1
<i>Penicillium aurantiogriseum</i>	3	2	-	2	-
<i>P. corylophilum</i>	4	4	-	2	2
<i>P. duclauxii</i>	2	2	-	1	1
Total isolates	73	62	30	19	13

NIT = number of isolates tested

NIP = number of isolates positive

DISCUSSION

The obtained results revealed that the average counts of molds were 98×10^3 and 65×10^3 colonies/ml in raw and pasteurized Rayeb respectively (Table 2). These results were lower than that obtained by Sabreen (2001) in raw Rayeb and higher than that obtained on pasteurized Rayeb, while Moustafa *et al.* (1988) reported various counts of yeasts and molds in the examined yoghurt samples. These results revealed that pasteurization of Rayeb allow the isolation of lower counts of molds specially yeasts, anyhow the high counts of mycoflora either in pasteurized or in raw Rayeb may contributed to the unhygienic measures during handling by attendants or failure to complete pasteurization process of equipments. It was observed that 12 and 18 samples of the 50 samples of tested raw & pasteurized Rayeb were completely free from fungal contamination (Table 1). It is appear from the results in Table 2 that eleven fungal species belonging to twenty genera were isolated from 50 samples of Rayeb. The most prevalent genus encountered in the examined samples was *Cladosporium* comprising 24% and 36% of the samples constituting 72.5% and 50.8% of the total fungi on raw and pasteurized Rayeb respectively. *Penicillium* occupied the second genus in frequency of occurrence. It was represented in 20% and 12% of the

samples of the two types of the product. Four species of the genus were identified of which *P. duclauxii* was the most prevalent species whereas *P. aurantiogriseum*, *P. corylophilum* and *P. oxalicum* were less common and these finding were almost in agreement with the results obtained by Morea (1980); Pitt and Hocking (1985) and Engel (1986).

Aspergillus (5 species) ranked the third in frequency of occurrence comprising 8% and 20% of the samples contributing 2% and 21.5% of the total fungi on raw and pasteurized Rayeb, respectively, (Table 2). All *Aspergillus* species isolated occurred in rare frequency of occurrence.

These results were in harmony with results obtained by Clavo *et al.* (1979) and Jordano *et al.*, (1989). They reported that *Aspergillus* was the most prevalent genus encountered in the previous examined samples, further more some species of this genus are mycotoxin producing molds which posses potential hazards to food safety and human health (Van Walbeek, 1973; Bullerman and Olivigni, 1974; Debeaupuis and La font, 1978; Charles *et al.*, 1979 and El-Shrief, 2000).

Acremonium (*A. strictum*), *Alternaria* (*A. alternata* and *A. tenuissima*), *Cunninghamella* (*C. elegans*), *Epicoccum* (*E. purpurascens*), *Fusarium* (*F. oxysporum*) and *Gibberella* (*G. Fufikuroi*) were also isolated from one substrate or in rare occurrence or absent on the other one. These molds were reported in the same trends by Sutic *et al.* (1979) and Leistner (1984).

Yeast results presented in Table 2 showed that 36% and 24% of the examined raw and pasteurized laban Rayeb proved to be contaminated with yeast, respectively and the total yeast count/ml of the examined samples comprised 91.71% and 73.5% of the total fungi, respectively. It is worth to mention that the high count of yeast in raw Rayeb is indicative to the neglected measures applied during processing and handling of the product, while presence of some yeasts and molds after pasteurization allow to conclude that the pasteurization conditions are not complete or may be post pasteurization contamination happens. In fact, the mold lies in raw and pasteurized Rayeb may be contaminated by some toxigenic fungal strains either direct or indirectly during manufacturing and storage. So that to safe gurd consumers from being infected and to obtain a finished product of good keeping quality strict hygienic measures should be applied during processing and storage.

Among 73 isolates tested to determine proteolytic activity about 84.9% of the isolates (62 isolates) could produce protease enzymes with variable degrees. From the positive strains 30 isolates (48.4%) exhibited

the highest strong protease production and these strains related to *Aspergillus niger*, *A. flavus*, *A. terreus*, and *A. sydowii*. Nineteen (30.6%) isolates of the positive isolates could produce enzyme with moderate degree including *Fusarium oxysporium*, *A. niger*, *Cladosporium* and *Penicillium* species and thirteen (21%) isolates are weakly producers (Table 3).

In this respect Moharram and El-Zyat (1989) tested the ability of different fungal isolates to produce proteolytic enzymes. They observed that most isolates were able to produce protease but in varying degrees. It was also observed that not only the species of a single genus differed in the production of this enzyme but also the different isolates within the same species. Omar *et al.* (1999) made an extensive study on the protease activity testing 96 isolates. They noticed that from 72 isolates, 28 isolates were highly proteolytic, 21 moderately degrading milk protein and the remaining isolates are weakly producers. These results agree to a great extent with findings reported by Robertsen (1984); Moharram and El-Zyat (1989); Mohawed *et al.* (1993); Singh *et al.* (1994); Abdel-Gawad (1997) and Wu and Hang (1998). They tested several species of fungi including the same species and noticed that these fungi have the ability to produce acid or alkaline proteases in different degrees.

In conclusion, fungal contamination of raw and pasteurized Rayeb samples indicated improper plant sanitation and neglected hygienic measures during production, packaging or storage. Also a large number of mold species and yeasts including mycotoxic fungi which produce mycotoxins are wide to contaminate several sources of foods rendering them unpalatable and unsafe for consumption (Munimbazi and Bullerman, 1996). So owing to the role played by fungi whether from economic or public health points of view, Laban Rayeb must be subjected to vigorous quality control program as well as, control of cultures and sanitation during processing and storage and employ HACCP practices in the handling and production stages of the product.

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