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LIPOLYTIC AND PROTEOLYTIC ACTIVITIES OF SOME FUNGI ISOLATED FROM RAW CAMEL'S MILK

(With 2 Tables)

By

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**قدرة التحلل الدهنى والبروتينى لبعض الفطريات المعزولة
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تمثل ألبان النوق مصدراً من مصادر الحياة لسكان المناطق الجافة وشبه الجافة لإحتوائها على جميع العناصر الغذائية الأساسية إضافة إلى كثير من المعادن والفيتامينات هذا بالإضافة إلى دورها العلاجى لكثير من الأمراض. لذا تزايد الإهتمام فى الآونة الأخيرة بجودة هذه الألبان وخلوها من بعض الملوثات الفطرية نظراً لإستهلاكها طازجة بعد الحلب مباشرة أو بعد ترويبها. لذا اهتمت هذه الدراسة بعزل بعض الفطريات الملوثة لهذه الألبان من ثلاثين عينة عشوائية من ألبان النوق جمعت من مركز بيع لبن الجمال بأسبوط وكذلك تم اختبار قدرة بعض هذه العزلات على إفراز أنزيمى الليبيز والبروتيز المحللين للدهون والبروتينات متسببة فى إفساد هذه الألبان من حيث الطعم والرائحة وتقصير فترة صلاحية منتجاتها. وقد أوضحت النتائج أن ٢٣ عينة كانت ملوثة بالفطريات وذلك بنسبة (٧٦,٧%) وقد عزل منها ثمانية وخمسون نوعاً من الفطريات التابعة لست فصائل والتي تمثلت فى الأسبرجلس والذى ضم الأسبرجلس نيجر ٢١ (٧٠%) والأسبرجلس فلافس ١٣ (٤٣,٣%)، الأيسيديا كوريمبفرا ٧ (٢٣,٣%)، الريزوبس استلونيفر ٤ (١٣,٣%) ، فصيلة الأيميرسيلائيديولاس ٣ (١٠%) والفيوزيرم بروليفرا ١ (٣,٣%) وأخيراً الخمائر بنسبة ٤ (١٣,٣%). وعند قياس نشاط قدرة الفطريات المعزولة على إفراز إنزيمى الليبيز والبروتيز وجد أن الأسبرجلس نيجر وفلافس لهما أعلى نسبتي إفراز لإنزيم الليبيز دون العزلات الأخرى حيث كانت ست وثلاث عزلات عالية الإفراز، وخمس وأربعة متوسطة الإفراز وثمان وست ضعيفة الإفراز للأسبرجلس نيجر وفلافس على التوالى. أما باقى العزلات التابعة للست فصائل فقد تمكنت من إفراز أنزيم الليبيز بدرجات متفاوتة. بالنسبة لإنزيم البروتيز فلم يتم إفرازه من العزلات السابقة بأى درجات وقد أعزى ذلك ربما لوجود مضادات البروتين الموجودة فى ألبان الجمال ذات التأثير المثبط لكثير من الميكروبات الضارة والمنسببة فى فساد الألبان ومنتجاتها. وقد ناقشت الدراسة الأهمية الصحية والاقتصادية لبعض الفطريات المعزولة والاحتياطات الواجبة لمنع تلوث الألبان ومنتجاتها.

SUMMARY

A total of 30 raw camel's milk samples randomly collected from a camel Milk Center in Assiut were subjected to fungal analysis and then screening the isolated fungi to enzymatic activations. Results of isolation revealed that: 23 (76.7%) of the examined samples were contaminated by 58 fungal species belonging to 6 genera. *Aspergillus*, *Absidia corymbifera*, *Rhizopus stolonifer*, *Emericella nidulans*, *Fusarium proliferatum* and yeasts. The most predominant species capable to secrete lipase enzyme were *A. niger* 21 (70%) and *A. flavus* 13 (43.3%) while the other fungal species could produce lipase enzyme with variable degrees. Protease enzyme could not be detected from all the isolated fungal species and this may be attributed to the protease inhibitors presented in camel's milk. The health and economic significances of the isolated fungi were also discussed.

Key words: *Camel's milk, lipase, protease enzymes.*

INTRODUCTION

Camel milk is an important factor in the economic life and for survival of many nomads in the drier areas and desert dwellers where no other animal can produce milk due to stress conditions. A she camel yields daily from 3.5 to 35 kg of milk containing all essential nutrients and exceeds in vitamins and minerals more than that found in milk from other species (Farah, 1993). Camel's milk has a large scale of medical treatment for number of diseases and illnesses from diarrhea to tuberculosis, reducing the coronary heart diseases and diabetes (Roae *et al.*, 1970 and Zagorski *et al.*, 1998). Traditional therapeutic uses of camel milk in folk medicine in different areas in the world were recorded due to its antibacterial and antiviral properties (Yagil, 1982). Camel's milk is gaining popularity by nomads who consumed it fresh immediately after milking in a raw state or just after mild souring (FAO, 1992) therefore, much attention has been given to produce high quality raw camel's milk with minimal microbial contamination (Robinson, 1990). The microbiological quality of raw camel's milk was studied by Al-Mohizea (1986), Mahmoud (1997), Teshager and Bayleyen (2001), Khedid *et al.* (2003) and Korashy *et al.* (2006). Fungi including the mycotoxic species are among the contaminating hazardous that influence the safety of raw milk. A potential relationship between human

consumption of moldy foods and various illnesses has been long suspected (Stoloff, 1976). Molds constitute a part of normal flora inhibit camel wool (Bagy and Abdel Hafez, 1985) and consequently may be a source of camel's milk contamination during the milking process. Recently, microbiologists focused their attention on yeasts as food spoilage causative organisms and the role of pathogenic yeasts affecting human health through consumption of raw milk (Spillman and Ceiges, 1983 and Hurley *et al.*, 1987). The occurrence of yeasts in raw milk was reported by some investigators in different countries (Bridge Cooke and Brazis, 1968, Haridy, 1992 and Mahmoud, 1997). Yeast and mould counts are usually used as a guide for proper sanitation and an index of the quality control of dairy products (Foster *et al.*, 1983).

Mycotoxins are fungal metabolites proved to be etiological agents in some outbreaks of foodborne diseases of human and animals (Wyllie and Morehouse, 1977). Many of fungi are mycotoxin producers. These mycotoxins are carcinogenic, mutagenic and teratogenic such as aflatoxins, ochratoxin A and sterigmatocystin (Chu, 1991). An outbreak of hepatitis infected 400 Indian people of whom 100 died resulted from aflatoxin contaminated maize ration fed to animals (Krishnamachari *et al.*, 1975). Although, some species of molds are used in manufacturing and ripening of certain varieties of some milk products they are responsible for lipolytic and proteolytic spoilage including discolouration, rancidity, off flavour and poor appearance which often result in downgrading of milk and milk products leading to economic losses (Jakobsen and Narvhus, 1996). Temporal bitterness in some soft cheese manufactured from camel's milk reported by Layer and Keller *et al.* (2003). So there is an increasing demand by the dairy industry for high quality raw milk free from pathogenic and spoilage microorganisms (Desmaures *et al.*, 1997). Braun *et al.* (2002), Ali and Abo-Al-Yazeed (2003) and Saxena *et al.* (2003) dealt with lipolytic and proteolytic activities of fungi produced from camel's milk. Lipase enzyme still to be the main factor responsible for camel's milk spoilage as the bacteriolytic nature of camel's milk protein including lysozymes, lactoferrin, lactoperoxidase, immunoglobulin and the N-acetylglucosaminidase which inhibit the growth of some pathogenic and spoilage organisms (Al-Naki, 1984). Protein inhibitors in camel's milk explain the traditional therapeutic uses of this milk in folk medicine in different areas in the world (Yagil, 1982).

In general, lipolytic and proteolytic activities expressed to the primary microbial spoilage enzymes of dairy products which are responsible for deleterious effects in milk and its products (Stelma, 1989). Both lipases and proteases are heat stable extracellular enzymes produced by spoilage microflora resulting in off flavour described as rancid, unclean, soapy and bitter (Conesa *et al.*, 2001) due to lipid and protein degradation (Downey, 1980) and adversely affects the processing properties of milk and the quality of milk products (Cormie, 1992) which consequently leading to economic losses through the unacceptability and reduction of the shelf-life time of the product. Much attention has been given to lipolytic activity screened by different microflora in camel's milk (Kotula *et al.*, 1982; Favale *et al.*, 1994; Abu-Tarboush *et al.*, 1998 and Ali and Abo Al-Yazeed, 2003).

Regarding to the health and economical points of fungi and their lipid activities this study was designed to:

- 1- Survey fungi (mould and yeast) which contaminate raw milk.
- 2- Screening the capabilities of the isolated fungi for lipase and protease enzymes production.

MATERIALS and METHODS

Collection and preparation of samples

A random collection of 30 raw camel's milk in clean, dry and sterile containers were transferred to the laboratory without delay and kept at 4°C until fungal analysis. Each sample was thoroughly mixed and subjected to storch test according to Lampert (1975) to exclude the heat treated samples.

Mycological analysis

Isolation of fungi: Using sabaroud dextrose agar (Difco Lab) according to Harrigan and McCance (1976) with antibiotic for characterization of the mycoflora contaminated the milk samples. The cultured plates were incubated at 25°C for 5-7 days. The developing fungi isolated and identified according to Raper and Fennel (1965) and Ellis (1971).

Enzymatic activities:

A- Lipolytic activity: was detected according to Ullman and Blasins (1974).

B- Proteolytic activity adopted according to Paterson and Bridge (1994).

RESULTS

The obtained results were tabulated in Tables 1 and 2.

Table 1: Incidence of mould and yeast in the examined samples of raw camel's milk.

No. of examined samples	+ve samples	%
30	23	76.7

Table 2: Incidence of fungal species and their lipolytic activity in the examined raw camel's milk samples.

Isolated fungal species	NIT	%	Lipolytic activity			
			NIP	H	M	W
<i>A. niger</i>	21	70	19	6	5	8
<i>A. flavus</i>	13	43.3	13	3	4	6
<i>A. terreus</i>	4	13.3	4	1	2	1
<i>A. fumigatus</i>	1	3.3	1	1	-	-
<i>Absidia corymbifera</i>	7	23.3	7	7	5	1
<i>Rhizopus stolonifer</i>	4	13.3	2	1	-	1
<i>Emericella nidulans</i>	3	10	3	3	1	
<i>Fusarium proliferatum</i>	1	3.3	1	1	-	1
Yeasts	4	13.3	4	4	2	1
Total	58		54	27	19	19

Total number of examined samples = No. 30

NIT= number of isolates tested.

NIP = number of isolates positive

H: high

M: moderate

W: weak

DISCUSSION

Table 1 showed that the incidence of fungi isolated from the thirty raw camel's milk samples were 23 (76.7%). Higher results were obtained by Mahmoud (1997) and Ali and Abo Al-Yazeed (2003), lower results were obtained by Khedid *et al.* (2003). The high incidence of fungi in the examined raw milk samples may be attributed to the widespread distribution of fungi in nature. Fungi are normal inhabitant of wool of the animal, also, ability of fungi to be adopted at wide range of temperatures (Nasser *et al.*, 1998). The high incidence of fungal contamination not only be responsible for the public health hazard through the wide variety of mycotoxin production affecting humans and

animals (Mossel, 1982) but also it may lead to economic losses through milk spoilage by lipase enzyme which leads to inferior quality of milk specially when long stored and when it is manufactured (Jakobsen and Narvhus, 1996). Table 2 clarified that raw camel's milk tested became molded by fifty eight fungal species which were belonging to six genera. *Aspergillus* was the common genus and it was represented by 4 species of which *A. niger* 21 (70%) and *A. flavus* 13 (43.3%), *A. terreus* 4 (13.3%) and *A. fumigatus* 1 (3.33%) were recorded with lower incidence. The same results were obtained by Ali and Abo Al-Yazeed (2003) who found that genus *Aspergillus* was the most prevalent in fifty raw camel's milk samples.

A. niger is one of the best known of all fungal species and it is regarded as a benign fungus. So, it is categorized as safe by the U.S. Governorate. It has been widely used in food processing since the toxin production does not appear to be common (Abarca *et al.*, 1994). *A. niger* is more prevalent in warmer climates in field saturation and stored foods (Ayerst, 1966).

On the other side, *A. flavus* is the most important natural source of aflatoxins in the world's food supplies of which are B₁, B₂, G₁ and G₂. The risk of B₁ and B₂ aflatoxins starts when they ingested by a lactating animal and a proportion is hydroxylated to M₁ and M₂ aflatoxin derivatives of lower toxicity but their significant due to the widespread consumption of mycotoxicated milk by infants (Frobish *et al.*, 1986). Aflatoxins produced by *A. flavus* exposed animals including man to acute and chronic toxicity distinguished in four forms: liver damage, liver cirrhosis, induction of tumours and teratogenic effects. A wide range of metabolites were produced by *A. terreus* and of these only territrems appear to have significant toxicity (Frisvad and Samson, 1991).

A. fumigatus has a marginal xerophile nature ranges from 12°C to 55°C (Ayerst, 1966). *A. fumigatus* produces number of toxins of which fumitremorgens, verruculogen and gliotoxin which is the only important in the invasion of the animal (Richard *et al.*, 1994). Other infections to eye, skin, sinusitis and lungs (pulmonary Aspergillosis) were reported by (Hog *et al.*, 2000) due to genus *Aspergillus*. *A. niger* and *A. flavus* recorded the highest degree of lipase production among the fifty eight fungal species tested for screening of lipase, Table 2. Nineteen and thirteen tested isolates representing *A. niger* and *A. flavus* respectively revealed 6 and 3 for high, 5 and 4 with moderate and 8 and 6 with weak production for lipase enzyme. The same results were recorded by Ali and

Abo Al Yazeed (2003) who tested ninety mold isolates for detection of lipase activity and they found that seventy isolates were positive and genus *Aspergillus* dominated the other genera for lipase production.

Following up Table 2, it is obvious that *Absidia corymbifera* occupied the second genus in occurrence 7 (23.3%). It was rarely to be isolated from milk and its products by several examiners. It is a widespread species in tropics. It could be isolated from group of cereals used in animal and human feeding (Dragoni *et al.*, 1979). *A. corymbifera* is a weak human and animal pathogen although its wide host range and capable of infecting many body organs (Lunn, 1977). Only 5 of the 7 tested isolates were moderate for lipase production. *Rhizopus stolonifer* scored 4 (13.3%), (Table 2). High and weak lipase production by *R. stolonifer* were recorded by one isolate for each. *Emmericella nidulans* scored 3 (10%). It could be isolated from milk and other dairy products by Korashy and Hussein (2005). It is a marginal thermophile has been isolated from a wide variety of sources (Moubasher *et al.*, 1972). It has been reported to produce sterigmatocystin and emestrin which is highly toxic (Terao *et al.*, 1990). High, moderate and weak production of lipase were represented by one isolate for each from the three *E. nidulans* tested isolates. *Fusarium proliferatum* recorded weak occurrence with weak production of lipase enzyme. It is worth to mention that lipase production did not differ among the different mold species but also among the isolates of the same species, (Table 2).

Yeasts derived from fungal analysis of raw camel's milk samples revealed 4 (13.3%). Different results concerning this line recorded by several examiners from raw milk as Haridy (1992), Mahmoud (1997), Sabry (2001), Al-Ganzoury (2002). High incidence of these organisms in raw milk is indicative of unsatisfactory sanitation during the milking journey as they gain entrance to raw milk from the contaminated air, water, dust, utensils. Hurley *et al.*, 1987 studied the pathogenesis of some yeast strain isolated from raw milk consumed by human. Lipase production by yeast species recorded one isolate for high and weak production and two isolates with moderate production. Ottogalli and Galli (1972) recorded lipolytic activity by yeasts which is responsible for rancid and off flavour attributed to the undesirable changes and inferior quality of milk specially during long storage and processing (Conesa *et al.*, 2001).

Protease activity could not be detected from all fungal species tested and this is attributed to the protease inhibitors which may be found

in camel's milk inhibiting the growth of saprophytic and pathogenic organisms (Al-Naki, 1984 and Farah, 1993). So, lipase enzyme still to be the main factor affecting the milk spoilage.

Finally, it could be concluded that raw camel's milk is strongly liable to be contaminated with several fungal species as they are normal inhabitant of the camel wool and adapt themselves at a wide range of environmental conditions. Strict hygienic measures must be applied during the milking process including, animal, milkers, utensils and equipments since, the nomad and the young camels consume this milk in its raw state. Greater attention must be focused on the antimicrobial property of camel milk to improve preservation conditions of milk specially when it is transformed to cheese or other fermented dairy products to creat the bright future demand to camel's milk.

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