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# HAZARDS ASSOCIATED WITH DROMEDARY CAMEL MILKS

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**ABSTRACT**
Fifty Egyptian camel milk samples were randomly collected under sterile condition from different camel herds at North and South Sinai, Sina Governorate, Egypt. Thirty percent of the examined samples were positive for *Aerobic spore former* count, 24% were positive for total *coliform* count and 20% were positive for total *Enterococcai* count. *Pseudomonas, Aeromonas,* coagulase positive *Staphylococcus aureus* were counted. Many mold species were isolated from the examined samples including *Aspergillus, Penicillium, Alternaria, Acremonium* and *Chrysosporium* species. Among one hundred and seven mold strains tested, eighty one isolates were able to produce lipase enzyme with varying percentages while none of the screened *A. flavus* and *A. parasiticus* strains were aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ producers. *Salmonella, Listeria monocytogenes* and *Bacillus cereus* could not be isolated from all the examined raw camel milk samples. The economical and public health importance of the isolated microorganism as well as control measures for improving the quality of camel milk were discussed.

**KEY WORDS**: camel milk, hazards and aflatoxin.

## INTRODUCTION

Camel milk is always an important basic food in the arid regions of Africa and growing countries. It may be used alone as single food for children and elderly people. It is highly nutritious and delicious, low in fat, lactose and cholesterol while rich in protein, lactoferrin, insulin, minerals as sodium, potassium, magnesium, iron, iodin and vitamins as vitamin C, $B_2$ and $B_{12}$ (Maefield and Tinson, 1997; Tefera & Gebreah, 2004 ; Lora *et al*., 2005 and EL-Ziny and AL-Turki, 2013). The use of camel milk for medicinal purposes is a recent exciting development, where it proved to fortify the immune system of the human body as well as effective in treating many diseases as malnutrition, jundice, chronic hepatitis, anaemia, diabetes, asthma, ulcers, milk allergy, Lactase deficiency and breast cancer (Elsayed *et al*., 1992; Gorban and Izzeldin, 2001; Guliye *et al*., 2002 and Farah & Fischer, 2004). Camel milk in addition of being nutritious it considered as favorable medium for multiplication of microorganisms. Microbial contaminants together with high temperature reduce the milk quality and cause economical and public health hazards. They could attack camel milk protein and fat leading to milk spoilage. The battle of milk spoilage is assisted by new information on enzyme production by spoilage fungi. It is proved to produce lipase and protease enzymes resulting off-flavours. As camel milk rich in specific protease inhibitors so lipase enzyme considered as the main factor affecting milk spoilage. Toxigenic fungi may constitute a real public health concern through production of secondary toxic metabolites known as mycotoxins (Shiller, 1990; Stevenson & Rowe, 1994; Agrawal *et al*., 2002 and Saxena *et al*., 2003). As camel milk is consumed in the raw state by desert nomads, therefore, our challenge has planned to evaluate the microbiological quality of Egyptian camel milk and testing for lipase enzyme as well as aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ produced by isolated mold strains.

## MATERIAL & METHODS

- **Collection of samples**

Fifty random raw camel milk samples were collected from different camel herds at North and South sinai, Sina Governorate, Egypt. The milk samples were kept at 4ºC in ice box until analyzed according to A.P.H.A., (1992).

**1. Organoleptic examination**

The collected milk samples were sensory scored using score cards for flavor and odor (45 points), color (20 points) and bacteria (35 points). The scores were averaged by five panelists according to Nelson and Trout, (1981). All collected milk samples were examined for titratable acidity (TA) expressed as lactic acid % and pH using pH meter (model 920 Orion Inc., Boston MA) according to Marshall (1992). Resazurine reduction test was carried out according to the method reported by Chalmers (1962).

**2. Microbiological examination**

2.1 Total colony count

One ml of decimimal dilutions were plated onto standard plate count agar media and incubated at 10ºC for 7 days, 37ºC for 48 h and 55ºC for 24 h for psychrophilic, mesophilic and thermophilic count respectively according to ICMSF (1996).

2.2 *Aerobic spore former* count according to A.P.H.A (1992)

2.3 *coliform* count (MPN/ml) according to A.P.H.A (1992).

2.4 *Enterococcal* count according to ICMSF (1996).

2.5 *Pseudomonas* and *Aeromonas* count as recommended by Collins *et al*. (1995).

2.6 *Staphylococcal* count (coagulase positive) according to Nathalie and Gueguen (1997).

2.7 Total *mold* and *yeast* count were perfomed according to Gourama and Bullerman, (1995). Isolated *molds* were subjected for identification according to their morphological and microscopical characters according to Pitt and Hocking (1997).

2.8 Detection of lipolytic activity of the isolated *molds* according to Setala and Garanina (1986) and Seeley *et al*. (1991).

2.9 Screening of the isolated *Aspergillus flavus* and *Aspergillus parasiticus* strains for aflatoxins production according to (Abramson and Clear, 1996).

**3. Isolation and identification of some pathogenic microorganisms**

3.1 Isolation of *Salmonella* according to D'Aust (1991).

3.2 Isolation of *Listeria monocytogenes* according to Fedio and Jackson (1992).

3.3 Isolation of *Bacillus cereus* according to Jenson and Moir (1997).

**4. Data analysis**

The obtained results were analyzed using computer program (SPSS and Excel office 2000).

## RESULTS

**TABLE 1**: Organoleptic properties of raw camel milk samples

| Criteria | Score | ± S.E.M. |
|---|---|---|
| Flavor and odor (45) | 44 | 0.01 |
| Color (20) | 19 | 0.14 |
| Bacteria (35) | 31 | 0.10 |
| Mean Acidity % | 0.16 | 0.01 |
| Mean pH value | 6.70 | 0.1 |
| Resazurine test | Lilac color | - |

**TABLE 2:** Total colony count in the examined raw camel milk samples (cfu/ml) (N* =50).

| Total colony count | No. positive | % | Min | Max | Mean | S.E.M. ± |
|---|---|---|---|---|---|---|
| PC | 20 | 40 | 10 | $7.3x10^4$ | $6.6x10^3$ | $3.3x10^2$ |
| MC | 35 | 70 | 20 | $6.6x10^6$ | $9.1x10^4$ | $4.2x\ 10^2$ |
| TC | 8 | 16 | 10 | $2.5x10^4$ | $4.7x10^3$ | $1.3x10^2$ |

* = Number of samples; PC = Psychrophilic count, MC= Mesophilic count, TC = Thermohilic count.

**TABLE 3:** Microbiological examination of raw camel milk samples (cfu/ml) (N* =50).

| Tests | No. | % | Min | Max | Mean | ± S.E.M. |
|---|---|---|---|---|---|---|
| *Aerobic spore former* | 15 | 30 | 10 | $5.1x10^5$ | $7.8x10^3$ | $0.1x10^2$ |
| *Coliform* count | 12 | 24 | 40 | $8.2x10^5$ | $9.5x10^4$ | $2.6x10^3$ |
| Total *Enterocoocai* count | 10 | 20 | 10 | $7.1x10^4$ | $3.2x10^3$ | $0.04x10^2$ |
| *Pseudomonas* & *Aeromonas* | 11 | 22 | 10 | $9.4x10^4$ | $4.1x10^3$ | $1.1\ x10^2$ |
| *Stapylococcus c*ount | 9 | 14 | 10 | $6x10^4$ | $5.3x10^3$ | $1.5\ x10^2$ |
| Total *mold & yeast* count | 50 | 100 | 10 | $4.7x10^6$ | $6\ x10^5$ | $2.9\ x10^3$ |

* = Number of samples

**TABLE 4:** Incidence of isolated mold strains and their lipolytic activity in the examined camel milk samples (N* = 50)

| Isolated strains | No. | % | Lipolytic activity | |
|---|---|---|---|---|
| | | | No. positive | % |
| Genus *Aspergillus* | 38 | 70 | 29 | 76.3 |
| *A. flavus* Link | 15 | 28 | 13 | 34.2 |
| *A. parasiticus* Speare | 13 | 26 | 10 | 26.3 |
| *A. sydowii* (Bain &Start) Thom | 7 | 14 | 5 | 13.2 |
| *A. ustus* (Bain &Start) Thom | 3 | 6 | 1 | 2.6 |
| Genus *Penicillium* | 27 | 54 | 22 | 81.4 |
| *P. aurantiogriseum* Dierckx | 11 | 22 | 10 | 37.0 |
| *P. funiculosum* Thom | 9 | 18 | 9 | 33.3 |
| *P. purpurogenum* Stoll | 6 | 12 | 3 | 11.1 |
| *P. viridicatum* Westling | 1 | 2 | 0 | 0 |
| Genus: *Acremonium* | 16 | 32 | 9 | 56.3 |
| *A. fusidiodes* w. Gams | 10 | 20 | 5 | 31.3 |
| *A. strictum* w. Gams | 6 | 12 | 4 | 25.0 |
| Genus *Alternaria* | 15 | 30 | 13 | 86.6 |
| *A. alternata* Keissler | 9 | 18 | 8 | 53.3 |
| *A.tenuissima* (Kunze ex Peris) Wilt | 6 | 10 | 5 | 33.3 |
| Genus *Chrysosporum* | 11 | 22 | 8 | 72.7 |
| *C. carmichilli* van oorschot | 7 | 14 | 6 | 54.5 |
| *C. keratinophilium* Frey carmichael | 4 | 8 | 2 | 18.2 |

* = No. of samples.

The organoleptic properties of raw camel milk samples were shown in Table (1). The examined samples were generally opaque-white in color with sweet watery taste. The flavor & odor, color and bacteria were scored 44 ± 0.01, 19 ± 0.14 and 31 ± 0.10 points respectively. Fresh raw camel milk samples have mean pH value 6.70 ± 0.1 while the mean acidity % was 0.16 ± 0.01. The resazurine test was lilac in color. The results given in Table (2) revealed that total *Psychrophilic, Mesophilic* and *Thermophilic* count were detected in 40%, 70% and 16% of the examined camel milk samples with a mean values $6.6x10^3$ $\pm 3.3x10^2$, $9.1x10^4$ $\pm$ $4.2x10^2$ and $4.7x10^3$ $\pm$ $1.3x10^2$ (cfu/ml) respectively. The results shown in Table (3) demonstrate that *Aerobic spore former* bacteria were isolated from 30% of the examined camel milk samples with a mean value of $7.8x10^3$ $\pm$ $0.1x10^2$ cfu/ml. *coliform* were presented in 24% of the examined camel milk samples with a mean value of $9.5x10^4$ $\pm 2.6x10^3$cfu/ml. The *Enteococcaal* count was existed in 20% of the examined camel milk samples with a mean value of $3.2x10^3$ $\pm$ $0.04x10^2$. *Pseudomonas* and *Aeromonas* count could be detected in 22% of the examined camel milk samples with a mean value of $4.1x10^3$ $\pm$ $1.5x10^2$ cfu/ml. Coagulase positive *Staphylococcus* was isolated from 14% of the examined camel milk samples with a mean value of $5.3x10^3 \pm 1.5x10^2$ cfu/ml. Mold and yeast were isolated from all examined camel milk samples with a mean value of $6x10^5$ $\pm$ $2.9x10^3$ cfu/ml. The data illustrated in Table (4) revealed that the isolated molds were distributed in five genera as follows: Genus *Aspergillus* (70%) which represented by *A. flavus* Link, (28%), *A. parasiticus* Speare (26%), *A. sydowii* (Bain and Start) Thom (14%) and *A. ustus* (Bain &Start) Thom (6%); Genus *Penicillium* (54%) which represented by *P. aurantiogriseum* Dierckx (22%), *P. funiculosum* Thom (18%), *P. purpurogenum* Stoll (12%) and *P. viridicatum* Westling (2%); Genus *Acremonium* (32%) which represented by *A. fusidiodes* w. Gams (20%) and *A. strictum* w. Gams (12%); Genus *Alternaria* (30%) represented by *A. alternata* Keissler (18%) and *A.tenuissima* (Kunze ex Peris) Wilt (10%) and Genus *Chrysosporum* (22%) which represented by *C. carmichilli* van oorschot (14%) and *C. keratinophilium* Frey carmichael (8%). Eighty one (out of one hundred and seven) mold isolates could produce lipase enzymes in a percentage of *A. flavus* Link (34.2%), *A. parasiticus* Speare (26.3%), *A. sydowii* (Bain &Start) Thom (13.2%); *A. ustus* (Bain &Start) Thom (2.6%), *P. aurantiogriseum* Direckx (37%), *P. funiculosum* Thom (33.3%), *P. purpurogenum* Stoll (11.1%), *A. fusidiodes* w. Gams (31.3%), *A. strictum* w. Gams (25%); *A. alternata* Keissler (53.3%), *A. tenuissima* (Kunze ex Peris) Wilt (33.3%), *C. carmichilli* van oorschot (54.5%) and *C. keratinophilium* Frey carmichael (18.2%). None of *A. flavus* and *A. parasiticus* strains proved to be aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ producers. *Salmonella*, *Listeria monocytogenes* and *Bacillus cereus* could not be detected in any of the examined camel milk samples.

## DISCUSSION

Camel milk considered as an important part of the life of desert dwellers providing them a source of protein and energy. Physical and microbiological characters are the main factors, which measure the camel milk quality. The data illustrated in Table (1) revealed that the organoleptic properties of raw camel milk samples were good and within the acceptable level. Nearly similar findings were reported by Mohamed, (1990); Elamin & Wolcox, (1992) and Farah, (1993). The type of feed and the availability of drinking water may affect the physical properties of camel milk (Robinson, 1990; Wilson, 1997 and Landis, 2004). The results given in Table (2) revealed that total *Psychrophilic, Mesophilic* and *Thermophilic* count were detected in 40%, 70% and 16% of the examined camel milk samples. The camel milk samples contain higher *Mesophilic* than *Psychrophilic* and *Thermophilic* count. Nearly similar findings were reported by Salwa and Heidy, (2003). Higher results were recorded by Sallam and Nagah (1993) while lower data was obtained by Elamin and Wolcox, (1992). This might be due to the physical environmental condition of dessert, which allows most of milk flora and pathogenic bacteria to grow and multiply (Varnam and Sutherland, 1994 and Ray, 1996). There is no Egyptian standard for raw camel milk. However, total colony count not to exceed 500000 cfu/ml is widely accepted standard for bovine milk (Boor and Murphy, 2002). Therefore comparing our results with the standard, it revealed that most of the examined samples proved to be satisfactory. Results shown in Table (3) demonstrate that *Aerobic spore former* bacteria were isolated from 30% of the examined camel milk samples. Nearly similar findings were reported by Salwa and Heidy (2003) and Lora *et al*., (2005). Higher count were reported by Al-Mohizea (1986) and Sallam and Nagah, (1993*). Aerobic spore former* bacteria are widely distributed in nature and may gain access to milk through various routes including air, water and utensils (John and Despencer, 2001). *coliform* were presented in 24% of the examined camel milk samples and may be used as indication of good milk sanitation. The presence of more than 750 coliform/ml indicates bad hygienic measure during milk production, handling and distribution. Moreover, contamination of raw camel milk with *coliform* might induce economical and public health hazard (WHO, 2000 and Boor & Murphy, 2002). The *Enteococcaal* counts were existed in 20% of the examined camel milk samples. They are normal inhabitants of the alimentary tract of man and animal and their presence indicates fecal contamination. Also sometimes food poisoning may occur particularly if milk heavily contaminated (Ray, 1996). *Pseudomonas* and *Aeromonas* count could be detected in 22% of the examined camel milk samples. They are commonly associated with milk spoilage through production of heat stable enzymes, protease and lipase inducing off-flavor and shorten shelf life time of milk (Celestino *et al*., 1996). Coagulase positive *Staphylococcus* was isolated from 14% of the examined camel milk samples. These results agreed to certain extend with those reported by Barbour *et al*., (1985) and Lora *et al*., (2005). It is potential pathogens causing mastitis and has been implicated in many foods borne intoxication associated with consumption of raw milk (Mostafa *et al*., 1987). Mold and yeast were isolated from all camel milk samples (Table 3). Genus *Aspergillus*, *Penicillium*, *Acremonium*, *Alternaria* and *Chrysosporum* could be isolated from the examined raw camel milk

samples at percentages of 70%, 54%, 32%, 30% and 22% respectively. Eighty one strains (out of one hundred and seven) could produce lipase enzyme in a percentages ranged from 2.6% - 54.5% which hydolyzed the fatty compound tween 80 (poly exoethylene sorbitan monooleated) forming oleic acid precipitated as calcium oleate (Table 4). Lipase production differs not only among the different mold species but also among the isolates in the same species. *Aspergillus flavus* Link, *P. aurantiogriseum* Dierckx, *P. funiculosum* Thom, *A. fusidiodes* w. Gams, *A. alternata* (Kunze ex Peris) wilt and *C. carmchilli* van oorschot isolates exhibited the highest lipase production. Nearly similar findings were reported by Braun *et al*. (2002) and Layer and Keller (2004). The examined camel milk samples showed high incidence of molds which may be attributed to either their widespread distribution in nature or their high ability to adopt at wide range of environmental conditions. They also may constitute part of normal flora of camel wool (Bagy and Abdel- Hafz, 1985; Laila et al., 1998 and Jakobsen, 2004). The presence of relatively high incidence of molds in raw camel milk could constitute a public health hazard or undesirable changes in milk. Lipolytic molds may cause spoilage through production of lipase enzyme which hydrolysis triglycerides into monoglycerides, diglycerides and free fatty acids leading to off- flavor (Sawaya *et al*., 1984; Hubbert *et al*., 1996; Günther *et al*., 2001 and Layer & Keller, 2004). The high level of lipase enzyme may be responsible for temporal bitterness noted in some soft cheese manufactured from camel milk (Farah and Streiff, 1994; Abu-Tarboush *et al*., 1998; Al-Saleh and Zahran (1999); Giardet *et al*., 2000; Attia *et al*., 2001 and Saima, *et al*., 2003). None of the screened isolates of *A. flavus* and *A. parasiticus* proved to be aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ producers. Although, they are worldwide distribution in nature, there are many factors restrict their aflatoxin production in camel milk. The most important factors for aflatoxin production are moisture, relative humidity and temperature. The optimal growth of *A. flavus* and *A. parasiticus* occur at 37- 40°C while the optimal aflatoxin production occurs at 24-29°C. Therefore raw camel milk samples are suitable for growth of fungi but not suitable for aflatoxin production (Abramson and Clear, 1996; Beuchat *et al*., 1998; Chapman, 2003 and Jakobsen, 2004). The ability of camel milk to inhibit the growth of pathogenic bacteria through the high lysozymes, lactoperoxidase, immunoglobulin and N-acetyl glucosaminidase (NAGase) were reported by several authors (Al-Nakli, 1984; Farah, 1993; Elagamy *et al*., 1996; Kappeler *et al*., 1998; Baars, 2000 and Merin *et al*., 2001). This may explain why *Salmonella*, *Listeria monocytogenes* and *Bacillus cereus* could not be recovered in all the examined camel milk samples. Nearly similar finding were reported by Conesa *et al*. (2001); Salwa and Heidy (2003) and Lora *et al.* (2005).

## CONCLUSION

The result of our study clearly showed the importance of raw camel milk examination, as revealed potential problems associated with milk quality. Although camel milk does not have pathogenic microorganisms but still harbor economical and public health hazard to the consumer. Therefore, good sanitation and hygiene during milking and handling of raw camel milk are important factors to prevent milk spoilage and protect the consumer health.

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