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CHEMICAL PROPERTIES AND MICROBIOLOGICAL QUALITY FOR HANDLING WHITE CHEESE AND EFFECT OF GOAT AND CAMAL MILK ON SOME PATHOGENIC BACTERIA

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ABSTRACT

In this study 30 samples each packet and unpacked white cheese (high, middle and low salt white cheese) were collected from different retail markets in Cairo. All examined samples were evaluated chemically and microbiologically. The results revealed that, highest contents of protein, carbohydrate, Ca and P were 12.70, 7.00, 1.72 and 1.85% in high salt packed white cheese, low salt unpacked white cheese, high salt unpacked white cheese and low salt packed white cheese respectively. Lowest contents of fat, fiber, ash, moisture ,NaCl. and pH value represented 21.70, 8.00, 1.29, 41.39, 0.85% and 3.52 in low salt packed white cheese , high salt unpacked white cheese, low salt unpacked white cheese, high salt unpacked white cheese , low salt unpacked white cheese, and low salt packed white cheese respectively. The highest total bacterial counts was similar in all examined samples as ranged from $6x10^4$ to $5.5x\ 10^6$ cfu/g in middle salt packed and unpacked white cheese thalaga samples. High salt packed (estanpoly) and low salt packed white cheese samples were free from total coliforms and *faecal coliforms* counts .*E. coli* was isolated from 90% and 70 % of unpacked (thalaga) and low salt white cheese, while 80% and 60% of packed and unpacked low salt white cheese (thalaga) respectively were contaminated with *Staph. aureus.*The camel milk showed most antibacterial effect more than goat milk against *Staph. aureus, E.coli* and *L. monocytogenes* .All the examined white cheese samples were free from aflatoxin M1, (AFM1).The fungal isolates from Estanpoly cheese were, *Aspergillus niger, Aspergillus candidus, Aspergillus fumigates, Fusarium copactum Fusarium spp. Emericella nidulans* and *Rhizopus spp.*

INTRODUCTION

Cheeses although they have been characterized, as one of the safest food products by some authors Little *et al*., (2008). In 2006 the consumption of contaminated cheese accounted for the 0.4% of the total food- borne outbreaks in EuropeEuropean (2006). Furthermore, the scientific literature has reported severe food posing outbreaks associated with various types of cheese Maria *et al.*, (2010). Cheeses are ready to eat (RTE) food products that do not undergo any further treatment to ensure their safety before consumption. Contamination of cheese with food- borne pathogens may occur at several stages. Moreover, the need for knowledge about the vectors and the routes of contamination into RTE food and quantitative data on recontamination to accurately establish microbial risk assessment has been also addressed Reij and Den Antrekker, (2004). A comprehensive reported on the presence of *L.moncytogenes* in RTE food has been conducted by

Lianou and Sofos, (2007). Various types of cheeses were contaminated with *L.monocytogenes*. *Staph. aureus*, *Salmonella spp*. *E coli* O_{157} H_7 Conedera *et al*., (2004). *E. coli* O_{157} H_7 is an emerging food- borne pathogen that has caused more food borne outbreaks related to consumption of raw and pasteurized milk Upton and Coia, (1994), compared to cheese. One inspection in Canada associated with the consumption of raw milk hard cheese Honish *et al*.,(2005) and another outbreak linked to fresh unpasteurized goat's cheese in France Espie *et al*., (2006). Microbial contamination of cheese may originate from various sources. Such sources during cheese production might be: starter culture, brine, flour and packaging material, cheese vat, cheese cloth and curd cutting knife, cold room and production room airTemelli *et al.* ,(2006) .Storage cooler have been also demonstrated to be the source of *L.monocytogenes* contamination of cheese made from pasteurized milk Brito *et al*., (2008). Contamination of milk cheese with *Staph aureus* by food handlers was also reported by Callon *et al*., (2008) and several studies have focused on the behavior of *L.monocytogenes* in a variety of cheeses including soft ripened. Contamination of raw milk with the pathogenic bacteria from the farm environment is also well documented Callon *et al.*, (2008)in particular *Listeria, Salmonella* and *E.coli* shed in the faces Van kassel *et al*.,(2004) prevalence of faced shedding of *Listeria spp*. has been, found to very substantially, with reported prevalence from 2% to 52% Husu, *et al*, (1990). The prevalence of food- borne pathogens in raw milk influenced by numerous factors such as farm size, number of animals on the farm, hygiene, farm management practices, geographical location, season but also types of samples evaluated differences in detection methods and variation on sampling Oliver *et al*., (2005). Cheese contaminated by bacteria that can survive outside the product, on equipments and storage facilities is well documented Mclauchlin, *et al*., (1990). Low pH and salt size two of most important factors contributing to inactivation of bacterial pathogens during the 60days curing process Johnson *et al*., (1990). Cheese made from goat's milk had a significantly ($p<0.05$) lower coliform and coagulase positive staphylococci as compared to cheese made from the untreated control goats milk Seifu *et al.,(* 2004). Aflatoxin M_1 (AFM) in the main of aflatoxin B_1 metabolite found in the milk of lactating animals that have ingested contaminated feed the population can therefore be indirectly exposed to aflatoxins by the consumption of milk a milk products such as cheese Pieter *et al.* (1997),the presence of AFM, in milk and milk products is considered to be undesirable because is a toxic and carcinogenicmetabolite Saitanu, (1997). If raw milk contains AFM, cheese made from such milk will also contain AFM, lopez(2001). Egyptian authorities have adopted 0.0 mg/ kg^{-1} of AFM, as the maximum residue limit from milk and milk products such as cheeseEgyptian Regulation (1990).The European Commissions EC(2006) have adopted 50 mg kg-1 as the maximum residue limit for milk and milk products. In this survey to detect the fungi flora, a wide variety of microorganisms are associated with cheese the kinds and abundance of these microorganisms depend on factors such as the climate, storage conditions, and the portion of the milk of which the products are composed. Hence, some molds growing in some materials under some conditions can

produce toxic compounds. Fungal toxins produce a wide range of injurious effects in animal, in addition to serving as food-borne hazards to humans. Lund (1996) showed that *Penicillium commune* and related species found in air samples in a cheese factory. Larsen (2003) found that, the cheese-associated fungi *penicillium commune, P. roqueforti, P.solitum, P. discolor* and *Aspergillus versicolor* have been investigated for production of volatile trepans for chemical identification, when grown on yeast extract agar. Lund *et al.* (2005) isolated and identified fungi from hard, semi-hard and semi-soft cheeses from Denmark, France, Greece, UK and other countries, 371 fungal isolates were identified of which 91% were *Penicillium* species. *Penicillium commune* was the most widespread and most frequently occurring (42%) species. Most of the isolates (88%) found on cheese belonged to the following species: *P. commune, P. nalgiovense, P. verrucosum, P. solitum, P. roqueforti, Aspergillus versicolor, P. crustosum, P. atramentosum, P. chrysogenum* and *P. echinulatum*. Mycological investigations in cheese factories showed that control of cheese smear contamination was important in an attempt to prevent mould growth on cheese. Some species showed a consistent ability to produce mycotoxins: *P. commune* produced cyclopiazonic acid, *P. verrucosum* produced ochratoxin A, *A. versicolor* produced sterigmatocystin and *P. crustosum* produced roquefortine C. Lund(2003)found that the diversity and distribution of *Penicillium commune* contaminants in two different cheese dairies, swab and air samples were taken from the production plants, the processing environment and contaminated cheeses. A total of 321 *Penicillium commune* isolates were characterized using morphotypes (colony morphology and colors) and secondary metabolite profiles. Based on production of secondary metabolites the *P. commune* isolates were classified into 6 groups. Pitt *et al.* (1986)revealed that moulds used in the manufacture of white cheeses, which are all classified in *P. camembertii*, and which also produce cyclopiazonic acid, are domesticated fungi derived from *P. commune*.

The objective of this study is to determine of microbial load and chemical composition of processed white cheese.

MATERIALS AND METHODS

Thirty random samples of each packed and unpacked white cheese were collected in sterile bags from different local markets and transported directly to the laboratory in ice box for bacteriological and chemical analysis , each samples were divided into 3 groups. The first group included, high salt white cheese Estanpoly the second group included middle salt, white cheese, Thalagha and the third group included low salt white cheese.

Bacteriological examination:

Each group was thoroughly mashed in a sterile mortar where eleven gram of prepared cheese sample were aseptically homogenized with 99 m/ of sterile2% sodium citrate solution at 40° C, sterile dilutions of the homogenates were prepared. The following analyses were performed Total bacterial count was carried out according to Berrang *et al.*, (2001).

Total coliform and faecal coliform counts were carried out according to Mercuri and Co (1979).

Total yeasts and moulds counts were carried out according to NMKL (1999).

Isolation of *E. coli* was carried out according to Collins *et al*., (1998). *E. coli* identification was carried out according to Kreig and Holt (1984) .

Isolation of *Staphylococcus aureus* was carried out according to Gouda (2002). Isolation of Campylobacter was carried out according to Osterom *et al.,* (1983).

Isolation of *Listeria monocytogenes* was carried out according to USDA-FSIS (1989).

Standard Inoculums:

Standard inoculums were prepared by inoculation of conical flask (100ml in volume)containing 50 m1 of tryptic soy broth (TSB) pH 7.2 with loop of *Listeria monocytogenes* isolates was incubated for 24hr at 30° C and another flasks containing 50ml of 1% buffered peptone water (pH7.2) with loop of *E.coli* or *Staph aureus and incubated* for 24 hr at 37° C. Achieved viable cells count were determined by serial dilution and subsequent enumeration on a palcam agar for *L.monocytogenes*, vogel-johnson agar for *Staph .aureus* and MacConkey agar for *E.coli.*

Effect of camel and goat milk on growth and survival of *Staph. aureus*, *E coli and L.monocytogenes*.

***In vitro*:**

Erlenmeyer flask (250ml) contained 50ml of trypticase soy broth 0.6% yeast extract were inoculated with 1ml of *L.monocytogenes* incoulum containing about 10^{13} cfu/ ml then added 1ml of each camel or goat milk to the flask separately. The flasks were incubated at 30° C for 24 hr on rotary shaker (100 rpm). Beside another flasks contained 50 ml of 1.0% buffered peptone water (pH 7.2) were inoculated with 1ml of each *Staph. aureus* inoculums containing about 10^{11} cfu/ ml or *E. coli* inoculums containing about 10^{12} / ml then added l ml of each camel or goat milk to the flask separately. The flasks were incubated at 37° C for 24hr on rotary shaker (100rpm). The controls without any addition of camel or goat milk were inoculated with each bacterial strain with the same experimental condition mentioned before.

Antimicrobial assay using the diffusion method:

The disc diffusion method Ericsson and sherries (1971) was used in screening of camel and goat milk for antimicrobial activity. The Petri plates were poured with 20 ml of either nutrient agar for *Staph. aureus* and *E. coli* or tryptic soy agar supplemented with 0.6% yeast (TSAYE) extract for *L.monocytogenes*, after inoculated it with microbial cultures. The microorganisms and growth media were mixed thoroughly to ensure uniform distribution of the microorganisms. All experiments were performed in duplicate. Sterilized filter paper discs (whatman type 1- 0.6cm in diameter) were placed on the surface of each nutrient agar TSAYE. Each one of the antimicrobial substances was tested by addition 100 µl of each camel and goat milk to each filter paper disc. Plates for antimicrobial activity test were incubated at 30° C for 24-48hr. the experiment was done three times. The

inhibition zone diameter was measured (including the filter paper disc 6 mm in diameters) using vernier callipers and expressed in millimetres.

Isolation and identification of fungi.

The isolated fungi were purified using hyphal tip techniques Riker and Riker, (1936), and then identified according to their morphological , macroscopically characters by used different media Czapek yeast autolysate agar medium (CYA) used for purification and identification of *Penicillium* spp., Czapek agar (CZ) medium used for identification of *Aspergillus* spp., potato sucrose agar (PSA) medium used for identification of *Fusarium* as described by Jens *et al.*, (1991) and confirmed by Fungal Taxonomy Dept., Plant Pathology Institute, ARC, Egypt.

Extraction and quantification of aflatoxin M1(AFM1).

The method used to extract AFM1 from cheese was carried out by Dragacci *et al*. (1995).

Determination of chemical compositions

Moisture, protein, fat, ash and sodium chloride were evaluated according to A.O.A.C (2006) carbohydrates were calculated by difference between moisture, protein, fat and ash

Calcium:

Calcium was determined by EDTA titration according to the method described in A.O.A. C(2006).

Phosphorus:

Ammonium molybeidate titration method was used for phosphorus determination as described in the A.O.A.C. (2006).

Measurement of pH value and titratable acidity:

The value of pH was measured and titratable acidity was determined according to Ling (1963).

RESULTS AND DISCUSSION

Chemical compositions and minerals contents of white cheese samples:

Data recorded in Tables(1-6) showed that high contents of protein, carbohydrate, Ca and P represented 12.70, 7.00, 1.72 and 1.85% in high salt packed white cheese, low salt unpacked white cheese, high salt unpacked white cheese and low salt packed white cheese respectively. Low contents of fat, fiber, ash, moisture. NaCl and pH value represented 21.70, 8.00, 1.29, 41.39, 0.85% and 3.52 in low salt packed white cheese, high salt unpacked white cheese, low salt unpacked white cheese and high salt unpacked white cheese, low salt unpacked white cheese low salt packed white cheese respectively.Obtained results nearly similar to those recorded by Mahfouz *et al*., (1986),whereas chemical composition of produced cheeses were fat in DM, 19.7 to 60.0%, Nacl in DM 6.2 to 7.9%, pH 5.2 to 5.7. Turkoglu *et al*., (2003)mentioned that the moisture, fat, protein, salt and acidity percentage in cheese were 52.25, 17.86, 19.96, 5.32 and 1.11% respectively. Patr (2000) reported that the pH value and acidity of cheese value were 4.92 and 1.09% respectively, the values for moisture and salt content in dry matter of the

cheese samples were 42.51 and 8.02%. Muir *et al.*,(1999) recorded that fat, protein and carbohydrate contents of processed cheese ranged from 12.3-22.8, 11.6-14.4 and 3.2-9.0g/100g respectively. Consuming more sodium than is recommended can raise blood pressure. Reducing sodium consumption to within recommended limits also helps those with hypertension to get and keep their blood pressure under control ,even if a person does not have high blood pressure, reducing sodium intake is important because the lower one's blood pressure in general, the lower the risk for heart disease and stroke. Because nearly 400,000 deaths each year are attributed to high blood pressure, reducing sodium intake could prevent many thousands of deaths annually Danaei *et al*., (2009) .

Table (1): Chemical composition of high salt packed white cheese (Estanpoly).

Samples	Protein %	Fat %	Fiber %	Ash %	Moisture %	Carbohydrates %	Ca %	P %	NaCl %	pH
1	12.5	27.5	8.08	4.30	44.15	3.07	1.16	0.82	3.89	4.34
2	11.9	23.47	8.16	4.32	50.72	1.45	1.58	0.93	3.05	4.09
3	10.9	28.45	8.12	4.31	45.66	2.56	1.19	0.74	3.97	4.22
4	12.70	24.20	8.08	4.42	47.39	3.21	1.38	0.94	2.15	4.12
5	10.50	24.39	8.13	4.40	49.62	2.96	1.32	0.88	3.79	4.33
6	11.30	28.23	8.11	4.35	48.00	1.91	1.10	0.80	2.28	4.40
7	6.20	25.33	8.43	5.83	47.70	6.51	0.53	0.43	4.50	3.71
8	5.52	24.22	9.23	11.30	48.31	1.36	0.63	0.10	9.15	3.67
9	5.79	24.12	8.50	7.29	49.50	4.80	0.79	0.74	6.08	3.90
10	9.70	27.22	8.63	5.84	46.61	2.94	0.77	0.67	4.08	4.21

Table (2): Chemical composition of high salt unpacked white cheese (Estanpoly).

Samples	Protein %	Fat %	Fiber %	Ash %	Moisture %	Carbohydrates %	Ca %	P %	NaCl %	pH
1	5.88	24.42	8.60	9.07	47.79	4.24	0.51	1.27	8.28	5.80
2	9.55	27.30	8.77	8.93	44.44	1.01	1.33	0.76	7.87	4.05
3	10.30	26.22	8.10	10.85	43.38	1.15	1.41	0.80	10.15	4.07
4	9.50	26.07	8.09	10.00	44.38	1.96	1.42	0.79	9.05	4.09
5	10.00	27.30	8.01	11.70	40.45	2.54	1.36	0.77	10.05	4.05
6	10.00	25.02	8.05	10.60	43.48	2.85	1.43	0.83	9.70	4.13
7	9.70	25.29	8.19	10.02	44.47	3.03	1.72	0.82	9.68	4.11
8	10.50	24.12	8.63	11.39	42.36	3.00	1.56	0.87	10.30	4.08
9	10.00	26.10	8.00	11.30	41.39	2.65	1.39	0.80	8.90	4.00
10	9.65	26.28	8.65	9.45	44.45	2.02	1.46	0.79	8.72	4.08

The 2005 Dietary Guidelines for Americans recommend limiting sodium to less than 2,300 mg per day (equal to about 1 teaspoon of table salt). The guidelines further recommend that specific populations (blacks, people with high blood pressure, and middle-aged and older adults) limit their intake to 1,500 mg per day (equal to about 2/3 teaspoon of table salt CDCP (2009). Castelo *et al*., (1996). mentioned that mean chemical composition in cheese was 36.53% moisture, 25.41% protein, 32.42% fat, 4.24 ash and 1.87% NaCl. Digrak *et al*., (1994). showed that mean value for chemical composition were fat 27.26, protein 16.91%, total ash 5.22% and salt 3.44%. Nazem and

Saleh, (1994), reported that the average percentages of acidity, moisture, fat and salt of cheese in DM were 2.22, 23.16, 49.01 and 5.84 respectively. Diaz-Cinco, *et al*., (1992), found that contained 43.7% fat, 49.2% protein, 2.7% salt and 45% moisture. PH was 5.25 in white cheese. Yalcn, (1986). Recorded that DM content averaged 38.66% with 15.19% fat, 16.12% total protein, 4.38% and 4.79% salt in pickled cheese.

Table (3): Chemical Composition of middle salt packed white cheese (Thalaga).

Samples	Protein %	Fat %	Fiber %	Ash %	Moisture %	Carbohydrates %	Ca %	P %	NaCl %	pH
1	9.40	23.31	8.03	3.84	47.85	3.73	0.49	0.32	1.92	6.67
2	9.50	21.79	8.14	3.86	50.81	2.90	0.49	0.37	1.70	6.68
3	9.30	22.30	8.15	3.88	51.90	4.47	0.39	0.24	2.82	6.63
4	9.00	25.80	8.09	3.90	47.88	5.33	0.86	0.81	2.64	6.60
5	6.50	23.22	8.07	4.38	52.72	5.11	0.44	0.28	2.64	6.66
6	6.90	22.40	8.11	4.32	53.67	4.6	0.78	0.53	2.87	6.62
7	6.30	23.55	8.12	5.98	52.78	3.27	0.67	0.42	2.04	6.65
8	5.50	24.33	8.05	6.98	53.03	2.11	0.70	0.74	5.08	6.60
9	9.40	22.46	8.06	3.85	50.18	2.76	0.84	0.86	1.85	6.65
10	6.50	23.05	8.13	5.76	52.50	4.97	0.64	0.73	2.50	6.70

Table (4): Chemical composition of middle salt unpacked cheese (Thalaga).

Samples	Protein %	Fat %	Fiber %	Ash %	Moisture %	Carbohydrate %	Ca %	P %	NaCl %	pH
1	9.30	25.03	8.90	4.4	47.29	5.04	0.42	0.91	3.35	4.80
2	9.20	25.73	8.22	4.35	47.30	5.20	1.50	0.89	3.32	5.50
3	9.70	28.43	8.55	3.35	44.77	5.20	0.55	1.11	3.32	5.82
4	6.85	25.24	8.67	4.35	49.43	4.50	0.48	1.26	2.20	5.77
5	7.95	26.44	8.87	4.45	47.22	5.07	1.43	1.20	3.26	5.82
6	5.90	25.82	8.93	3.93	48.80	6.62	1.54	1.23	2.21	5.80
7	7.88	27.77	8.29	2.37	47.70	5.55	0.49	1.15	0.0	5.79
8	6.94	26.02	8.88	5.83	46.93	5.40	0.51	1.23	4.01	5.85
9	12.07	26.33	8.04	5.73	45.30	2.52	0.47	1.19	4.71	5.69
10	10.00	27.00	8.09	5.00	48.00	1.91	0.53	1.16	4.91	5.72

Table (5): Chemical composition of low salt packed white cheese.

Samples	Protein %	Fat %	Fiber %	Ash %	Moisture %	Carbohydrates %	Ca %	P %	NaCl %	pH
1	5.40	25.39	8.13	3.95	52.60	4.53	0.67	0.43	2.98	3.63
2	6.20	21.70	8.12	2.63	56.83	4.52	0.65	0.42	2.90	3.63
3	6.30	25.47	8.44	4.17	49.78	5.84	0.66	0.40	1.55	3.95
4	6.40	25.23	8.35	3.53	51.60	4.89	0.59	0.70	4.50	4.03
5	6.40	25.11	9.10	3.77	50.26	5.36	0.77	0.69	3.15	3.69
6	6.50	26.00	9.07	4.40	49.30	4.73	0.89	1.80	2.50	3.72
7	5.79	24.30	8.50	4.65	53.52	4.24	1.13	1.66	2.78	3.92
8	6.20	24.30	8.60	4.40	50.44	6.02	1.31	1.85	3.08	3.52
9	6.10	25.20	8.40	3.65	51.80	4.45	0.88	0.18	2.88	3.59
10	6.60	23.80	8.20	4.35	49.90	5.60	1.10	1.57	3.57	3.80

Table (6): Chemical composition of low salt unpacked white cheese.

Samples	Protein %	Fat %	Fiber %	Ash %	Moisture %	Carbohydrate %	Ca %	P %	NaCl %	pH
1	9.12	28.81	8.22	1.30	48.83	3.72	1.35	1.57	0.0	4.00
2	10.15	26.23	9.00	1.33	46.63	6.66	1.42	0.87	0.0	4.02
3	8.30	28.25	8.45	1.32	46.92	6.76	1.27	0.74	0.0	4.03
4	8.40	27.44	8.17	1.30	47.70	6.99	0.60	1.13	0.0	5.43
5	8.70	25.35	8.20	2.95	48.79	6.01	0.57	1.26	1.92	5.45
6	6.95	25.83	8.87	3.49	47.93	7.00	0.49	1.24	1.57	5.87
7	10.00	25.00	8.74	2.29	48.72	5.30	0.65	1.28	1.14	5.86
8	10.12	25.92	8.95	1.85	47.50	5.93	0.95	0.96	0.85	4.63
9	8.55	26.39	8.19	2.15	47.85	6.65	0.59	1.20	0.95	5.44
10	8.85	26.65	8.19	1.29	48.20	5.35	0.95	1.35	0.0	4.75

M0icrobiological determinations of white cheese samples:

Results recorded in Tables (9,10) showed that T.B.C. ranged from 2×10^3 to 2×10^5 cfu/g and T.F from 5×10^2 to 2×10^5 cfu/g while T.C, F.C, yeasts, *Campylobacter* and *Staph. aureus* were completely disappeared but *L.monocytogenes* and *E.coli* found at 30%and at10% of the examined high salt packed white cheese samples respect. T.B.C. ranged from 5×10^2 to 4.5×10^6 cfu/g, yeasts from 5×10^3 to7×10^6 ,T.F from 2×10 to 1×10^5 cfu/g, while *Campylobacter* was completely disappeared but T.C, F.C, *L. monocytogenes*, *E.coli* were found at 10% and *Staph. aureus* at 20% of the examined high salt unpacked white cheese samples. T.B.C. ranged from 3×10^3 to 10×10^4 cfu/g, T.F from 1×10 to 5×10^5 cfu/g, while yeasts and *Campylobacter* were completely disappeared but TC, FC. *L.monocytogenes* and *E.coli* were detected at 10% and *Staph. aureus* at 80% of the examined middle salt packed white cheese samples Tables (11,12).The results in Tables (13,14) revealed that TBC was ranged from 15×10^2 to 8×10^6 cfu/g, T.C from 7×10^2 to 15×10^5cfu/g, FC. from 9×10 to 2×10^5 cfu/g, total yeasts from 5×10^3 to 10×10^6 cfu/g and T.F from 1×10 to 6×10^5 cfu/g, while *Compylobacter* and *L.monocytogenes* were not detected , whereas *E.coli* was isolated from 90% and *Staph. aureus* from 60% of the examined middle salt unpacked white cheese samples. The examined low salt packed white cheese samples in Tables (15,16) were contaminated with 10% for campylobacter and *L.monocytogenes* and 40% for *E.coli* ,on the other hand T.C, F.C, yeasts and *Staph. aureus* were completely disappeared whereas T.B.C. ranged from 6×10^2 to 7×10^5 cfu/g, T.F from 5×10^2 to 3×10^5 cfu/g.

Regarding to the examined low salt unpacked white cheese samples data in Tables (17,18) showed that TBC was ranged from 7×10^4 to 5.5×10^6 cfu/g, T.C from 5×10^2 to 2×10^6 cfu/g, F.C from 2×10^2 to 4×10^5 cfu/g, total yeasts from 10×10^2 to 12×10^5 cfu/g, and T.F from 4×10^2 to 3×10^6 cfu/g, while *Campylobacter* was found at 60%, *L.monocytogenes* at 10%, *E.coli* at 80% and *Staph. Aureus* at70%.These results are in agreement with those reported by several investigations i.e. the prevalence of *staph aureus* in various types of cheese was reported range from 0% to 25% Maria *et al.,(*2010). However *Staph aureus* pose a risk with respect to staphylococcal food poisoning with concentration levels are higher than 5 $\log_{10}$cfu/g, lair *et al*., (2003) *L.monocytogenes* was found most frequently in

cheese samples obtained from retail outlets, 91 out of 1818 samples (5.0%) tested positive to *L.monocytogenes* compared with 29out of 1936 (1.5%) milk samples Greenwood *et al.,(*1991) Differences in prevalence rates of *L.monocytogenes* between cheese made from raw milk and pasteurized milk have been documented Beckers, *et al*., (1987) Loncarevic *et al*.,(1995) Higher prevalence of the pathogen has been reported in soft and semi-soft cheese compared with hared cheese Filiousis *et al*.,(2008) The *coliform* bacteria counts in cheese are expected to decrease during ripening due to low pH and antagonistic action of lactic acid bacteria Babel, (1977) Total bacterial count (T.B.C.), *coliforms*, *E.coli*, *staphylococcus aureus* and *psychrotrophic* bacterial counts significantly ($p > 0.05$) decreased during storage, while yeasts and moulds increased as storage time progressed Osman and Omer(2008) *E.coli* o_{157}: $_{H7}$ has not been detected in any of the 37 cheese samples in a survey conducted in 15 dairy processing plants in U.S.A Ansay and Kaspar(1997) Doyle *et al*.,(1987) found that *L.monocytogenes* survived in high temperature short time pasteurization. Belessi *et al*.,(2008) reported that survival of *L.innocua* in feta cheese when addition al cheese contamination with high loads of fungi increased p/t of Feta cheese. Raw milk is a potential source of cheese contamination Andre *et al*.,(2008).

Tondo *et al*. ,(2000) found that 10 samples out of 3200 dairy products were *Staph aureas* positive, 90.4% (19/21) samples of raw milk were contaminated with *Staph. aureus* and 35.2% (19/51) of food handlers were asymptomatic carriers of *Staph aureus*. *Staph..aureus* is considered one of the most prevalent pathogen causing intra-mammary infections in dairy cows Nickerson, (2002) and may be excreted in milk in level up to 8 $\log_{10}$ cfu/ml Sanaa *et al* .,(1993)*E.coli* O_{157}: H_7 infections have not been documented, Van Kessel *et al*., (2004). The pathogens spread from cow to cow through the milking machines, the milking installations and applied milking practices Bergonier *et al*., (2003). Positives samples *E.coli* O_{157} H_7 have not found in any of the 739 cheese samples made of raw milk purchased from retail premises in a survey conducted in Scotland during the period 1997-1999 Coia *et al.,* (2001).In Feta cheese Litopoulou *et al.*, (1993); Vafopoulou *et al.,* (1990) or in white – brined cheese made from caprine milk Tzanetakis and litopulou, (1992), yeasts are not among the predominate micro floras; by contrast , Yanai *et al*., (1977) reported that coliforms were found in white - brined cheese. Turkoglu *et al.*,(2003) and Patr *et al*., (2000). Reported that coliforms staphylococcus and yests and moulds in cheese samples collected in elazg turkey were 9.8×10^6, 4.4×10^5 and 2.9×10^5 cfu/g, respectively. Castelo, *et al*., (1996) recorded that mean mircrobial counts (log cfu/g) were 7.88 aerobic mesophils and 3.52 yeasts / moulds but no faecal coliforms and *Salmonella spp*. Digrak *et al*., (1994). Showed that mean value for count of microorganisms in cheese obtained from count of microorganisms in cheese obtained from shops were (perg) total count 1.8×10^9, *Staphylococcus aureus* 3.5×10^4 yeasts and fungi 3.6×10^6 and *listeria monocytogenes* 3.2×10^4. Counts of *coliform* bacteria ranged from 240 to 2400/g. Yalcn, (1986). Reported that total bacterial count and *coliforms* in pickled cheese were 2.7×10^8 and 3.1×10^6/g, respectively. Results of occurrence and frequency of the associated fungi are presented in high salt in packed cheese samples factory

(Estanpoly)Table (7). Identification trials showed that the isolated fungi belong to4 genera such as *Aspergillus niger, Aspergillus cndidus,Fusarium spp.,Rhizopus spp. and Emericella nidulans.* Moreover *Emericella nidulans* was found only in sample no.4 these results were harmony with Lund *et al.,* (2005),Luís *et al.,* (2002) and Larsen(2003) . On the other hand the associated fungi are presented in middle salt in a cheese samples (Thalaga),Table (11) results indicated that the isolated fungi were identified *Aspergillus niger, Aspergillus fumigates, Fusarium copactum* from 10 collected samples, no difference was observed between fungi flora in industrial dairy farms middle salt and white cheese samples factory Table (15) mycological investigations in cheese factories showed that control of cheese smear contamination was important in an attempt to prevent mould growth on cheese Abbas and Dobson(2011) Luís *et.al* (2002). Some species showed a consistent ability to produce mycotoxins such as *A. versicolor* produced ochratoxin A. isolates fungi with a common origin and the distribution of these isolates in the processing environment indicated possible contamination points in the cheese dairies Lund *et al.*(2003) and Karan *et al* (2005). Data presented in Table (9) showed that isolated fungi from (Estanpoly) such as *Penicillium Spp., Aspergillus cndidus, Aspergillus niger, Aspergillus ochraceus Aspergillus spp.* and *Aspergillus fumagatus.* The moulds used in the manufacture of white cheeses, which are all classified in *P. camembertii*, and which also produce cyclopiazonic acid, are domesticated fungi derived from *P. commune* Pitt *et al.,*(1986).and Ayres *et al.,* (1980) Some species that belong to these genera are known as potential producers of different toxic substances such as ochratoxins that exhibit toxic, mutagenic, teratogenic and carcinogenic effects in humans and animals. Our results revealed that, *Aspergillus ochraceus* was found in two samples No.1and No.3this species is ability to produce mycotoxins as ochratoxinTable(13),these findings are in harmony with Lund *et al.*(2003). On the other hand, the rest of isolated fungi such as *Fusarium copactum , Fusarium graiminum, Alternaria Spp. Penicillium Spp., Aspergillus cndidus. Aspergillus Spp,and* and *Rhizopus spp.*were found to be associated with most of the processing white cheese Table(17), These results are in agreement with those obtained by Abbas and Dobson(2011) Luís *et al.,* (2002). Raw milk contaminated with foodborne pathgens and introduced into dairy processing plant constitute a risk to human health if used unpasteurized for the production of some types of cheeses or in case of cross contamination with pathogens. Infected mammary glands with clinical or subclinical staphylococcal mastitis are the main source of *S.aureus* contamination of raw milk whereas *Salmonella, E.coli* O_{157}: H_7 and *L.monocytogenes* contaminate raw milk from the farm environment, e.g faces. Thus, to avoid raw milk contamination at farm, good farm practices (e.g.)animal and waste management, water treatment, good hygienic conditions during milking and mastitis control are essential to prevent the accumulation, survival and transmission of pathogens Fox, (1999).

Table (7): Microbial load of high salt packed white cheese (Estanpoly)(cfu/g).

Microorganisms / Samples No.	T.B.C	T.C	Yeasts	F.C	T.F	Identification of fungi
1	2×10^5	–	–	–	2×10^5	*Aspergillus niger* and *Rhizopus sp.*
2	10×10^4	–	–	–	1×10^5	*Aspergillus niger*
3	7×10^3	–	–	–	1×10^5	*Rhizopus spp.*
4	3×10^3	–	–	–	1×10^3	*mericella nidulance* and *spergillus candidus*
5	9.5×10^4	–	–	–	3×10^3	*Fusarium spp.*
6	2×10^4	–	–	–	2×10^4	*Aspergillus spp* and *Aspergillus niger*
7	7×10^5	–	–	–	7×10^2	*Fusarium spp.* and,*Aspergillus niger*
8	2×10^5	–	–	–	3×10^4	*Aspergillus niger* and *Fusarium spp*
9	2×10^3	–	–	–	1×10^3	*Aspergillus niger*
10	16×10^3	–	–	–	5×10^2	*Aspergillus niger*

T.B.C: Total bacterial counts T. C: Total Coliform F. C.: Faecal coliform
T. F.: total fungi

Table (8): Isolation of pathogenic bacteria from high salt packed white cheese (estanpoly).

Microorganisms / Samples No.	*Campylobacter spp.*	*L. monocytogenes*	*E. coli*	*Staph. aureus*
1	–	–	–	–
2	–	–	–	–
3	–	+	–	–
4	–	–	–	–
5	–	–	+	–
6	–	–	–	–
7	–	+	–	–
8	–	+	–	–
9	–	–	–	–
10	–	–	–	–

Positive: (+) Negative: (-)

Table (9): Microbial load of high salt unpacked white cheese (Estanpoly) (cfu / g).

Microorganisms / Samples No.	T. B. C	T. C	F. C	Yeasts	T. F	Identification of fungi
1	4.5×10^5	4×10^5	$3X10^{-5}$	$6x10^{-4}$	$9x10^2$	*Penicillium spp.*
2	4.5×10^6	-	-	7×10^6	$1x10^4$	*Aspergillus candidus*
3	3×10^4	-	-	$5x10^3$	$1x10^4$	*Aspergillus niger*
4	3.5×10^5	-	-	$5x10^5$	$2x10^3$	*Aspergillus niger*
5	5×10^2	-	-	-	$1x10^4$	*Aspergillus ochraceus*
6	4×10^4	-	-	$3x10^{-5}$	$1x10^4$	*Aspergillus spp.*
7	5×10^{-4}	-	-	$4x10^6$	$1x10^5$	*Penicillium spp.*
8	2×10^6	-	-	4x10	2x10	*Aspergillus fumigatus* and *A.ochraceus*
9	3×10^5	-	-	$3x10^5$	$2x10^4$	*Aspergillus spp*
10	4×10^4	-	-	$4x10^4$	$2x10^3$	*Penicillium spp*

T.B.C: Total bacterial counts T. C: Total Coliform F. C.: Faecal coliform
T. F.: total fungi

Table (10): Isolation of pathogenic bacteria from high salt unpacked white cheese (Estanpoly).

Microorganisms / Samples No.	*Campylobacter spp.*	*L. monocytogenes*	*E. coli*	*Staph. aureus*
1	–	+	+	–
2	–	–	–	–
3	–	–	–	–
4	–	–	–	+
5	–	–	–	–
6	–	–	–	–
7	–	–	–	+
8	–	–	–	–
9	–	–	–	–
10	–	–	–	–

Positive: (+) **Negative: (-)**

Table (11): Microbial load of middle salt packed white cheese (Thalaga) (cfu/g).

Microorganisms / Samples No.	T. B. C	T. C	F. C	Yeasts	T. F	Identification of fungi
1	4×10^4	25×10^{-2}	–	–	2×10^2	*Aspergillus spp.*
2	6×10^4	–	–	–	1×10	*Aspergillus niger*
3	5×10^4	–	–	–	5×10^5	*Rhizopus spp.*
4	9×10^3	–	–	–	2×10^4	*Emericella nidulanc* and *A. niger,Aspp.*
5	5×10^3	–	–	–	1×10^4	*Rhizopus spp.*
6	3×10^3	–	–	–	–	*Aspergillus fumigates* and *F.compactum*
7	10×10^4	–	–	–	3×10^4	*Aspergillus niger* and *A.spp.*
8	6×10^4	–	–	–	1×10	*Aspergillus niger and Rhizopus spp.*
9	8×10^4	–	–	–	5×10^4	*Aspergillus niger* and *A.spp.*
10	7×10^3	–	–	–	4×10^3	*Aspergillus niger*

T.B.C: Total bacterial counts **T. C: Total Coliform** **F. C.: Faecal coliform**
T. F.: total fungi

Table (12): Isolation of pathogenic bacteria from middle salt packed white cheese (Thalaga).

Microorganisms / Samples No.	*Campylobacter spp.*	*L. monocytogenes*	*E. coli*	*Staph. aureus*
1	–	+	+	+
2	–	–	–	+
3	–	–	–	+
4	–	+	–	+
5	–	–	–	–
6	–	–	–	+
7	–	–	–	+
8	–	–	–	–
9	–	–	–	+
10	–	–	–	+

Positive: (+) **Negative: (-)**

Table (13): Microbial load of middle salt unpacked white cheese (Thalaga) (cfu/g).

Microorganisms / Samples No.	T. B. C	T. C	F. C	Yeasts	T. F	Identification of fungi
1	$3x10^5$	-	-	-	$2 x 16^3$	*Aspergillus ochraceus.*
2	$10x10^5$	$7x10^{-2}$	-	$5x10^3$	14x10	*Aspergillus. Spp.*
3	$15x10^2$	-	-	$5x10^{-5}$	$3x10^{-2}$	*Aspergillus ochraceus.*
4	$5x10^5$	$7x10^4$	-	$2x10^6$	4x10	*Aspergillus spp.*
5	$16x10^5$	$4x10^5$	-	$10x10^6$	2x10	*Aspergillus spp*
6	$8x10^5$	$9x10^4$	$2x10^2$	-	$6x10^5$	*Emericella nidulans*
7	$18x10^4$	$8x10^4$	-	$5x10^{-5}$	3x10	*Aspergillus spp*
8	$8x10^6$	$4x10^5$	$2x10^5$	$8x10^{-4}$	$1x10^{-4}$	*Aspergillus spp.*
9	$2x16^5$	$15x10^{-5}$	$10x10^{-5}$	-	$1x10^{-4}$	*Emericella nidulans*
10	$3.5 x 10^6$	$13 x 10^5$	9x10	$6x10^5$	1x10	*A.flavus* and *Penicillium spp.*

T.B.C: Total bacterial counts T. C: Total Coliform F. C.: Faecal coliform
T. F.: total fungi

Table (14): Isolation of pathogenic bacteria from middle salt unpacked white cheese Thalaga).

Microorganisms / Samples No.	*Campylobacter spp.*	*L. monocytogenes*	*E. coli*	*Staph. aureus*
1	–	–	–	–
2	–	–	+	+
3	–	–	+	–
4	–	–	+	+
5	–	–	+	–
6	–	–	+	–
7	–	–	+	+
8	–	–	+	+
9	–	–	+	+
10	–	–	+	+

Positive: (+) Negative: (-)

Table (15): Microbial load of low salt packed white cheese (cfu / g).

Microorganisms / Samples No.	T. B. C	T. C	F. C	Yeasts	T. F	Identification of fungi
1	$3x10^3$	-	-	-	$2 x 10^3$	*A.spp* and *F.compactum*
2	$10x10^2$	-	-	-	$14x10^5$	*Fusarium spp.*
3	$7x10^5$	-	-	-	$2x10^3$	*Rhizopus spp.*
4	$29x10^2$	-	-	-	$3x10^5$	*Aspergillus niger ,A. ochraceus*
5	$7x10^3$	-	-	-	$5x10^2$	*Aspergillus niger* and *A. ochraceus*
6	$8x10^2$	-	-	-	$6x10^2$	*Aspergillus niger*
7	$6x10^2$	-	-	-	$2x10^5$	*Aspergillus spp.*
8	$3x10^5$	-	-	-	$1x10^3$	*Fusarium compactum,* and *A spp.*
9	$8x10^2$	-	-	-	$3x10^3$	*Aspergillus niger*
10	$5x10^3$	-	-	-	$2x10^5$	*Aspergillus spp* and *Fusarium spp*

T.B.C: Total bacterial counts T. C: Total Coliform F. C.: Faecal coliform
T. F.: total fungi

Table (16): Isolation of pathogenic bacteria from low salt packed white cheese.

Microorganisms / Samples No.	*Campylobacter spp.*	*L.monocytogenes*	*E. coli*	*Staph. aureus*
1	–	–	–	–
2	–	–	–	–
3	–	–	+	–
4	–	–	+	–
5	–	–	–	–
6	–	–	–	–
7	+	–	+	–
8	–	+	–	–
9	–	–	+	–
10	–	–	–	–

Positive: (+) **Negative: (-)**

Table (17): Microbial load of low salt unpacked white cheese.

Microorganisms / Samples No.	T. B. C	T. C	F. C	Yeasts	T. F	Identification of fungi
1	$8x10^5$	$5x10^2$	$3x10^2$	$12x10^5$	$1x10^3$	*F. compactum* and *A spp.*
2	$2x10^5$	$10x10^3$	$3x10^2$	$4x10^5$	$10x10^4$	*A. niger* and *P. spp.*
3	$15x10^4$	$13x10^2$	$7x10^2$	$14x10^4$	$4x10^4$	*Aspergillus niger*
4	$5.5x10^6$	$16x10^4$	$4x10^4$	$10x10^2$	$9x10^2$	*Rizopus Spp.*
5	$3x10^6$	$2x10^6$	$4x10^5$	-	$3x10^3$	*Pencillium Spp.*
6	$4x10^6$	$8x10^5$	$3x10^5$	$25x10^4$	$12x10^4$	*Alternaria Spp.*and *F.graminum*
7	$10x10^4$	$6x10^4$	$6x10^3$	-	$3x10^6$	*A. candidus* and *F. compactum*
8	$5x10^6$	$4x10^5$	$6x10^4$	$2x10^5$	$2x10^5$	*A. niger* and *Penicillium Spp.*
9	$6x10^5$	$3x10^4$	$7x10^3$	$6x10^3$	$2x10^3$	*F. compactum* and *A. Spp.*
10	$7x10^4$	$5x10^2$	$2x10^2$	-	$4x10^2$	*Penicillium Spp.*

T.B.C: Total bacterial counts **T. C: Total Coliform** **F. C.: Faecal coliform**
T. F.: total fungi

Table (18): Isolation of pathogenic bacteria from low salt unpacked white cheese.

Microorganisms / Samples No.	*Campylobacter spp.*	*L. monocytogenes*	*E. coli*	*Staph. aureus*
1	–	–	+	–
2	–	–	+	–
3	–	–	+	+
4	+	+	–	+
5	+	–	+	+
6	+	–	+	+
7	+	–	+	+
8	+	–	+	+
9	–	–	–	–
10	+	–	–	–

Positive: (+) **Negative: (-)**

Effect of camel and goat milk on growth and survival of *Staph. aureus* , *E.coli* and *L.monocytogenes* in vitro:

Data reported in Table (19) obviously showed that the effect of camel and goat milk on growth of *Staph aureus, E.coli and L.monocytogenes* in vitro paid to decreasing of *Staph. aureus* counts from 5×10^{11} to 2×10^5 and $15 \times$

10^5 cfu/ml, , *E.coli* counts from 2×10^{12} to 4×10^7 and 8×10^7 cfu/ml, and *L.monocytogenes* counts from 2×10^{13} to 4×10^7 and 2×10^9 cfu/ml, for camel and goat milk respectively. The same observation regarding effect goat milk and raw milk on pathogenic bacteria were reported by Brooks *et al.* (2012) ,and Silanikova(2010).

Table (19): Effect of camel and goat milk on growth and survival of *Staph. aureus*, *E. coli* and *L. monocytogenes* in Vitro.

Milk type / Microorganisms	Camel	Goat
Staph. aureus	$2x10^5$	$15x10^5$
E. coli	$4x10^7$	$8x10^7$
L. monocytogenes	$4x10^7$	$2x10^9$

- **The used inoculums of *staph aureus* was 5×10^{11} cfu/ml**
- **The used inoculums of *E. coli* was 2×10^{12} cfu/ml**
- **The used inoculums of *L. monocytogenes* was $2x10^{13}$ cfu/ml**

Determination of aflatoxin M1 in the white cheese samples.

All the examined cheese samples were free from aflatoxin M1.These results are accepted by Egyptian Regulations which demonstrated that during products such as cheese samples should be free aflatoxin M1.

Conclusion

At farm level to prevent raw milk contamination it is essential to implement good farm practices, at cheese processing level the risk from the pathogenic bacteria is eliminated by the pasteurization of raw milk. The quality of the raw milk, heat treatment of the milk, activity of the starter culture and salting process – together with the storage in brine- are the most important control points for the preventation of growth / survival of undesirable micro – organisms during the manufacture of white brined cheeses.

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الخواص الكيميائية والجودة الميكروبيولوجية للجبن الابيض المتداول وتأثير لبن الماعز والجمال على بعض البكتيريا المرضية

نعمات إبراهيم بسيوني ، أحمد فريد عبد السلام ، زينب محمدعبد الغنى ، محمد فتحى أبو العلا ، عادل محمد محمد القرماني و محمد عبد المطلع عطوة

المركز الأقليمي للأغذية والاعلاف- مركز البحوث الزراعية

فى هذة الدراسة تم تجميع ٣٠عينة من كل من الجبن الابيض المعلب والغير معلب من الانواع العالية والمتوسطة والمنخفضة الملح من محال البيع المختلفة بالقاهرة و تم تقييم كل العينات المختبرة كيماويا وميكروبيولوجيا. وأوضحت النتائج أن:

أعلى نسبة من البروتين والكربوهيدرات والكالسيوم والفوسفور كانت ١٢,٧٪ ،٠, ٧٪، ٧٢, ٪١، ٨٥, ٪١ - وذلك فى الجبن الابيض المعلب عالى الملح والجبن الابيض الغير معلب منخفض الملح والجبن الابيض الغير معلب عالى الملح والجبن الابيض المعلب منخفض الملح على التوالى.

المكونات المنخفضة من الدهن،الآلياف،الرماد،الرطوبة،ملح الطعام، رقم الحموضة تمثل٧٠,٪٢١، ٠, ٨ ٪، ٢٩ ,١ ٪ ، ٩ ٣, ٪٤١ ،٨٥, ٠٪، ٥٢, ٣ ٪ وذلك في الجبن الابيض المعلب منخفض الملح والغير معلب عالى الملح و الغير معلب منخفض الملح و الغير معلب عالى الملح و الغير معلب منخفض الملح و المعلب منخفض الملح على التوالى.

- كان أعلى عدد كلى للبكتريا متشابها فى كل العينات المختبرة ويتراوح من ٦ وحدة خلية /جم. $x^{٦}١٠$ ٥,٥ الى $x^{٤}١٠$
- الجبن الابيض المعلب عالى الملح (الاسطنبولى) و المعلب منخفض الملح خالى من البكتيريا القولونية الكلية و البكتيريا القولونية البرازية.
- تم عزل ميكروب العنقود الذهبى بنسبة٩٠٪' ٧٠ ٪ من الجبن الابيض الغير معلب(الثلاجة) والغير معلب منخفض الملح على التوالى بينما الجبن الابيض المعلب (الثلاجة) والغير معلب منخفض الملح ملوث بميكروب الاسيتافيلوكوكس أوريس بنسبة ٨٠ ٪' ٦٠ ٪ على التوالى.
- أظهرت ألبان الجمال تأثيرا مضادا لأنواع البكتيريا المختبرة (الاسيتافيلوكوكس أوريس وميكروب العنقودى الذهبى واليستريا مونوسيتوجينس) أكثر من البان الماعز .
- كانت كل العينات المختبرة من الجبن الآبيض خالية من الآفلاتوكسين(M1)
- أما بالنسبة للفطريات المعزولة من أنواع الجبن المختبرة فكانت اسبرجلس نيجر، أسبرجلس كانديدس، أسبرجلس فيوماجاتس، فيوزاريوم كومباكتم،ايمارسيلا نيودلنس و أنواع من الريزوبيس. ,

قام بتحكيم البحث

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