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Evaluation of chemical and microbiological quality of white pickled soft cheese consumed in Minia governorate .

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ABSTRACT

This study aimed to evaluate the chemical and microbiological quality of white pickled cheese consumed in some districts of Minia governorate.

In this study 24 samples of unpacked white pickled soft cheese were collected from different local markets in Minia Governorate. Samples were divided into six brands , each brand includes four samples, and analyzed chemically and microbiologically.

Chemical analysis were 4.2-4.6 for pH , 3.0 -3.7 % titratable acidity , 43.3-54 % total solids , 18.1-25.9 % fat , 19.43-19.8% protein , 7.3 -11.3% salt , 7.0-8.6% ash , 0.25-0.27 % soluble nitrogen , 312- 1651 mg/100g for calcium and 263-368 mg/100g phosphorus .

Microbiological analysis data showed that the average of total aerobic bacteria was $4.8x10^5 \pm 1\times10^5$ cfu /ml . Lactococci , Lactobacilli , molds & yeasts and *E.coli* were $5.7 \pm 1\times10^3$, $1.6 \pm 0.2\times10^5$, $5.1 \pm 0.35\times10^5$ and $3.7 \pm 0.1\times10^1$ cfu /ml, respectively . Results revealed that 58. 3 and 41.5 % of samples were positive for Coliform and Enterobacteriacae . The identified microbial groups also cleared that *salmonella* was the predominant of about 9% of the total tested groups . In conclusion, it should be taken in considered the urgent need to manufacture white soft pickled cheese with a criteria or standard method.

KEYWORDS: Soft cheese , pathogenic bacteria , titratable acidity and unpacked.

1. INTRODUCTION

Cheese is considered as an essential daily food for many people around the world. It has a nutritional value that provides an essential source of digestible protein and minerals, including calcium and phosphorus (Hammam *et al*.,2020). Domiati or Damietta cheese is the most popular soft white cheese variety in Egypt. It is consumed fresh or ripened in salted whey for 4-8 months . It differs chiefly from other pickled varities in that , the salt is added directly to the milk before renneting . It is produced from buffalo or cow milk or mixture from them (Hamad , 2015) or sheep's and goats milk or reconstituted whole or skimmed milk (Hellmuth and Brink ,2013) or ultrafiltrated whole cow and buffalo milk. The percentage of the salt differs to the season of manufacture and the ripening temperature of the cheese . In general salt is added at a level of 8-10 % in winter and 12-14% in the summer. Milk coagulated at 38˚C and coagulation take time took about 2-3 h . The chemical composition in these cheese is primarily influenced by the type of milk used (Robinson and Tamime ,1991 and Ayad ,2009).

As raw milk is generally utilized in this cheese manufacture , the produced cheese should not be eaten except after 60 days of storage to inactivate the pathogens but these conditions could not kill all of the pathogens (Shehata *et al*., 2007).

The developments in processing technology packaging sales techniques have led to a widening of choice of fresh and pickled white cheese available to the consumers. Unfortunately , there is no data reported about the chemical and microbiological of pickled white soft cheese consumed in Minia governorate , so the objective of this study was carried out to evaluate the chemical and microbiological quality of white pickled cheese consumed in some districts of Minia governorate.

2. MATERIAL AND METHODS

2.1 Materials:

In this study 24 random samples of unpacked white pickled soft cheese samples were collected from

different local markets in Minia Governorate, the samples were put in sterile plastic bags and transferred directly to an insulated ice box. The samples (200 gm) were divided into two parts (100 gm each). The first part was examined for chemical analysis while the second part was microbiologically examined. Samples were divided into six brands ,each brand include four samples .

2.2 Chemical analysis:-

Moisture, fat and ash contents were determined according to A.O.A.C (2012).

Titratable acidity and pH was measured by the method described by (Hooi *et al*. 2004).

Total nitrogen (TN) and Soluble nitrogen (SN) contents were determined using kjeldahl & semi - micro kjeldahl method as described in A.O.A.C (2012), respectively.

Calcium was determined using the EDTA titration method according to Pearce (1979).

Ammonium molybdate method was used for Phosphorus determination as described by A.O.A.C (2012).

2.3 Microbiological analysis:-

One gram of each sample of white pickled soft cheese was serially diluted with 9 ml of 0.1 % peptone water. Appropriate dilutions were plated on MRS agar (for lactobacilli), and incubated anaerobically at 37˚ C for 72 hrs; and on M17(for lactococci) incubated aerobically at 37˚ C for 48 hrs The total vible counts were recorded (Standard method for the examination of dairy products , 1978). Aerobic plate count was determined according to APHA, (1998). The number of colony forming units (cfu/g) was determined by surface-spread technique onto Sorbitol MacConkey agar (Oxoid) for enumeration of *E. coli* plates were incubated at 37˚ C for 24–48 hrs and then counted for viable organisms (Roberts and Greenwood, 2003). Yeasts and moluds were enumerated in pour-plates of oxytetracycline potato dextrose agar (Oxoid) after incubation at 25 ˚ C for 3–5 days. Total yeasts and moulds counts were carried out according to NMKL (1999). *Enterobacteriaceae* was detected according to APHA, (1998). Coliform were carried out according to APHA (1998). *Salmonella spp* was detected by enrichment technique according to the procedure outlined in ISO (2001).

3. RESULTS AND DISCUSSION

3.1 Gross chemical composition

Compositional analysis of white pickled soft cheese for each brand is summarized in Table (1) and Table (2). Data show variation in chemical composition between brands. pH ranged from 4.00 - 4.3 , averaged 4.2 in brand No (5) while, pH for band No(1) is similar to brand No(3) , the average PH was 4.4. Also brand No (2) and brand No (6) the average of pH was 4.6 .The titratable acidity (%) of the brand No (5) was the lowest value compared to other brands, while brand No (6) recorded the highest value 3.3%. These results nearly similar by Hamad (2015), who found that ripening of white cheese lead to decreasing in pH values from 4.3 to 3.32 after storage at 3 and 6 months , respectively.

Table 1. Gross composition analysis of white pickled soft cheese samples in Minia governorate

Brand No	pH	Titratable acidity%	Fat %	Ash %	TS total solids %
1	4.413±0.065 (4.300-4.600)	2.888±0.264 (2.200-3.350)	23.75±1.451 (20.00-27.00)	6.961±0.2722 5.690-7.830	44.50±2.164 39.15-49.73
2	4.645±0.175 (4.350-5.100)	2.513±0.253 (2.050-3.000)	18.13±1.586 (15.00- 22.50)	8.563±0.7729 4.960-10.32	43.47±2.308 37.83-49.11
3	4.355±0.049 4.280-4.490)	2.863±0.226 (2.200-3.200)	21.75±1.315 (18.00-24.00)	8.379±0.4875 6.160-10.25	42.85±1.676 39.60-47.15
4	4.318±0.039 4.230-4.420)	3.225±0.286 (2.650-4.000)	23.50±3.323 (14.00- 29.50)	7.109±0.5127 4.950-8.880	43.35±4.521 31.79-53.10
5	4.210±0.078 (4.010-4.340)	2.350±0.509 (1.400-3.750)	25.88±2.249 (19.50- 30.00)	7.814±0.4510 6.750-10.10	48.72±2.144 44.80-54.00
6	4.618±0.344 (4.180-5.640)	3.313±0.157 (3.000-3.750)	18.88±2.105 (15.50-25.00)	7.956±0.4916 5.750-9.110	41.52±1.040 38.65-43.33

Data in parenthesis are minimum and maximum values of four samples, Results are expressed as mean value ± standard error.

The obtained data for fat content showed that , the lowest content was observed in brand No2 (18.1%) and 18.9% for brand No 6, while , the highest fat content was 25.9 for brand No 5. Values for fat content were similar to the results obtained by Abdou *et al* (1976) who found that fat content of pickled white cheese after four months storage was about 25%. Also, the same results obtained by Neamat *et al*, (2012). According to the data obtained by Hamouda (2020), the increase of fat content is due to the increase in total solids .

Data in Table (1) showed that there was no variation among brands in protein content . protein content ranged between 19.4-19.9 % for each brands .These results were higher than those reported by Neamat *et al* (2012) for Estanpoly unpacked white cheese who found that the average protein content was 5.88-10.5% for samples collected from different markets in Cairo. The present data also showed that protein content was higher than that obtained by Hamouda (2020) for white pickled cheese at 30 days storage being 15.6%. The lowest value of ash content was 7% in brand No1 while the highest value was 8.6% in brand No 2 , results were higher than those obtained Hamad (2015) who reported that ash content was 4.55, 5.01 and 6.12 after 1,3 and 6 months of storge . TS total solids content were quite large being as low as 41.5 in brand No 6 high in brand No 5 (48.74%). These results were lower than those obtained Hamad (2015) who found that TS was 53.6 after 1 month of storage in white soft pickled cheese.

According to the Egyptian Standard, (EGSQ, 2005), the moisture content of Domiati cheese must not be higher than 60% and the salt content must not be more than 9%. In current study , twenty one of the tested samples were met the EGSQ, 2005 in the category of moisture while only seven samples met the EGSQ, 2005 in salt content .

Total nitrogen percentages of white pickled soft cheese are shown in Table (2). For all tested cheese the average of total nitrogen was between 2.9-3.15% , total nitrogen in brand No 1 was the highest value compared to the other brands, recorded 3.15%, while in brand No 6 was the lowest being 2.9% . Data also showed that the soluble nitrogen content ranged between (0.25-0.27) for all tested cheese .These data were lower than reported by Abdou *et al* ,(1976), who found that the soluble nitrogen was 0.5%in 4 months old Domiati cheese made by coagulating the milk with calf rennet. The differences in soluble nitrogen in the present data compared to the others could be due to different factors such as type of milk , age of cheese , coagulant agent and percentage of salt and moisture content . Low percentage of soluble nitrogen was due to restricted proteolysis ,may be due to the high concentration of salt in cheese (Table 2). The soluble proteolysis products resulted from the Domiati cheese during ripening mixed with the brine to keep the stability with their content in the cheese (Hayaloglu, 2017).

Table 2. Total nitrogen (TN) , Water soluble nitrogen(WSN) , salt , calcium and phosphorus content of white pickled soft cheese in Minia governorate :

Brand No	TN %	SN%	SN/TN	Salt NaCl%	Calcium mg/100g	Phosphorus mg/100g
1	3.15±0.17 (2.94-3.36)	0.26±0.028 N ib (0.23- 0.29)	8.36±0.649 6.990-10.00)	9.78 ±4.28 (6.55-11.09)	1651±3.86 (1648-1656)	368±60.6 (268-411)
2	3.05±0.21 (2.8-3.29)	0.250±0.036 (0.20-0.294)	8.133±0.876 6.250-10.53)	10.44±1.40 (9.23-11.66)	400±86.0 (300-510)	268±1.29 (267-270)
3	3.12±0.15 (2.94-3.29)	0.27±0.023 (0.24 -0.29)	8.47±0.309 7.610-8.970)	9.11±0.61 (8.26-9.72)	1042±1.89 (1041-1045)	278±14. 69 (270-300)
4	2.98±0.41 (2.65-3.30)	0.27±0.022 (0.23- 0.28)	8.70±0.909 6.120-10.53)	7.29±2.86 (4.73-10.20)	312±2.51 (310-316)	263±3.82 (260-268)
5	3.08±0.12 (2.94-3.22)	0.26±0.18 (0.23- 0.28)	8.31±0.418 (7.610-9.520)	11.30±0.46 (10.66-11.66)	1042±16.07 (1036-1050)	279±14.8 (270-300)
6	2.90±0.32 (2.85-3.22)	0.26 ±0.035 (0.22- 0.29)	8.91±1.122 6.840-11.55)	10.08±1.14 (9.23-11.66)	1410±4.56 (1008-1016)	310±9.39 (299-322)

Data in parentheses are minimum and maximum values of four samples, Results are expressed as mean value ± standard error

Data in Table (2) also showed that the percentages of SN/TN for all tested brands were between 8.13- 8.91%

Robinson and Tamime (1991) concluded that the maturity of index of cheese is mainly due to the protein breakdown occurred by the growth and activities of microflora and or proteolysis with proteolytic enzymes .

The obtained data for calcium content shown that the highest average was observed in brand No1 (1651 mg/100g) followed by 1410 mg/100g in brand No5 and 1042 in brand No3, the lowest content was 312 mg/100g for brand No 4 . Omar and Buchheim, (1983) found that the calcium content was 960 mg/100g for white pickled soft cheese in 2 months old made from cow's milk .

Phosphorus content of cheese showed that the lowest value was 263 mg/100g in brand No 4 , despite the highest average of Phosphorus (368) was in brand No 1. These results were lower than that recorded by Neamat *et al* (2012) who found that phosphorus content was from 760 to 1270 mg/100g in unpacked white soft pickled cheese .

3.2 Microbiological examination of servied cheeses:

Cheese samples were analyzed for their microbiological quality. The quality and safety of Domiati cheese are strictly related to their microbial content. The microbial counts of cheese samples are shown in (Table 3). The APC ranged between 1×10^4 and 1.9×10^6 cfu/g (Table 3). Aly *et al*. (2007) , Sayed *et al*. (2011) and El-kholy *et al*.,(2014) reported mean APC in Domiati cheese as 2.6×10^5, 1.1×10^6 and 7.55×10^4 cfu/g, respectively. In this study, the mean APC was $4.8 \pm 1 \times 10^5$ which turned out to be similar to the previously mentioned studies.

Table 3. Microbiological examination of white pickled soft cheese in Minia governorate :

Microrganisms	Positive samples		Min	Max	Mean ±SE
	Number	%			
Aerobic plate count (APC)	24	100	1×10^4	1.9×10^6	$4.8\pm1X10^5$
Lactococci	24	100	1×10^2	2×10^4	$5.7\pm1\times10^3$
Lactobacilli	24	100	1×10^4	3.5×10^5	$1.6\pm0.2\times10^5$
Molds and yeasts	19	79	1×10^3	6×10^5	$5.1\pm0.35\times10^5$
E.coli	7	29	1×10^1	2×10^2	$3.7\pm0.1\times10^1$
Coliform	14	58.3	ND	ND	ND
Enterobacteriacae	10	41.5	ND	ND	ND
Samonella	2	9	ND	ND	ND

ND= Not Determined , Results are expressed as mean value ± standard error

The mean value of lactococci in cheese samples was $5.7\pm1\times10^3$ however, lactobacilli was found in higher level $1.6\pm0.2\times10^5$.

Adding 7.5% of salt to the milk resulted in the presence of lactococci as the predominant of LAB in Domiati cheese, which is recently substituted by lactobacilli .Increasing the addition of salt to 15% led to the presence of micrococci and lactobacilli as the majority of LAB. Enterococci were isolated from Domiati cheese, which exhibits high esterolytic activities (Litopoulou-Tzanetaki, 2007).

From the obtained results, it is obvious that mean value of yeast and mold counts of cheese samples was $5.1\pm0.35\times10^5$, which is higher than the Egyptian Standards Limits (mold content must not exceed 10 cfu /g , while yeast must not exceed 400 cfu/g) . The high incidence may attribute to the numerous sources of cheese contamination. It may be contaminated through milk used, washing water, environment, utensils and equipment, as well as through persons taking part in manufacturing and handling the product (Mullan, 2007). The public health importance of moluds has been emphasized as certain species can produce mycotoxins, which may induce food poisoning and neoplastic diseases including leukemia and other cancers among consumers. Also, some species of *Penicillium* have been associated with pulmonary and urinary tract infections in man (Brown, *et al*., 2007).

Coliforms were detected in 58.3 % of the examined Domiati cheese samples (Table 3). These values were lower than those obtained by Meshref and Hassan (2009) who found 78% of their samples were contaminated with coliform bacteria and higher than those reported by Amin *et al*. (2001), Aly *et al*. (2007) and Sayed *et al*. (2011) as they found 38, 52.5 and 20% of the samples were contaminated with coliforms, respectively. High levels of coliforms in cheese may sometimes give rise to early blowing or gassing of the product, which is characterized by large gas holes and a spongy texture of the cheese that

generally occurs 1–2 days after it was manufactured (Bintsis , 2006).

The existence of coliforms may not necessarily indicate a direct fecal contamination of Domiati cheese, but is more likely as an indicator of poor manufacturing conditions and lack of hygiene especially during cheese marketing. The survival of coliforms in Domiati cheese is affected by the level of salt added to the cheese milk. Not less than 9.5% NaCl should be added to the milk to suppress the growth of coliforms in the product, especially in cheeses made from raw milk (Abd-El Salam and Benkerroum, 2006). In this study, the salt concentration of the cheese ranged from 4.37 to11.66% and the average value was 9.77% (Table 2). It seems that the salt content of Domiati cheese is favorable for the growth of coliforms, these results are in accordance with those obtained by El-kholy *et al*., (2014) .

E. coli and coliforms are often used as indicator microorganisms and the presence of *E. coli* implies a risk that other enteric pathogens may be present in the sample. *E. coli* was isolated from seven samples constituting about (29%) of the total samples (Table 3). Moreover, the mean count of *E. coli* was $3.7\pm0.1\times10^{1}$cfu/g (Table 3). The contamination rate in cheese samples was generally higher than those reported by El-Gamal and Abdel-Khalek (1997), Meshref and Hassan (2009) Sayed *et al*. (2011) and El-kholy *et al*., (2014) as they found 16, 6,4 and 26% of their samples were contaminated by *E. coli*, respectively. On the contrary, higher incidence rates were reported by Kaldes (1997) and Amin *et al*. (2001) as they found 60 and 37.89% of the samples were contaminated with *E. coli*, respectively. According to the Egyptian Standards (2005), *E. coli* should be absent in Domiati cheese; all positive samples failed to meet the national standard. The presence of *E. coli* in Domiati cheese samples indicates an extensive deficiency of satisfactory sanitary practices during cheese manufacturing and/or post-production handling.

Salmonella was detected in 2 samples represent 9% of the tested samples . In contrast , the same microbial group was not detected in a similar survey carried out in Cairo and Giza regions studied by Aly *et al*.,(2007). The presence of *Salmonella* in some tested samples may be due to the unhygienic conditions in the production.

The present different types of microbial groups in this type of cheese was expected as it was manufactured from raw milk .

From the foregoing results , the obvious need for criteria or standard methods for white pickled soft cheese is necessary .

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الملخص العربي

تقييم الجوده الكيمياويه والميكروبيولوجيه للجبن الابيض الطري المخزن والمستهلك في محافظه المنيا

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تهدف هذه الدراسه الي تقييم الصفات الكيميائيه والجوده الميكروبيولوجية لعينات من مناطق مختلفه بمحاغظه المنيا ،اجريت الدراسه علي ٢٤ عينه جبن ابيض مخزن جمعت عشوائيا من أماكن مختلفه في محافظه المنيا وقسمت هذه العينات الي ٦ مناطق كل منطقه اشتملت علي ٤ عينات وتم تحليل هذه العينات كيماويا وميكروبيولوجيا.

اوضحت نتائج التحليل الكيميائي ان الأس الهيدروجيني كان ٤,٢–٤,٦ ، ٣,٢–٣,٧ جموضه كليه ، ٤٣,٣–٥٤ % جوامد صلبه كليه ، ١٨,١–٢٥,٩% دهن ، ١٩,٤٣–١٩,٨ % بروتين ، ٧,٣–١١,٣% ملح ، ٧–٨,٦ رماد ، ٠,٢٥ –٠,٢٦ % نيتروجين ذائب ، ٣١٢– ١٦٥١ ملليجرام /١٠٠ جم كالسيوم و ٢٦٣–٣٨٦ ملليجرام / ١٠٠جم فوسفور .

كما اوضحت دراسه نتائج المحتوي الميكروبي ان متوسط عدد البكتريا الكليه الهوائيه كان $4.8 \pm 1\times10^{5}$ مستعمره / مل و عدد Lactobacilli, Lactococci كانوا $1.6 \pm 0.2\times10^{5}$ و $5.7\pm1\times10^{3}$ علي التوالي وعدد الفطريات والخمائر وبكتريا القولون $5.1\pm0.35\times10^{5}$ و $3.7\pm0.1\times10^{1}$ علي التوالي. وأظهرت النتائج ايضا ان ٥٨,٣% و ٤١,٥% من العينات المختبره كانت موجبه لكلا من Enterobacteriacae Coliform , ، علي التوالي. وأن حوالي ٩ % من العينات احتوت علي بكتريا ال . *Salmonella*

واستخلصت الدراسه الحاجه الماسه لتصنيع الجبن الابيض الطري المخزن الجبن بطرق متمشيه مع المعايير و المواصفات القياسيه.