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MICROBIOLOGICAL, CHEMICAL AND ORGANOLEPTIC STUDIES TO PRODUCE DESIRABLE AND SAFETY DOMIATI CHEESE

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Key words: *Staph. aureus, E. coli, Ent. faecalis*, starter, vells milk; Domiati cheese and Pickling.

ABSTRACT

One hundred and ten milk samples (50 ml each) were examined for the presence of contaminants and related microorganisms. Four samples were found free from *Staphylococcus aureus, Escherichia coli,* and *Enterococcus faecalis.* Free samples were mixed, and enriched by successive inoculation in skim milk media to further use as a natural starter for Domiati cheese made from pasteurized milk. Vells milk were also used in cheese production to achieve the same objective.

Vells milk were treated with three salting levels 10, 15, 20% with or without vells tissue, then incubated at refrigerator or room temperature. The pathogenic indicators were detected in vells milk mixed with vells tissue with a 10% salt concentration up to 8 weeks of storage. The survival of indicators were decreased at higher salt concentrations, on the contrary lower storage temperatures prolonged their survival. RCT of vells milk treatment and the above microbial results led to use vells milk treated with 15% salt without tissue which storage at room temperature as a good rennet source in manufacture of cheese.

The total viable counts of cheese samples were sharply decreased by time in samples treated with the prepared starter, especially with the increase of starter percent. *Staph. aureus. E. coli* and *Ent. faecalis* were disappeared within 2-3 months in samples made from raw milk either by using powder rennet or vells milk.

The use of 6% starter in addition of vells milk or powder rennet accelerated the pickling of Domiati cheese and led to increase the W.S.N., N.P.N. and T.V.F.A. in the produced cheese, also lead to give the best organoleptic properties compared to the other cheese treatments.

INTRODUCTION

White soft cheese known as Domiati is the most popular cheese which is craved for all socioeconomic classes in Egypt and widely

produced in many Mediterranean and Balkan countries **(Shazly, 2001).** Vells milk (Coagulated milk found inside the vells) is used as a coagulant for cheese making in small primitive plants **(El-Assar and Abeid 1998).** The use of local wild starter to improve the flavour in manufacture of venaco soft cheese was carried out by **Casalta and Zennaro (1997).** The microflora and enzymes occurred in raw and vells milk are responsible for the favorable flavour of Domiati cheese. In the same time both raw milk and vells milk used in cheese making play the main role in cheese contamination with all undesirable and pathogenic bacteria **(Naguib *et. al.*, 1979 b and Sharaf *et. al.*, 1989).** One of the major defects that reported in this type of cheese is gas blowing during pickling period mainly in the early stage, the gas holes in cheese has been attributed to the presence of coliform bacteria. **(El-Shibiny *et. al.*, 1988).**

The presence of coliforms or *E. coli* in milk samples collected from different locates in Egypt, were thoroughly investigated. Coliform bacteria were found in 100% of milk samples **(Abou-Elkheir 1985, Hassan and Badran 1986, and Gamal El-din 1994),** whereas *E. coli* was found in 22-66.6% from collected samples **(Moustafa *et. al.*, 1975 and Ahmed and sallam 1991).** The incidence of *E. coli* and coliform group in Domiati cheese were found by many investigators to be from 10 to 100% of studied samples **(Zaodhof *et. al.*, 1984; Ahmed *et. al.*, 1988; El-zayat, 1988; El-Kholy 1989 and Gamal El-din 1994).** Although production of Domiati cheese at sanitary condition is demanded, the favorable flavour produced by raw and vells milk will disappear with pasteurization according to the new Egyption standards **(Egyption Standard laws, 2000).**

The aim of this study is to produce a starter, which can be used with safety vells milk, for Domiati cheese production from pasteurized milk with a desirable flavour.

MATERIALS AND METHODS

Materials

1- Vells sample

Eight fresh vells were obtained from slaughterhouse in Fayoum City to be used as a source of vells milk and vells tissues.

2- Powder rennet

Powder rennet **(Stabo, 1290)** was obtained from CHR. HANSEN-Denmark.

3- Milk.

3-1 Collection of milk samples for preparation of starter

One hundred and ten milk samples (50 ml each) were randomly collected from farms in Fayoum Gavernorate. All milk samples were transferred to laboratory in polyethylene bags within 3 hr., each milk sample was considered as an individual sample and held in the refrigerator at 5-7 ^{0}C, until testing was begun.

3-2 Buffaloes milk

Fresh raw buffaloes milk were obtained from a private farm in Fayoum (Fat 8.4%, pH 6.64, acidity 0.16-0.17% expressed as lactic acid, moisture 82.2% and total protein 4.41%)

Methods

1- Starter Preparation

Raw milk samples proved to be free from undesirable microorganisms were maintain at 5-7^{0}C till the enrichment steps were begun. The lactic acid bacteria (*Lactococcus spp*, *Lactobacillus spp*) and flavour producing bacteria *Leuconostoc spp* occurred naturally in non contaminated milk samples were successively cultivated and subcultured three times on skim milk broth media at 37^{0}C for 6 hr. to produce enough amount of the natural starter.

2- Preparation of vells milk and vells tissues

Eight vells were transferred to the laboratory and rinsed with water, the superfluous tissues and fat were removed.

Diagram (1) shows all possible vells milk experiment treatments, which the rennet producer followed, the microbiological examinations (for *Stahylococcus aureus, Escherichia coli* and *Enterococcus faecalis*) and rennet coagulation time were weekly tested for eight weeks.

3- Cheese making

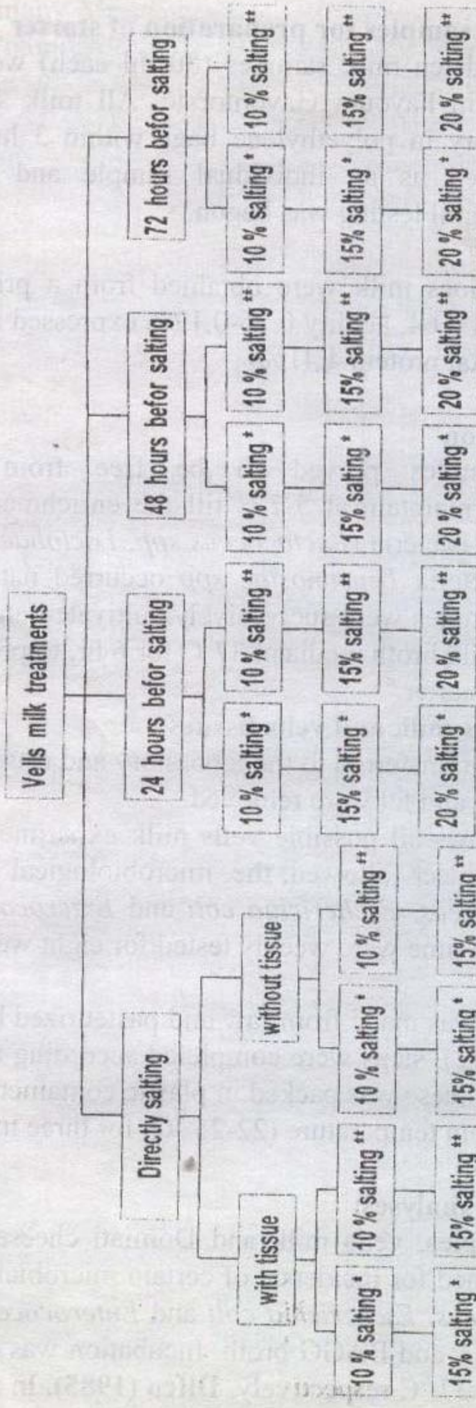
Domiati cheese was made from raw and pasteurized buffaloes milk as shown in diagram (2), steps were completed according to **Fahmi and Sharara (1950)** The cheese was packed in plastic containers with its own whey and stored at room temperature (22-25 ^{0}C) for three months.

4- Analyses of cheese

4-1- Microbiological analyses:

Raw milk samples, vells milk and Domiati cheese produced by raw milk were examined for incidence of certain microbial contaminants *i.e Stahylococcus aureus, Escherichia coli* and *Enterococcus faecalis* on Baird Parker agar, E.C. and BAGG broth. Incubation was carried out for 24 hr. at 37, 45.5 and 37 ^{0}C respectively, **Difco (1985).** In addition of the mentioned bacteria, total viable count were also detected monthly (three

Diagram (1) illustrates different vells milk treatments



*Keeping under chilling (5-7 °C)
**Keeping at 22-25 °C

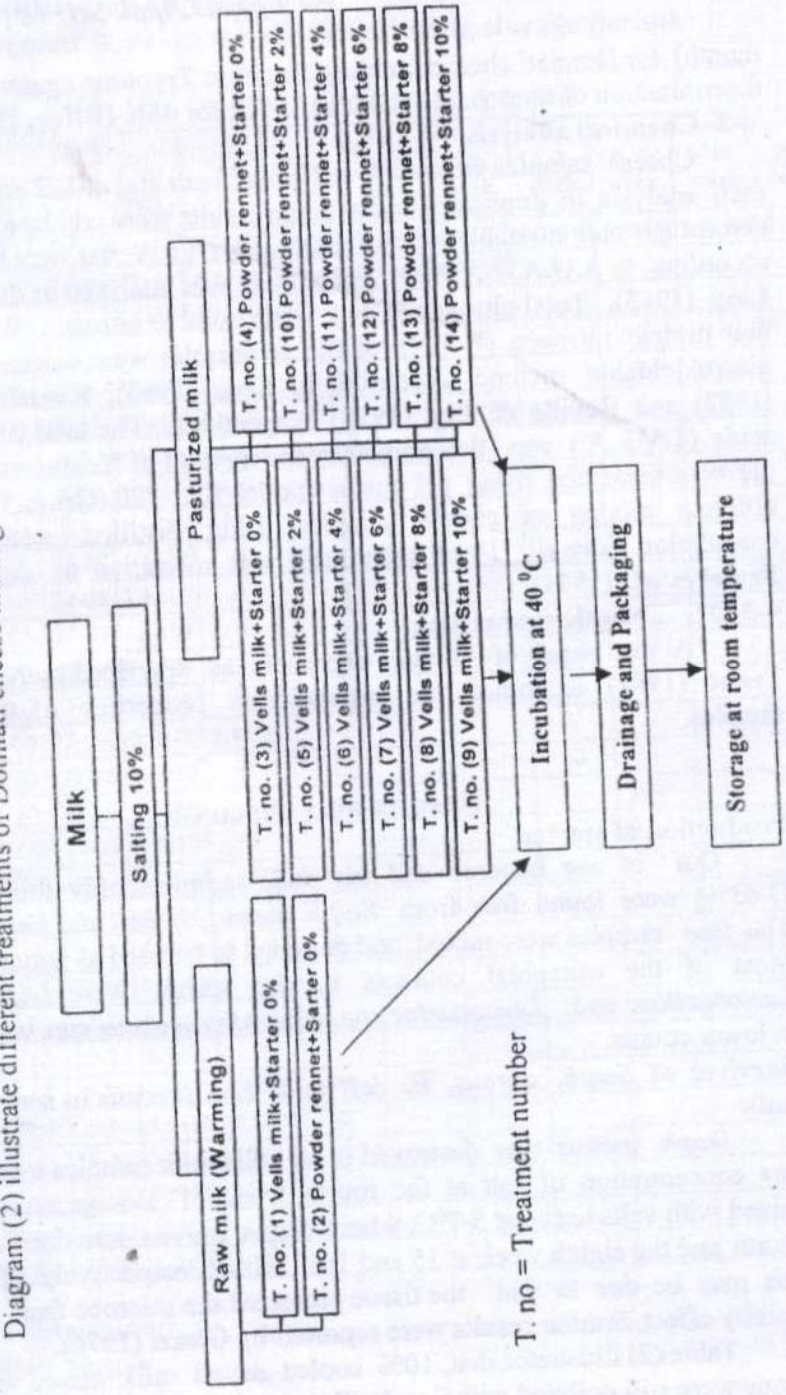


Diagram (2) illustrate different treatments of Domiati cheese making

month) for Domiati cheese samples. Proteose Tryptone agar was used for determination of total viable count at 35 °Ct for 48h. **(Difco, 1985)**

4-2- Chemical analysis

Cheese samples were analysed when fresh and at 1, 2 and 3 month, each analysis in duplicate and average results were tabulated. Moisture percentage and titratable acidity percentage (T.A %) were determined according to **A.O.A.C., (1990).** Fat content was analysed as described by **Ling (1963).** Total nitrogen (T.N.), water soluble nitrogen (W.S.N.) and non protein nitrogen (N.P.N) of cheese samples were determined using macrokjeldahle method according to **Ling (1963), Kuchroo & Fox (1982)** and **Reville & Fox (1978)** respectively. The total volatile fatty acids (T.V.F.A.) was determined by the method of **Kosikowski (1997),** pH was measured using pH meter model SA 720 (Orion, USA) after efficient mixing of cheese samples using distilled water. Rennet coagulation time (RCT) of vells milk was measured as described by **Samel *et. al.*, (1971).**

4-3-Organoleptic evaluation:

A test panel of 11 staff members as described by **Nelson and Trout (1981)** examined the organoleptic properties of the cheese samples.

Results and Discussion.

Production of starter

Out of one hundred and ten milk samples, only four samples (3.63%) were found free from *Staph. aureus, E. coli* and *Ent. faecalis.* The free samples were mixed, and enriched to be used as natural starter. Most of the microbial contents in the starter were *Lactococcus*, *Lactobacillus* and *Leuconostoc spp.* Also *Micrococcus spp*, were found in lower counts.

Survival of *Staph. aureus, E. coli* and *Ent. faecalis* in treated vells milk

Staph. aureus was destroyed in all vells milk samples treated with any concentration of salt at the fourth week of storage except, those mixed with vells tissue at 5-7°C, where *Staph. aureus* remained after the fourth and the eighth week at 15 and 10% salting, respectively (Table 1), this may be due to that the tissue protected the microbe from adverse salinity effect. Similar results were reported by **Ghazi (1976).**

Table (2) illustrates that, 10% cooled salted milk mixed with vells tissue were still polluted with*E. coli* till the end of the eighth week, whereas

Table (1) Effect of different treatments on survival of *Staph. aureus* count (CFU\g) in vells milk during storage period

Treatment				Storage period								
Time	Salt %	Tissue	Temp °C	Zero time	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	7 th week	8 th week
0	10	(+)	5-7	$9.2x10^4$	$4.6x10^3$	$1.1x10^3$	$7.9x10^2$	$4.9x10^2$	$4.9x10^2$	$7.9x10^2$	$1.8x10^2$	2.5x10
0	15	(+)	5-7	$9.2x10^4$	$5.2x10^2$	4.4x10	$3.6x10^2$	$1.3x10^2$	0	-	-	-
0	20	(+)	5-7	$9.2x10^4$	$1.1x10^2$	3.7x10	0	-	-	-	-	-
0	10	(+)	22-25	$9.2x10^4$	4.9x10	1.7x10	2.9x10	0	-	-	-	-
0	15	(+)	22-25	$9.2x10^4$	2.1x10	5.2x10	0	-	-	-	-	-
0	20	(+)	22-25	$9.2x10^4$	2.5x10	0	-	-	-	-	-	-
0	10	(-)	5-7	$1.1x10^2$	6.4x10	1.7x10	5.6	0	-	-	-	-
0	15	(-)	5-7	$1.1x10^2$	1.4x10	1.2x10	0	-	-	-	-	-
0	20	(-)	5-7	$1.1x10^2$	4.9x10	0	-	-	-	-	-	-
0	10	(-)	22-25	$1.1x10^2$	0	-	-	-	-	-	-	-
0	15	(-)	22-25	$1.1x10^2$	0	-	-	-	-	-	-	-
0	20	(-)	22-25	$1.1x10^2$	0	-	-	-	-	-	-	-
24	10	(-)	5-7	$9.5x10^2$	$4.6x10^2$	1.3x10	0	-	-	-	-	-
24	15	(-)	5-7	$9.5x10^2$	$1.5x10^2$	0	-	-	-	-	-	-
24	20	(-)	5-7	$9.5x10^2$	5.8x10	0	-	-	-	-	-	-
24	10	(-)	22-25	$9.5x10^2$	0	-	-	-	-	-	-	-
24	15	(-)	22-25	$9.5x10^2$	0	-	-	-	-	-	-	-
24	20	(-)	22-25	$9.5x10^2$	0	-	-	-	-	-	-	-
48	10	(-)	5-7	$8.7x10^2$	$9.5x10^2$	6.4x10	0	-	-	-	-	-
48	15	(-)	5-7	$8.7x10^2$	5.8x10	0	-	-	-	-	-	-
48	20	(-)	5-7	$8.7x10^2$	3.5x10	0	-	-	-	-	-	-
48	10	(-)	22-25	$8.7x10^2$	0	-	-	-	-	-	-	-
48	15	(-)	22-25	$8.7x10^2$	0	-	-	-	-	-	-	-
48	20	(-)	22-25	$8.7x10^2$	0	-	-	-	-	-	-	-
72	10	(-)	5-7	$5.9x10^2$	$1.7x10^2$	4.6x10	0	-	-	-	-	-
72	15	(-)	5-7	$5.9x10^2$	1.1x10	0	-	-	-	-	-	-
72	20	(-)	5-7	$5.9x10^2$	2.2x10	0	-	-	-	-	-	-
72	10	(-)	22-25	$5.9x10^2$	0	-	-	-	-	-	-	-
72	15	(-)	22-25	$5.9x10^2$	0	-	-	-	-	-	-	-
72	20	(-)	22-25	$5.9x10^2$	0	-	-	-	-	-	-	-

- = not determined
(+) = With tissue
(-) = Without tissue

Table (2) Effect of different treatments on survival of *E. coli* count (MPN/g) in vells milk during storage period

Treatment				Storage period								
Time	Salt %	Tissue	Temp °C	Zero time	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	7 th week	8 th week
0	10	(+)	5-7	$2.6x10^4$	$8.1x10^2$	$9.5x10^2$	$8.4x10^2$	$8.1x10^2$	$7.0x10^2$	$9.2x10^2$	$8.1x10^2$	$7.0x10^2$
0	15	(+)	5-7	$2.6x10^4$	$3.9x10^2$	2.6x10	0	-	-	-	-	-
0	20	(+)	5-7	$2.6x10^4$	9.5x10	0	-	-	-	-	-	-
0	10	(+)	22-25	$2.6x10^4$	0	-	-	-	-	-	-	-
0	15	(+)	22-25	$2.6x10^4$	0	-	-	-	-	-	-	-
0	20	(+)	22-25	$2.6x10^4$	0	-	-	-	-	-	-	-
0	10	(-)	5-7	$7.8x10^2$	0	-	-	-	-	-	-	-
0	15	(-)	5-7	$7.8x10^2$	0	-	-	-	-	-	-	-
0	20	(-)	5-7	$7.8x10^2$	0	-	-	-	-	-	-	-
0	10	(-)	22-25	$7.8x10^2$	0	-	-	-	-	-	-	-
0	15	(-)	22-25	$7.8x10^2$	0	-	-	-	-	-	-	-
0	20	(-)	22-25	$7.8x10^2$	0	-	-	-	-	-	-	-
24	10	(-)	5-7	$4.9x10^2$	0	-	-	-	-	-	-	-
24	15	(-)	5-7	$4.9x10^2$	0	-	-	-	-	-	-	-
24	20	(-)	5-7	$4.9x10^2$	0	-	-	-	-	-	-	-
24	10	(-)	22-25	$4.9x10^2$	0	-	-	-	-	-	-	-
24	15	(-)	22-25	$4.9x10^2$	0	-	-	-	-	-	-	-
24	20	(-)	22-25	$4.9x10^2$	0	-	-	-	-	-	-	-
48	10	(-)	5-7	$9.2x10^2$	0	-	-	-	-	-	-	-
48	15	(-)	5-7	$9.2x10^2$	0	-	-	-	-	-	-	-
48	20	(-)	5-7	$9.2x10^2$	0	-	-	-	-	-	-	-
48	10	(-)	22-25	$9.2x10^2$	0	-	-	-	-	-	-	-
48	15	(-)	22-25	$9.2x10^2$	0	-	-	-	-	-	-	-
48	20	(-)	22-25	$9.2x10^2$	0	-	-	-	-	-	-	-
72	10	(-)	5-7	$7.0x10^2$	1.2x10	0	-	-	-	-	-	-
72	15	(-)	5-7	$7.0x10^2$	9.8	0	-	-	-	-	-	-
72	20	(-)	5-7	$7.0x10^2$	3.0	0	-	-	-	-	-	-
72	10	(-)	22-25	$7.0x10^2$	1.6 x10	0	-	-	-	-	-	-
72	15	(-)	22-25	$7.0x10^2$	5.8	0	-	-	-	-	-	-
72	20	(-)	22-25	$7.0x10^2$	1.8	0	-	-	-	-	-	-

- = not determined
(+) = With tissue
(-) = Without tissue

samples kept at room temperature for 72 hours before salting still polluted till the beginning of the second week. The microbe was not found after 1 week in the rest of treatments. Also, there is no noticeable difference between salting percentage treatments (10,15 and 20%) on survival of *E. coli.*

As shown in table (3), *Ent. faecalis* was disappeared at the third and the second week at 15, 20% salted vells milk mixed with vells tissue respectively. Whereas the microbe remained till the end of the eighth week in samples salted with 10%, *Ent. faecalis* was destroyed at the first week in all the rest treatments.

Generally, the pathogenic indicators were detected in 10% salted samples of vells milk mixed with vells tissue up to 8 weeks of storage. The survival of indicators were decreased at higher salt concentrations. On the contrary lower storage temperatures prolonged their survival, which may be due to that the low temperature inhibited the growth of lactic acid bacteria, consequently lowered the acidity effect. These finding are in line with those reported by **Naguib *et al.*, (1979 a)**

RCT of vells milk treatment and the above microbial results led to use vells milk treated with 15% salt without tissue which storage at room temperature as a good rennet source in manufacture of cheese.

Total viable counts of cheese

Table (4) reveals that, the total viable counts in all treated samples were decreased till the end of experiment. Counts were decreased sharply in samples with high ratios of starter (6, 8 and 10%) compared with the other samples. It may be due to that the lactic acid produced by the starter. *Staph. aureus* were disappeared at 3 months in samples which were made of raw milk (either by using powder rennet or vells milk). *E. coli* and *Ent. faecalis* were not found at 2 month in samples manufactured with raw milk and powder rennet, whereas, the mentioned two microbes remained one month longer in case of samples manufactured with raw milk (vells milk which used as a coagulant).

Changes in cheese composition during pickling:

Moisture, yield, protein and fat content.

Results in table (5) revealed the yield of different cheese treatments. The yield of cheese obtained from pasteurized milk was higher than raw milk. This is in accordance with **El Koussy *et. al.*, (1977)** and Nizamlioglu *et. al.* (1998), as they found that heat treatment of milk improved the quality and yield of cheese. On the other hand, yield of cheese was increased as a result of increasing starter ratio. Such

Table (3) Effect of different treatments on survival of *Ent. faecalis* (MPN/g) in vells milk during storage period

Treatment				Storage period								
Time	Salt %	Tissue	Temp °C	Zero time	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	7 th week	8 th week
0	10	(+)	5-7	$9.5x10^4$	$8x10^2$	7.8x10	3.2x10	5.9x10	1.1x10	2.1x10	1.3x10	1.1x10
0	15	(+)	5-7	$9.5x10^4$	3.3x10	1.1x10	0	-	-	-	-	-
0	20	(+)	5-7	$9.5x10^4$	8.4x10	0	-	-	-	-	-	-
0	10	(+)	22-25	$9.5x10^4$	0	-	-	-	-	-	-	-
0	15	(+)	22-25	$9.5x10^4$	0	-	-	-	-	-	-	-
0	20	(+)	22-25	$9.5x10^4$	0	-	-	-	-	-	-	-
0	10	(-)	5-7	$7.8x10^2$	0	-	-	-	-	-	-	-
0	15	(-)	5-7	$7.8x10^2$	0	-	-	-	-	-	-	-
0	20	(-)	5-7	$7.8x10^2$	0	-	-	-	-	-	-	-
0	10	(-)	22-25	$7.8x10^2$	0	-	-	-	-	-	-	-
0	15	(-)	22-25	$7.8x10^2$	0	-	-	-	-	-	-	-
0	20	(-)	22-25	$7.8x10^2$	0	-	-	-	-	-	-	-
24	10	(-)	5-7	$4.0x10^2$	0	-	-	-	-	-	-	-
24	15	(-)	5-7	$4.0x10^2$	0	-	-	-	-	-	-	-
24	20	(-)	5-7	$4.0x10^2$	0	-	-	-	-	-	-	-
24	10	(-)	22-25	$4.0x10^2$	0	-	-	-	-	-	-	-
24	15	(-)	22-25	$4.0x10^2$	0	-	-	-	-	-	-	-
24	20	(-)	22-25	$4.0x10^2$	0	-	-	-	-	-	-	-
48	10	(-)	5-7	$4.1x10^2$	0	-	-	-	-	-	-	-
48	15	(-)	5-7	$4.1x10^2$	0	-	-	-	-	-	-	-
48	20	(-)	5-7	$4.1x10^2$	0	-	-	-	-	-	-	-
48	10	(-)	22-25	$4.1x10^2$	0	-	-	-	-	-	-	-
48	15	(-)	22-25	$4.1x10^2$	0	-	-	-	-	-	-	-
48	20	(-)	22-25	$4.1x10^2$	0	-	-	-	-	-	-	-
72	10	(-)	5-7	$1.7x10^2$	0	-	-	-	-	-	-	-
72	15	(-)	5-7	$1.7x10^2$	0	-	-	-	-	-	-	-
72	20	(-)	5-7	$1.7x10^2$	0	-	-	-	-	-	-	-
72	10	(-)	22-25	$1.7x10^2$	0	-	-	-	-	-	-	-
72	15	(-)	22-25	$1.7x10^2$	0	-	-	-	-	-	-	-
72	20	(-)	22-25	$1.7x10^2$	0	-	-	-	-	-	-	-

- = not determined
(+) = With tissue
(-) = Without tissue

Table (4) Counts of different microorganisms in various cheese treatments (CFU/g)

Sample No.	Treatment			Total viable counts (CFU)				*Staph. aureus* (CFU)				*E. coli* (MPN)				*Ent. faecalis* (MPN)			
				Pickling period (months)				Pickling period (months)				Pickling period (months)				Pickling period (months)			
	Milk	Rennet	Starter %	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
1	Raw	Vells milk	0	$1.4x10^7$	$6.2x10^6$	$1.1x10^6$	$5.9x10^5$	$2.5x10^5$	$1.4x10^2$	9x10	0	$1.8x10^4$	$5.4x10^2$	3.6	0	$2.2x10^2$	9.2x10	2.2	0
2		Powder		$2.8x10^6$	$9.0x10^5$	$1.9x10^5$	$1.0x10^5$	$9x10^4$	$2.3x10^2$	4	0	$2.2x10^3$	$1.2x10^2$	0	-	$1.4x10^2$	4.8x10	0	-
3	Pasteurized	Vells milk		$2.6x10^6$	$7.2x10^5$	$5.4x10^5$	$2.1x10^5$	0	-	-	-	-	-	-	-	-	-	-	-
4		Powder		$4.6x10^4$	$1.4x10^3$	$1.4x10^3$	$1.1x10^3$	0	-	-	-	-	-	-	-	-	-	-	-
5		Vells milk	2	$1.4x10^7$	$1.6x10^6$	$4.7x10^5$	$1.8x10^5$	0	-	-	-	-	-	-	-	-	-	-	-
6			4	$1.2x10^7$	$7.4x10^6$	$7.2x10^5$	$6.2x10^5$	0	-	-	-	-	-	-	-	-	-	-	-
7			6	$4.0x10^7$	$3.2x10^5$	$8.2x10^4$	$9.2x10^4$	0	-	-	-	-	-	-	-	-	-	-	-
8			8	$3.4x10^7$	$1.4x10^5$	$1.7x10^3$	$5.4x10^3$	0	-	-	-	-	-	-	-	-	-	-	-
9			10	$2.1x10^7$	$8.4x10^4$	$5.2x10^3$	$4.7x10^3$	0	-	-	-	-	-	-	-	-	-	-	-
10		Powder	2	$5.4x10^5$	$8.2x10^4$	$1.7x10^4$	$1.8x10^4$	0	-	-	-	-	-	-	-	-	-	-	-
11			4	$1.6x10^5$	$1.3x10^4$	$7.4x10^4$	$5.5x10^4$	0	-	-	-	-	-	-	-	-	-	-	-
12			6	$2.2x10^5$	$9.2x10^3$	$4.8x10^3$	$3.7x10^3$	0	-	-	-	-	-	-	-	-	-	-	-
13			8	$1.4x10^5$	$6.2x10^4$	$5.2x10^3$	$2.1x10^3$	0	-	-	-	-	-	-	-	-	-	-	-
14			10	$9.0x10^5$	$9.0x10^3$	$9.3x10^3$	$2.0x10^3$	0	-	-	-	-	-	-	-	-	-	-	-

- = not determined

Table (5) Effect of different treatments on yield, moisture, protein and fat content of Domiati cheese during pickling period.

Sample No.	Treatments			Yield	Moisture %				Total protein %				Fat %			
	Milk	Rennet	Starter %		Pickling period (months)				Pickling period (months)				Pickling period (months)			
					Fresh	1	2	3	Fresh	1	2	3	Fresh	1	2	3
1	Raw	Vells milk	0	38.3	59.75	57.03	55.97	55.05	13.70	14.52	15.12	15.89	20.0	21.3	22.5	24.8
2		Powder		38.0	59.36	56.13	55.82	54.79	13.96	15.11	16.09	16.73	20.2	21.5	23.0	25.9
3	Pasteurized	Vells milk		38.5	60.16	57.78	56.91	56.25	13.71	14.74	15.49	16.11	19.7	21.2	22.2	24.0
4		Powder		38.4	59.97	57.59	56.59	56.09	13.74	14.90	15.55	16.36	19.8	20.5	22.0	23.3
5		Vells milk	2	38.7	61.05	58.74	57.33	56.92	13.70	14.47	15.00	15.61	19.5	20.2	21.5	22.0
6			4	40.5	61.78	59.06	57.88	57.00	13.46	14.29	15.02	15.45	19.1	21.2	21.5	21.9
7			6	42.0	62.00	59.21	58.84	57.84	12.83	14.83	14.60	15.11	19.0	21.0	21.6	21.9
8			8	42.9	63.58	60.39	59.88	58.81	12.58	13.97	14.25	14.88	18.8	19.9	20.8	21.5
9			10	43.5	63.88	61.68	59.22	59.08	12.05	13.38	13.82	14.06	18.2	19.5	20.5	21.3
10		Powder	2	38.5	60.74	58.15	57.01	56.63	13.82	14.69	15.28	15.73	20.2	21.3	22.0	22.3
11			4	39.0	61.14	58.36	57.30	56.80	13.63	14.60	15.31	15.77	20.1	21.5	21.8	21.9
12			6	40.7	61.86	58.07	57.52	57.17	13.27	13.93	14.68	15.30	19.6	21.8	22.2	22.0
13			8	42.1	62.75	59.76	58.28	57.83	12.69	13.70	14.35	14.90	19.2	20.3	20.9	22.0
14			10	43.2	63.70	60.22	58.94	58.18	12.13	13.55	13.89	14.23	18.6	19.8	20.3	21.1

increase could be due to the high moisture content retained by the curd. Similar results were reported by **El-Ghandour *et. al.*,(1983) and El-Shafie (1994).**

It is clear that the moisture content of cheese treatments gradually decreased during pickling period. This might be due to the whey exudation during pickling (Table 5). These results agreed with those of **Ibrahim and Amer (1969).**

Results indicate that cheese made from pasteurized milk using vells milk or rennet powder had slightly higher moisture content than those made from raw milk (Table 5). Also addition of a starter led to appreciable in moisture content, especially with vells milk compared to rennet powder, Domiati cheese made from milk containing 10% starter and vells milk had the highest percentage of moisture content comparing with powder rennet.

Protein and fat content of cheese of different treatments gradually increased till the end of pickling period, and has opposite trend of cheese moisture content (Table 5).

Titratable acidity% and pH.

T.A and pH of various resultant Domiati cheese are presented in table (6). Results show that, slight differences were observed in T.A of fresh cheese in all treatments. Also, results indicate that the T.A gradually increased during pickling period progressed up to 90 days. Addition of starter led to increase in T.A, increasing starter ratio led to development of acidity of resultant cheese. This is attributed to lactose fermentation and production of lactic acid. pH-value had an opposite trend of T.A during the pickling period. The pH of cheese made from pasteurized milk (without starter) was higher than that made from raw milk especially with vells milk comparing to powder rennet. Thus, vells milk could be considered as an important source for supplying Lactobacilli (Naguib *et. al.* 1979 a). On the other hand, addition of starter (at different ratios) to milk led to production of Domiati cheese lower in pH values.

Water Soluble Nitrogen; Non Protein Nitrogen and Total Volatile Fatty Acids

The changes in W.S.N., N.P.N. and T.V.F.A. of various Domiati cheese treatments are presented in table (7). Results indicate that, the concentrations of W.S.N., N.P.N. and T.V.F.A. were higher in Domiati cheese made from raw than that made from pasteurized milk. this may be due to the activity of indigenous milk enzymes (Proteases and lipases) in

Table (6) Effect of different treatments on pH and acidity of Domiati cheese during pickling period.

Sample No.	Treatments			pH values				Acidity %			
	Milk	Rennet	Starter %	Pickling period (month)				Pickling period (month)			
				Fresh	1	2	3	Fresh	1	2	3
1	Raw	Vells milk	Non	6.1	5.6	4.8	4.1	0.285	0.55	0.97	1.28
2		Powder		6.1	5.6	4.8	4.3	0.285	0.45	0.88	1.00
3	Pasteurized	Vells milk		6.2	5.6	4.0	4.3	0.275	0.45	0.87	1.00
4		Powder		6.3	5.9	5.01	4.5	0.28	0.40	0.60	0.95
5		Vells milk	2	6.1	5.4	4.9	4.6	0.30	0.65	0.73	1.23
6			4	6.0	5.4	4.8	4.6	0.30	0.70	0.78	1.28
7			6	6.0	5.2	4.5	4.3	0.33	0.73	0.93	1.43
8			8	5.8	4.9	4.3	4.1	0.34	0.75	1.05	1.55
9			10	5.6	4.6	4.01	4.7	0.37	0.87	1.48	1.94
10		Powder	2	6.2	5.8	5.2	4.4	0.28	0.60	0.78	1.15
11			4	6.2	5.8	5.1	4.2	0.28	0.68	0.80	1.15
12			6	6.0	5.5	5.0	4.1	0.30	0.70	0.88	1.04
13			8	5.8	5.0	4.5	4.0	0.30	0.75	0.93	1.22
14			10	5.8	4.8	4.2	3.9	0.32	0.80	1.25	1.32

addition of lactic acid bacteria of raw milk. The W.S.N. and N.P.N. of all treatments gradually increased during pickling period, this indicate continuos degradation of cheese protein, similar findings were found by **Hamed *et. al.*, (1992) and Osman (2003).** This may be due to the proteolysis activities of cheese bacteria and enzymes. Also T.V.F.A. had the same trend of W.S.N. and N.P.N., which may be due to the excessive lipolyses occurred during pickling **(Shakeel *et. al.*,2000).** The use of starter with vells milk or powder rennet accelerated the pickling of Domiati cheese and the increasing of starter ratio led to increase in W.S.N., N.P.N. and T.V.F.A. of the resultant cheese.

Table (7) **Effect of different treatments on water soluble nitrogen, non protein nitrogen and total volatile fatty acids of Domiati cheese during pickling period.**

Sample No.	Treatment			W.S.N. %				N.P.N. %				T.V.F.A.	
	Milk	Rennet	Starter %	Pickling period (month)				Pickling period (month)					
				Fresh	1	2	3	Fresh	1	2	3	Fresh	3 month
1	Raw	Vells milk	Non	0.19	0.36	0.70	1.18	0.11	0.33	0.63	0.80	0.94	3.66
2		Powder		0.17	0.26	0.53	0.80	0.09	0.27	0.42	0.55	0.72	3.51
3	Pasturized	Vells milk	Non	0.13	0.28	0.61	1.02	0.05	0.28	0.35	0.65	0.68	3.55
4		Powder		0.10	0.25	0.43	0.71	0.04	0.18	0.32	0.51	0.52	3.20
5		Vells milk	2	0.11	0.33	0.62	0.99	0.08	0.19	0.59	0.66	0.70	3.94
6			4	0.19	0.55	0.94	1.46	0.09	0.24	0.63	0.72	0.76	3.95
7			6	0.18	0.56	1.01	1.33	0.09	0.32	0.67	0.93	0.80	4.50
8			8	0.20	0.60	1.11	1.47	0.09	0.43	0.79	1.06	0.85	4.68
9			10	0.23	0.63	1.50	2.10	0.09	0.53	0.89	1.13	0.85	5.01
10		Powder	2	0.17	0.27	0.50	0.81	0.06	0.19	0.40	0.52	0.67	3.65
11			4	0.17	0.34	0.62	0.97	0.08	0.22	0.44	0.59	0.70	3.77
12			6	0.19	0.44	0.72	1.00	0.09	0.29	0.49	0.63	0.70	3.92
13			8	0.19	0.49	0.84	1.16	0.09	0.36	0.60	0.80	0.73	4.16
14			10	0.20	0.53	0.96	1.31	0.09	0.35	0.74	0.89	0.78	4.73

Organoleptic evaluation

As shown in table (8), it is clear that cheese made from pasturized milk lacks the full flavour compared to traditional cheese made from raw milk and vells milk, these results agreed with those of **Shakeel *et. al.*,(1999) and Shakeel *et. al.*,(2000).** The best treatment was no. (7) with 6% starter at 3 months compared to 8 and 10% starter (treatment 8 and 9 respectively). The increasing addition of starter till 8 or 10 % either using

powder rennet or vells milk were not acceptable because the produced cheese had pasty texture and sharp acid tast. At 3 months treatment No. (7) gained score (87) more than treatment No. (3) [Pastuerized milk + Vells milk] (81). So, it is recommended to add starter within limit 6% either using powder rennet or vells milk. Comparing the treatments in which powder rennet was used to that which vells milk was used as a coagulant, it was found that, using vells milk gave better results. Also the 10% starter could be used with vells milk as a coagulant after one month which reduced the time of pickling period. The above recommendations are in accordance with the standard lows and also kept the lovely characteristic flavour for the popular Domiati cheese.

Table (8) Effect of different treatments on organoleptic properties of Domiati cheese during pickling period (degree per 100)

Sample N No.	Treatment			Pickling period (month)		
	Milk	Rennet	Starter %	1	2	3
1	Raw	Vells milk	Non	90	95	98
2		Powder		83	84	90
3	Pasteurized	Vells milk	Non	76	82	81
4		Powder		73	79	79
5		Vells milk	2	81	82	81
6			4	82	84	84
7			6	85	87	89
8			8	86	86	80
9			10	87	87	81
10		Powder	2	70	79	80
11			4	75	79	82
12			6	79	82	85
13			8	76	85	82
14			10	79	86	81

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