Studies on the Proteolytic and Lipolytic Bacteria in Butter

Saad, A. H.; Salama, E. M.;*Takwa H. Ismail;*Zeinab N. Qandeel
*Department of Food Hygiene, Faculty of Vet. Med., Suez Canal University, *Animal Health Research Institute, Ismailia Lab.*

Abstract
A total of 50 random samples of butter were collected from Ismailia governorate. The samples were divided according to the storage temperature into three groups, (5°C, 15°C and 25°C). All samples were examined sensory and bacteriologically. Some defects were found in different proportions in flavor, body, texture and appearance. There was a decrease in the values of the apparent properties at temperatures of 15°C and 25°C but did not affect their apparent validity. The results showed that mean value of total psychrotrophs count (cfu/g) at storage temperature 5, 15, 25°C were $5.6\times10^5 \pm1.1\times10^5$, $1\times10^6 \pm1.8\times10^5$, $2.3\times10^6 \pm3.2\times10^5$ respectively. Some proteolytic and lipolytic psychrotrophs as *Achromobacter* , *Acinetobacter, Alcaligens*, *Bacillus*, *Citrobacter, Enterobacter*, *E.coli, Flavobacter*, *Klebsiella, Micrococcus*,*Pseudomonas*, *Proteus*, *Serratia* and *Staphylococcus spp*. were isolated with different percentage.

Introduction

Storage temperature, processing and transportation of milk plays a major role in the diversity of microorganisms which can grow and leading to spoilage of milk. Psychrotrophs are able to grow at low temperatures and alter the milk by producing heat resistant proteolytic enzymes which induce degradation of casein with production of many off-flavors as musty, fruity, rancid, bitter and even putrid flavors. Lipolytic enzymes could be produced also from psychrotrophs which have spoilage effects as rancid flavors in milk and dairy products that make a subsequent reduction of their shelf-life and the products become unacceptable to consumers ***(Shah, 1994)***. Lipolysis is the enzymatic hydrolysis of milk lipids to free fatty acids and partial glycerides, is a constant concern to the dairy industry because of its harmful effects on milk and milk products. However, free fatty acids also contribute to the desirable flavor of milk and milk products when present at low concentrations. ***(Deeth, 2002)***. During cold storage of raw milk, psychrotrophic bacteria dominate the microflora and their extracellular enzymes mainly proteases and lipases contribute to the spoilage of dairy products. So, psychrotrophs which can grow at 7°C, play a leading role in spoilage of refrigerated milk and milk

products. The number of psychrotrophs that develop after milk collection depend on the storage temperature and time. ***(Elionora and Malka 2007)***. The highly nutritious nature of dairy products makes them especially good media for the growth of microorganisms. ***(Loralyn and Robert, 2009)*** Proteolysis is the enzymatic hydrolysis of the milk proteins to amino acids which catalyzed by hydrolytic enzymes. The proteases from psychrotrophs also find application in food and molecular biology. ***(Ramesh , 2010).***

Therefore, the present study was planned out to determine the influence of psychrotrophic bacteria and their lipolytic and proteolytic enzymes on the quality of some milk products stored at various temperatures.

Material and Methods

Samples Collection: A total of 50 random samples of butter were collected randomly and transferred immediately without delay to laboratory under complete aseptic condition in clean vessels.

Sensory evaluation of butter: Quality score card for sensory evaluation which determined by comparing the properties or characteristics of each product with their accepted standard of perfection.

Preparation of samples for bacteriological examination (APHA, 1992): Each sample was softened by placing the container in water-bath adjusted at $40^{\pm}1^{o}$C. 11g. of each prepared butter sample was added separately to 99 ml of hot sterile buffer Peptone water in a sterile bottle and thoroughly homogenized to prepare a dilution of 1/10. From original homogenate, 1 ml was transferred to a series of sterile test tubes containing 9 ml of 0.1% sterile buffered peptone water and well mixed to prepare decimal serial dilutions of sample homogenate up to 10^{-7}.

Enumeration of Psychrotrophic bacteria: according to **(APHA, 2004)**

Enumeration of the total proteolytic Psychrotrophic bacterial count: according to ***(APHA, 2004)***

Enumeration of the total lipolytic Psychrotrophic bacterial count: according to ***(APHA, 2004)***

Results and Discussion:

Spoilage of butter may result from the presence of heat resistant proteases and lipases produced by psychrotrophic bacteria during storage of raw milk or cream even after death of spoilage organisms. ***(Kornacki et al.2001)***

1. <u>Sensory examination</u>:

Flavour: Data presented in Table (1) showed that 30% of examined butter samples stored at 5°C had no flavour criticisms. The incidence of slight flavour defects was 50% and the incidence of definite flavour defects was 20%. While 22% of examined butter samples stored at 15°C had no flavour criticisms

(Table 2). The incidence of slight flavour defects was 48% and the incidence of definite flavour defects was 30%. While 12% of examined butter samples stored at 25°C had no flavour criticisms (Table 3). The incidence of slight flavour defects was 40% and the incidence of definite flavour defects was 48%.

Body and texture: The data presented in Table (1) showed that 60% of examined butter samples stored at 5°C with no body and texture criticisms. The incidence of slight body and texture defects was 24% and the incidence of definite body and texture defects was 16%. While 56% of examined butter samples stored at 15°C with no body and texture criticisms (Table 2). The incidence of slight body and texture defects was 26% and the incidence of definite body and texture defects was 18%. While 50% of examined butter samples stored at 25°C with no body and texture criticisms (Table 3). The incidence of slight body and texture defects was 32% and the incidence of definite body and texture defects was 18%.

Appearance: The data presented in Table (1) showed that 54% of examined butter samples stored at 5°C with no appearance criticisms. The incidence of slight appearance defects was 30% and the incidence of definite appearance defects was 16%.While 52% of examined butter samples stored at 15°C with no appearance criticisms (Table 2). The incidence of slight appearance defects was 32% and the incidence of definite appearance defects was 16%.While 44% of examined butter samples stored at 25°C with no appearance criticisms (Table 3). The incidence of slight appearance defects was 38% and the incidence of definite appearance defects was 18%.

2. <u>Bacteriological examination:</u>

It's evident from results recorded in table(4) that the mean psychrotrophic count in examined butter samples at storage temperature 5, 15, 25°C were $5.6\times10^5 \pm 1.1\times10^5$, $1\times10^6 \pm 1.8\times10^5$ and $2.3\times10^6 \pm 3.2\times10^5$ CFU /ml respectively.

The obtained results were nearly similar to those reported by ***So et al. (1992)*** 1.2×10^6/ml, ***El-Sayed (1996)*** 3.0×10^6 ml, ***Abdou (1997)*** $1.01\times10^6 \pm 4.42\times10^5$ / ml, ***Morsy (1998)*** $5\times10^6 \pm 2\times10^5$/g. and ***Amer et al. (2005)*** 3.20×10^6/ml **.**

Results given in table (5) revealed that the mean lipolytic psychrotrophic count in examined butter samples at storage temperature 5, 15, 25°C were $3\times10^4 \pm 4.7\times10^3$, $7.4\times10^4 \pm 1.3\times10^4$ and $1.7\times10^5 \pm 3\times10^4$ CFU /ml respectively. Comparatively lower counts were obtained by ***Morsy (1998)*** $3\times10^3 \pm 2\times10^2$/g.

It's evident from results recorded in table (5) that the mean proteolytic psychrotrophic count in examined butter samples at storage temperature 5, 15, 25°C were $2.1\times10^4 \pm 3.6\times10^3$, $3.4\times10^4 \pm 5.4\times10^3$ and $7.7\times10^4 \pm 1.3\times10^4$

CFU /ml respectively. These results is nearly similar to that obtained by ***El-Sayed (1996)*** 3.0×10^4 /ml, while comparatively lower counts were obtained by ***Abdel- Hakim and Sabreen (1995)*** 6.5×10^3 cfu/ml.

Results obtained in figure (1) showed that the incidence of proteolytic psychrotrophs isolated from examined butter samples at 5°C were *Achromobacter spp*. 1 (2.44%); *Acinetobacter spp*. 2 (4.88%); *Alcaligens spp*. 4 (9.76%); *Bacillus spp.* 4 (9.76%); *Citrobacter spp*. 3 (7.31%); *Enterobacter spp*. 4 (9.76%); *E.coli* 2 (4.88%); *Flavobacter spp.* 3 (7.31%); *Klebsiella spp*. 3 (7.31%); *Micrococcus spp*. 4 (9.76%); *Pseudomonas spp*. 4 (9.76%); *Proteus spp*. 2 (4.88%); *Serratia spp*. 3 (7.31%) and *Staphylococcus spp*. 2 (4.88%), while the incidence of lipolytic psychrotrophs at 5°C were *Achromobacter spp.* 2 (3.84%); *Acinetobacter spp*. 3 (5.77%); *Alcaligens spp*. 3 (5.77%); *Bacillus spp.* 5 (9.62%); *Citrobacter spp*. 5 (9.62%); *Enterobacter spp*. 3 (5.77%); *E.coli* 1 (1.92%); *Flavobacter spp.* 4 (7.69%); *Klebsiella spp*. 4 (7.69%); *Micrococcus spp*. 3 (5.77%); *Pseudomonas spp*. 6 (11.53%); *Proteus spp*. 3 (5.77%); *Serratia spp*. 5 (9.62%) and *Staphylococcus spp*. 5 (9.62%).

The results obtained in figure (2) showed that the incidence of proteolytic psychrotrophs isolated from examined butter samples at 15°C were *Achromobacter spp*. 1 (2.27%); *Acinetobacter spp*. 3 (6.82%); *Alcaligens spp*. 4 (9.09%); *Bacillus spp.* 5 (11.37%); *Citrobacter spp*. 3 (6.82%); *Enterobacter spp*. 4 (9.09%); *E.coli* 2 (4.54%); *Flavobacter spp.* 4 (9.09%); *Klebsiella spp*. 2 (4.54%); *Micrococcus spp*. 3 (6.82%); *Pseudomonas spp*. 5 (11.37%); *Proteus spp*. 3 (6.82%); *Serratia spp*. 2 (4.54%); and *Staphylococcus spp*. 3 (6.82%), while the incidence of lipolytic psychrotrophs at 15°C were *Achromobacter spp*. 3 (5.26%); *Acinetobacter spp*. 4 (7.02%); *Alcaligens spp*. 5 (8.77%); *Bacillus spp.* 6 (10.53%); *Citrobacter spp*. 5 (8.77%); *Enterobacter spp*. 4 (7.02%); *E.coli* 1 (1.75%); *Flavobacter spp.* 3 (5.26%); *Klebsiella spp*. 3 (5.26%); *Micrococcus spp.* 2 (3.51%); *Pseudomonas spp*. 8 (14.04%); *Proteus spp*. 4 (7.02%); *Serratia spp*. 3 (5.26%); and *Staphylococcus spp*. 6 (10.53%)

The results obtained in figure (3) showed that the incidence of proteolytic psychrotrophs isolated from examined butter samples at 25°C were *Achromobacter spp*. 3 (5.46%); *Acinetobacter spp*. 2 (3.63%); *Alcaligens spp*. 5 (9.09%); *Bacillus spp.* 5 (9.09%); *Citrobacter spp*. 5 (9.09%); *Enterobacter spp*. 3 (5.46%); *E.coli* 2 (3.63%); *Flavobacter spp.* 5 (9.09%); *Klebsiella spp*. 3 (5.46%); *Micrococcus spp.* 3 (5.46%); *Pseudomonas spp.* 6 (10.90%); *Proteus spp*. 3 (5.46%); *Serratia spp*. 5 (9.09%); and *Staphylococcus*

spp. 5 (9.09%), while the incidence of lipolytic psychrotrophs at 25°C were *Achromobacter spp*. 5 (7.57%); *Acinetobacter spp*. 5 (7.57%); *Alcaligens spp*. 4 (6.06%); *Bacillus spp.* 8 (12.12%); *Citrobacter spp*. 6 (9.09%); *Enterobacter spp*. 3 (4.54%); *E.coli* 3 (4.54%); *Flavobacter spp.* 4 (6.06%); *Klebsiella spp*. 2 (3.03%); *Micrococcus spp*. 3 (4.54%); *Pseudomonas spp*. 11 (16.66%); *Proteus spp*. 2 (3.03%); *Serratia spp*. 2 (3.03%); and *Staphylococcus spp*. 8 (12.12%). These finding agree, to a certain extent, with those reported by ***So et al. (1992), Abdel-Hakim and Sabreen (1995), El-Sayed (1996), Abdou (1997), Aiad (1998), Morsy (1998), Stepaniak (2002), Amer et al. (2005), Parkash et al. (2007) and Boubendir et al. (2011)***

The microflora of butter reflects the quality of cream, the sanitary conditions of equipment used to manufacture the butter and the environmental and sanitary conditions during packaging and handling of such product ***(Richter et al., 1992).***

Table 1 *:Score card for sensory evaluation of butter at 5°C.*

Flavour criticisms	**Score**		**No. of samples**		**%**	
No criticism	10		15		30	
	Intensity of defect					
	Slight		**definite**		**pronounced**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Defect	25	50	10	20	0	0
Body & texture	**Score**		**No. of samples**		**%**	
No criticism	5		30		60	
	Intensity of defect					
	Slight		**definite**		**pronounced**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Defect	12	24	8	16	0	0
Appearance	**Score**		**No. of samples**		**%**	
No criticism	5		27		54	
	Intensity of defect					
	Slight		**definite**		**pronounced**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Defect	15	30	8	16	0	0

Table 2: *Score card for sensory evaluation of butter at 15°C.*

Flavour criticisms	**Score**		**No. of samples**		**%**	
No criticism	10		11		22	
	Intensity of defect					
	Slight		**definite**		**pronounced**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Defect	24	48	15	30	0	0
Body & texture	**Score**		**No. of samples**		**%**	
No criticism	5		28		56	
	Intensity of defect					
	Slight		**definite**		**pronounced**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Defect	13	26	9	18	0	0
Appearance	**Score**		**No. of samples**		**%**	
No criticism	5		26		52	
	Intensity of defect					
	Slight		**definite**		**pronounced**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Defect	16	32	8	16	0	0

Table 3: *Score card for sensory evaluation of butter at 25°C.*

Flavour criticisms	**Score**		**No. of samples**		**%**	
No criticism	10		6		12	
	Intensity of defect					
	Slight		**definite**		**pronounced**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Defect	20	40	24	48	0	0
Body & texture	**Score**		**No. of samples**		**%**	
No criticism	5		25		50	
	Intensity of defect					
	Slight		**definite**		**pronounced**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Defect	16	32	9	18	0	0
Appearance	**Score**		**No. of samples**		**%**	
No criticism	5		22		44	
	Intensity of defect					
	Slight		**definite**		**pronounced**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Defect	19	38	9	18	0	0

Table 4: *Statistical analytical results of psychrotrophs count / ml. in examined butter samples at storage temperature 5, 15, 25°C .*

Temp	No. of samples	Min.	Max.	Mean ± S.E.
At 5°C	50	2×10^4	3×10^6	$5.6\times10^5 \pm 1.1\times10^5$
At 15°C	50	5×10^4	5×10^6	$1\times10^6 \pm 1.8\times10^5$
At 25°C	50	8×10^4	7×10^6	$2.3\times10^6 \pm 3.2\times10^5$

Table 5: *Statistical analytical results of lipolytic and proteolytic psychrotrophic count /ml. in examined butter samples at storage temperature 5, 15, 25°C.*

		No. of samples	Min.	Max.	Mean ± S.E.
At 5°C	**lipolytic**	50	3×10^2	1×10^5	$3\times10^4 \pm 4.7\times10^3$
	Proteolytic		2×10^2	8×10^4	$2.1\times10^4 \pm 3.6\times10^3$
At 15°C	**lipolytic**	50	6×10^2	3×10^5	$7.4\times10^4 \pm 1.3\times10^4$
	Proteolytic		2×10^2	1×10^5	$3.4\times10^4 \pm 5.4\times10^3$
At 25°C	**lipolytic**	50	9×10^2	7×10^5	$1.7\times10^5 \pm 3\times10^4$
	Proteolytic		7×10^2	3×10^5	$7.7\times10^4 \pm 1.3\times10^4$

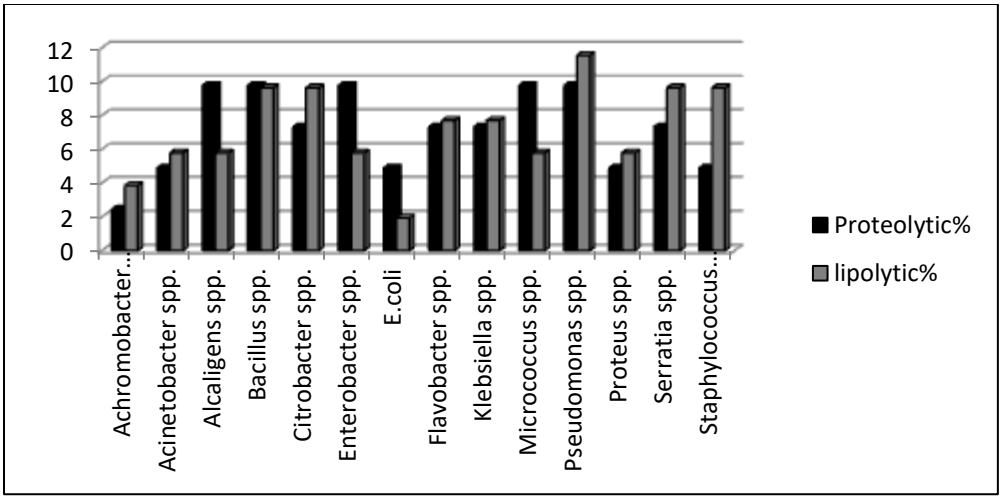

Figure 1: *Incidence of proteolytic and lipolytic psychrotrophs isolated from examined butter samples at 5°C:*

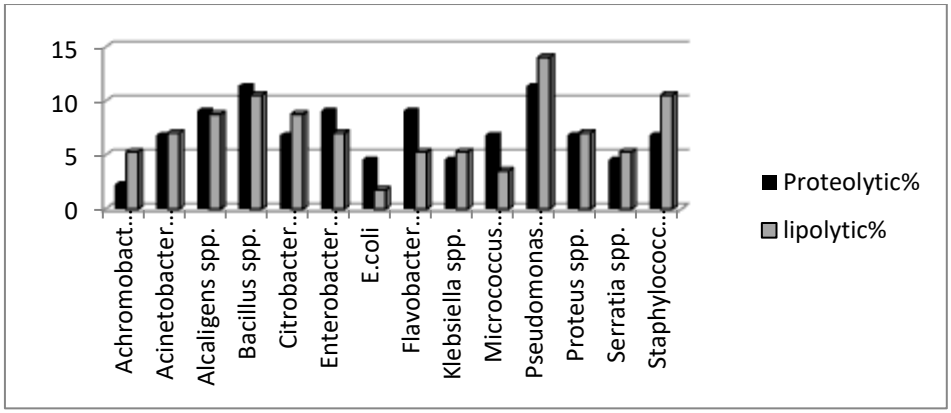

Figure 2: *Incidence of proteolytic and lipolytic psychrotrophs isolated from examined butter samples at 15°C:*

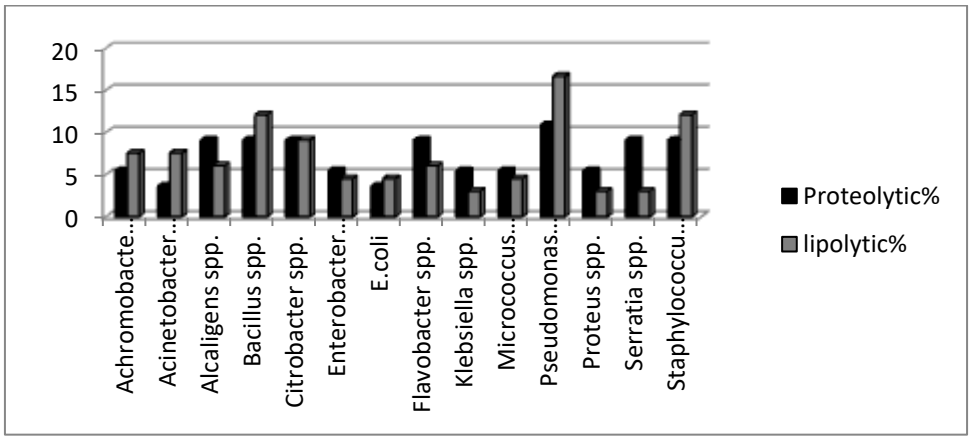

Figure 3: *Incidence of proteolytic and lipolytic psychrotrophs isolated from examined butter samples at 25°C:*

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الملخص العربي

دراسات على البكتيريا المحللة للبروتين والدهون فى الزبدة

احمد حسن سعد، ايهاب محمد سلامة، تقوى حسين اسماعيل*، زينب نبيل قنديل سليمان*
قسم الرقابة الصحية على الاغذية ، كلية الطب البيطري ، جامعة قناة السويس
**معهد بحوث صحة الحيوان- الاسماعيلية.*

أجريت الدراسة على خمسون عينة من الزبدة من محافظة الإسماعيلية. تم تقسيم العينات وفقا لدرجة حرارة التخزين إلى ثلاث مجموعات عند 5 ^{0}م و15 ^{0}م و25 ^{0}م. وتم تقيمها ظاهريا و ميكروبيولوجيا. وقد وجدت بعض العيوب بنسب مختلفة فى النكهة والتماسك والمظهر وقد حدث انخفاض فى قيم الخواص الظاهرية عند درجات الحرارة 15 ^{0}م و 25 ^{0}م ولكنها لم تؤثر على صلاحيتها ظاهريا. وأوضحت النتائج ان عند درجات تخزين 5 ^{0}م و15 ^{0}م و25 ^{0}م كان متوسط العدد الكلى للبكتريا المحبة للبرودة فى عينات الزبدة $5{,}6\times10^5 \pm 1{,}1\times10^5$, $1\times10^6 \pm 1{,}8\times10^5$, $2{,}3\times10^6 \pm 3{,}2\times10^5$خلية /جرام علي التوالى. وقد تم عزل بعض البكتريا المحبة للبرودة المحللة للبروتين والدهون مثل اكروموباكتر, اسينيتوباكتر, الكاليجينز, باسيلس, سيتروباكتر, انتيروباكتر, ايشيريشيا كولاى, فلافوباكتر, كليبسيلا, ميكروكوكس, سيدوموناس, بروتيس, سراتيا, العنقوديات, بنسب مختلفة.