PART II

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Introduction

The ripening of cheese is a costly process mainly due to extended refrigerated storage. Furthermore, impending legislation may impose mandatory pasteurization in some countries or heat treatments of milk to incorporate the whey proteins into cheese, with the consequence of slowing down the process of maturation **(Trepanier *et al.,* 1992b)**. Therefore, there is a need to identify an accelerated ripening process capable of producing a cheese with characteristics equivalent to standard matured cheese **(El-Soda *et al.,* 1991; Trepanier *et al.,* 1991, 1992a,b).** Among the numerous methods described for the acceleration of cheese ripening, especial attention has been recently given to attenuated starter and more particularly heat and freeze-shocked cells **(Ezzat and El-Shafie, 1991).** *Lb. helveticus* was selected for this study because it has higher activity of amino peptidases, dipeptidase and proteinase enzymes that may be useful to accelerate the cheese ripening. Therefore, **the aim of this part is** to study the effect of freeze and heat-shocked *Lb. helveticus* on proteolysis, lipolysis and flavor development in Gouda cheese during ripening, consequently shortening of its ripening period.

Experimental procedure:

80 kg of fresh buffalo's and cow's milk were mixed at the rate of (1:1) and standardized to 3.5% fat. The raw mixed milk was divided into two parts:

1 - The first part consisted of 60 kg milk which was heated to 72ºC for 15-20sec. and directly cooled to 31ºC then divided into 3 equal portions (20 kg each) in 3 stainless steel cheese vats:

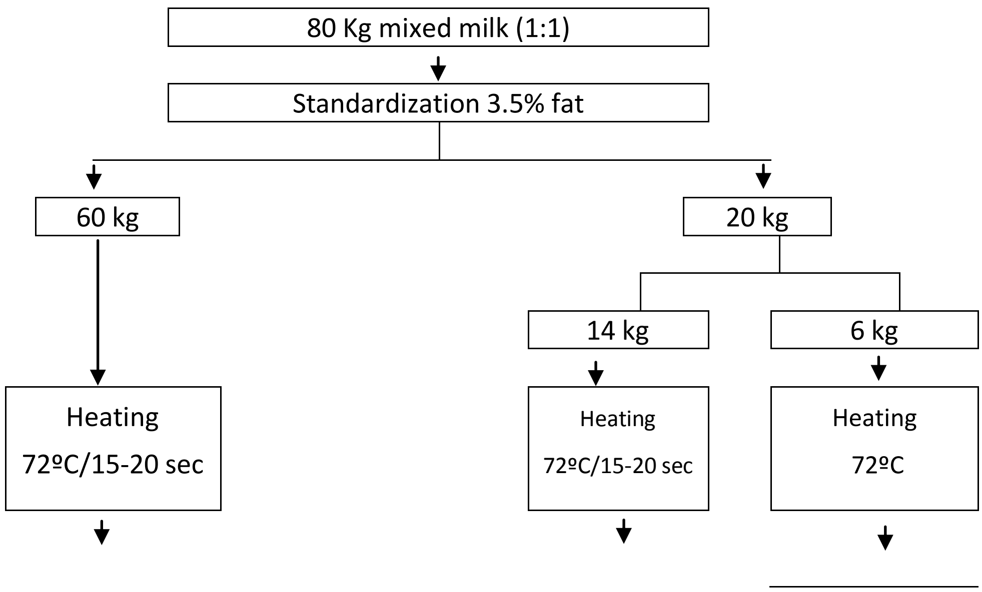
a - The first portion was mixed with about 720 g of reconstituted skim milk powder (11.5% T.S) and made into Gouda cheese which served as a control (treatment A).

b - The second portion was mixed with about 720 g of reconstituted skim milk powder (11.5% T.S) inoculated with 2% *Lb. helveticus* and manufactured to Gouda cheese (treatment B).

c - The third portion was mixed with about 720g of reconstituted skim milk powder (11.5% T.S) then inoculated with 2% freeze shocked *Lb. helveticus* and made into Gouda cheese (treatment C).

2 - The second part consisted of the 20 kg milk which was divided into two portions. The first portion was 14 kg milk out of 20 kg was heated to 72ºC for 15-20 sec. and directly cooled to 9ºC. The second portion was 6 kg milk which was heated to 72ºC then mixed with about 720g of reconstituted skim milk powder (11.5% T.S) inoculated with 2% *Lb. helveticus* and the milk temperature was maintained at 72ºC for 18sec. which rapidly cooled about 37ºC by adding the first portion of milk(14Kg milk at 9ºC). Meanwhile, heat shocked *Lb. helveticus* was obtained then converted into Gouda cheese (treatment D). Gouda cheese making was completed by the procedure described under materials and methods. The resultant cheese was ripened at 10-12ºC and 85-95% relative humidity for 3 months. Treatments were carried out in triplicate and each analysis in duplicate. The average results were tabulated and figured. Representative samples of cheese were taken when fresh and after 30, 60

and 90 days of ripening, while the rest of cheese was recoated. Cheese samples were analyzed chemically, microbiologically and rheologically. Meanwhile, cheese of all treatments was organoleptically evaluated. According to the prior analyses, the best treatment of cheese (treatment C) with control cheese (treatment A) were analyzed after 90 days of ripening by gas liquid chromatography (GLC) and electrophoresis for determination of fatty acids content and protein breakdown, successively. The results were statistically analyzed. Also, all analysis was carried out as mentioned under materials and methods.



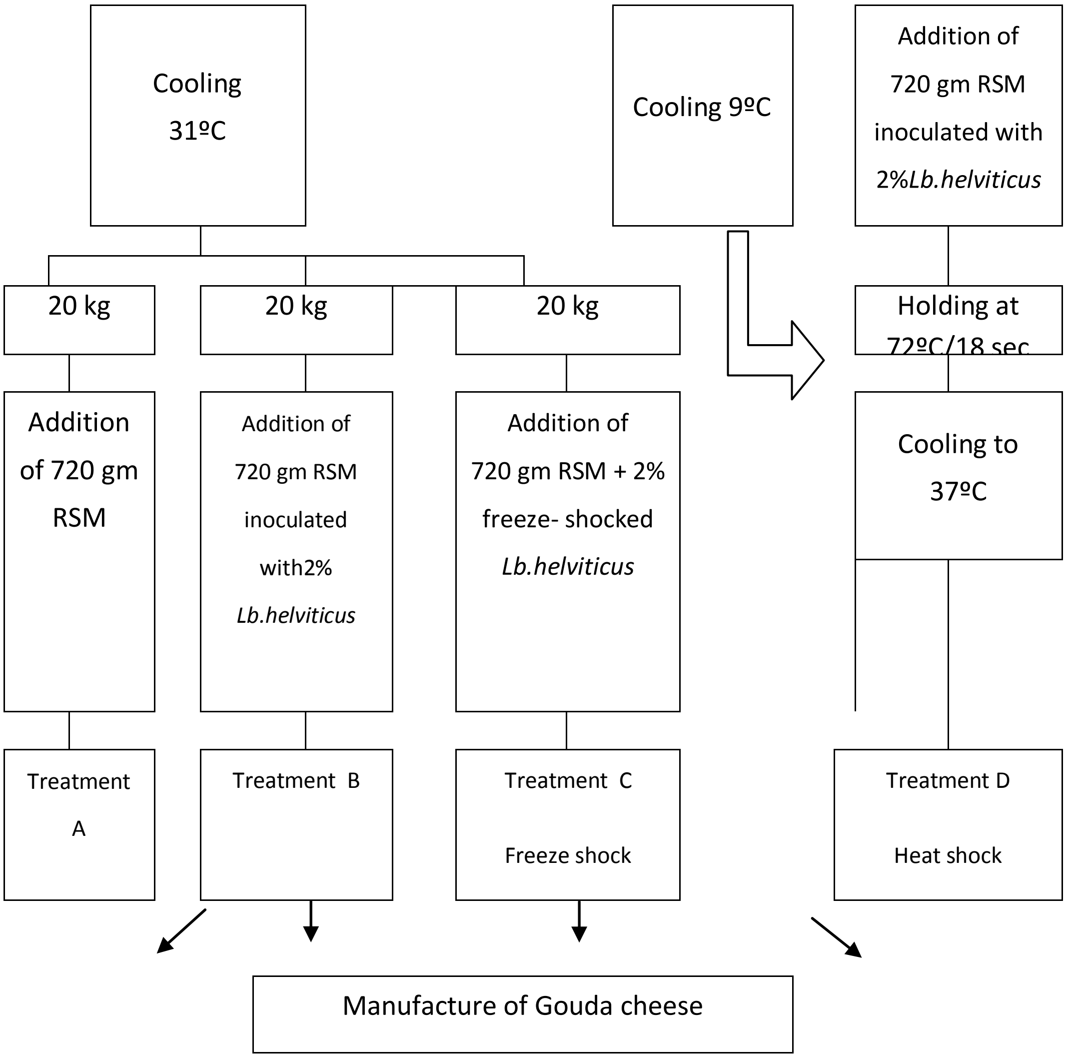


Fig. (10): Flow diagrams of Gouda cheese manufacture from different treatments.

Results and Discussion

1. Chemical composition of milk:

The chemical composition of raw mixed milk used for the production of Gouda cheese is presented in Table (4).

Table (4): Gross chemical composition of raw mixed milk used for making Gouda cheese**.**

Constituents %	Raw mixed milk

Moisture	86.69
Fat	3.5
Protein	3.41
Ash	0.93
Lactose	5.47
Acidity	0.17
pH value	6.78

2. Chemical composition of cheese:

2.1. Moisture content:

Data given in Table (5) and Fig (11) show the changes in moisture content of Gouda cheese made from different treatments during ripening for 90 days. It is clear from these results that the moisture content for fresh cheese was 48.14, 47.97, 48.10 and 48.12% for different treatments A, B, C and D, respectively. The moisture content of all cheese treatments sharply decreased to be 42.03, 41.16, 41.79 and 41.78%, successively at 30 days. Then, a gradual decrease in all cheese treatments reaching to 38.06, 37.27, 37.96 and 38.06% for different treatments in the same order at the end of ripening period. The decrease in moisture content of Gouda cheese during ripening may be due to the biochemical changes and lactic acid development which result in curd contraction and expulsion of aqueous phase of cheese. In addition evaporation of moisture during ripening. These results are in agreement with those obtained by **El-Demerdash (1996)**; **Mostafa (1999) and El-Sonbaty *et al.,* (2002)**. Also, these results reveal that the addition of freeze shocked *Lb. helveticus* or heat shocked *Lb. helveticus* did not remarkably affect the moisture content of Gouda cheese. These results confirmed by **Bartels *et al.,* (1987 a and b)** who reported that the use of heat and freeze shocked starter did not affect the moisture content of Gouda cheese**.** Also**, Spangler *et al.,* (1989)** reported that the using of a liposome entrapped enzyme and freeze shocked lactobacilli did not influence the moisture content of UF Gouda cheese. Finally, **Kebary *et al.,* (1996)** reported that the addition of lactobacilli, either freezed or heated shock did not significantly affect the moisture content of low fat Ras cheese. While, cheese treated with *Lb. helveticus* (treatment B) had the lowest moisture content throughout the ripening period compared to other treatments. This can be explained on the basis that viable whole cells of *Lb. helveticus* with starter culture increase lactic acid development which result in increasing curd contraction and expulsion of extra aqueous phase of cheese. These results are confirmed by **Abdel-Halem (2007).**

The statical analysis of variance for moisture content shows highly significant differences ($P \leq 0.01$) at 30 days of ripening, while there were significant differences ($P \leq 0.05$) after 60 and 90 days of ripening between Gouda cheese treatments.

Table (5): **The effect of addition of freeze and heat shocked *Lb. helveticus* culture to cheese milk on moisture content of resultant Gouda cheese during ripening period.**

Treatments	Moisture %			
	Ripening period (days)			
	Fresh	30	60	90
A	48.14	42.03^{A}	40.02^{A}	38.06^{A}
B	47.97	41.16^{B}	38.62^{B}	37.27^{B}
C	48.10	41.79^{A}	39.92^{A}	37.96^{A}
D	48.12	41.78^{A}	39.85^{A}	38.06^{A}
LSD (5%)	----*	0.3996	0.8616	0.5994

* : Not significant
A : Control cheese with commercial starter.
B : Cheese treated with *Lb. helveticus*.
C : Cheese treated with freeze-shocked *Lb. helveticus*.
D : Cheese treated with heat-shocked *Lb. helveticus*.

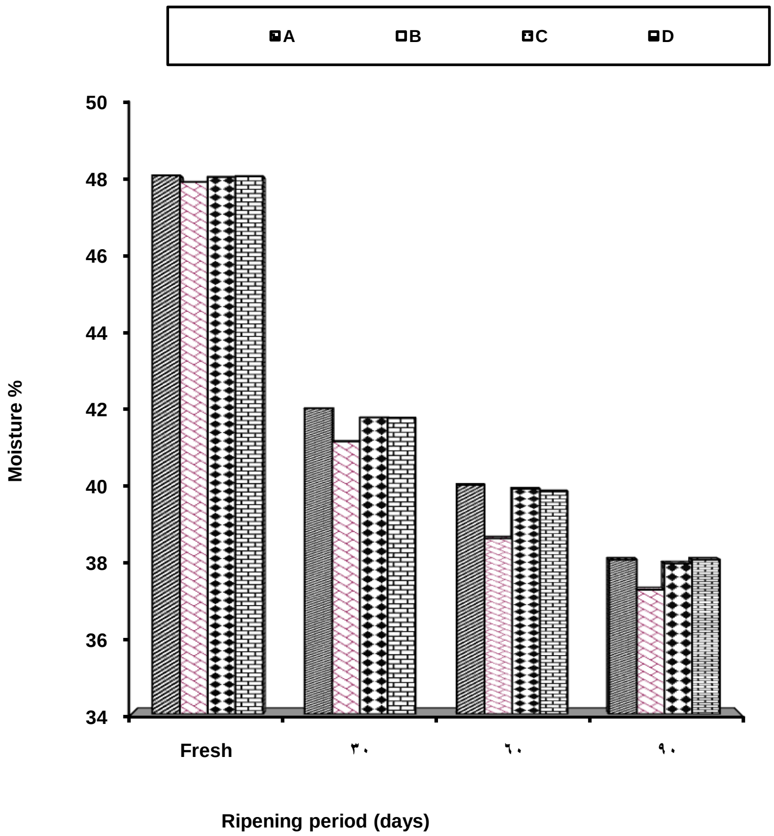


Fig. (11): **The effect of addition of freeze and heat shocked *Lb. helveticus* culture to cheese milk on moisture content of resultant Gouda cheese during ripening period.**

2.2. Fat content:

Results in Table (6) and Fig (12) show the changes in fat content and fat on dry matter basis (Fat/DM%) of Gouda cheese treated with either whole cells of *Lb. helveticus* or freeze and heat shocked *Lb. helveticus* during ripening period. The fat content of fresh cheese was 25.67, 26.00, 25.63 and 25.70% for treatments A, B, C and D, respectively. The fat content of different treatments markedly increased during the first month of ripening, then slightly gradually increased up to the end of ripening period to be 31.00, 31.70, 31.07 and 31.10% for different cheese treatments in the same order. On the other hand, fat/DM content of fresh cheese was 49.49, 49.97, 49.35 and 49.54 for treatments A, B, C and D, respectively. The fat/DM content slightly increased during the ripening period to be 50.05, 50.93, 50.07 and 50.21% in the same order at the end of ripening period. These increases in fat/DM content could be attributed to the gradual increase of fat content throughout the ripening period. Similar results were obtained by **Ammar (1995); El-Shafie *et al.,* (2003) and Ismail *et al.,* (2004)** who reported that there was slight increase in fat content of Edam cheese due to evaporation of water during ripening period. The results clear that neither the addition of freeze shocked *Lb. helveticus* nor heat shocked *Lb. helveticus* noticeable affected the fat or fat/dry matter content of Gouda cheese. These results are confirmed by **Aly (1990)** who stated that the addition of freeze shocked *Lb. helveticus* at levels of 1% and 2% to Ras cheese did not considerably affect the gross chemical composition of cheese. **Kim *et al.,* (1994a)** reported that the addition of fungal proteases and freeze shocked *Lb. helveticus* had no effect on fat content of Gouda cheese**. El-Baz (2001)** illustrated that the addition of different levels of freeze or heat shocked *Lb. helveticus* did not significantly affect the fat/DM content of UF Edam cheese during ripening period**.** While, concerning cheese treated with *Lb. helveticus* (treatment B) had slight increase in fat content and fat/DM content compared to other treatments with the increase in ripening period. This may be due to that cheese treated with viable whole cells of *Lb. helveticus* had the lowest moisture content throughout the ripening period.

From the statical analysis, it is clear that there were no significant differences between the treatments of Gouda cheese in fat and fat/DM content when fresh or during ripening period.

Table (6): **The effect of addition of freeze and heat shocked** *Lb. helveticus* **culture to cheese milk on fat and fat/DM content of resultant Gouda cheese during ripening period.**

Parameters	Ripening period (days)	Treatments				L.S.D (5%)
		A	B	C	D	
Fat%	Fresh	25.67	26.00	25.63	25.70	-----*
	30	28.70	29.50	28.83	28.90	-----
	60	29.90	30.83	29.93	30.13	-----
	90	31.00	31.70	31.07	31.10	-----
Fat/DM%	Fresh	49.49	49.97	49.35	49.54	-----
	30	49.50	50.14	49.57	49.64	-----
	60	49.85	50.84	49.82	50.10	-----
	90	50.05	50.93	50.07	50.21	-----

-----* : Not significant
A : Control cheese with commercial starter.
B : Cheese treated with *Lb. helveticus*.
C : Cheese treated with freeze-shocked *Lb. helveticus*.
D : Cheese treated with heat-shocked *Lb. helveticus*.

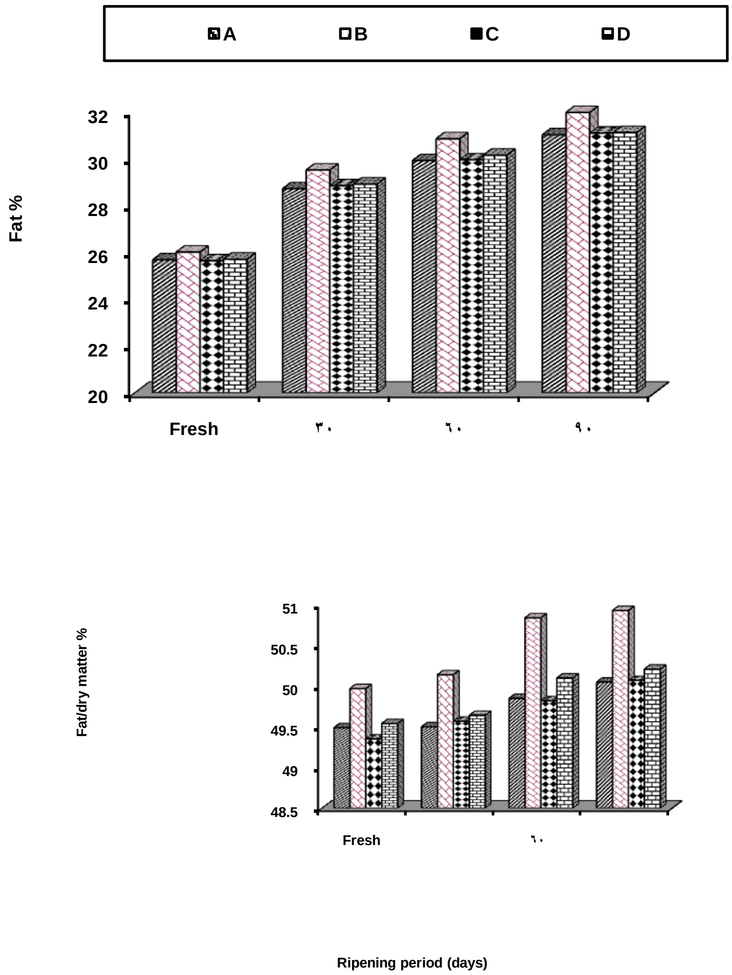


Fig.(12): The effect of addition of freeze and heat shocked *Lb. helveticus* culture to cheese milk on fat and fat/DM content of resultant Gouda cheese during ripening period.

2.3. Salt content:

Data given in Table (7) and Fig (13) indicate the changes in salt content and salt on moisture basis of Gouda cheese made from different treatments during ripening period. At the beginning of the ripening period, salt content of cheese was 1.57, 1.70, 1.63 and 1.63% for treatments A, B, C and D, respectively. While, the corresponding values

for salt on moisture content were 3.26, 3.54, 3.40 and 3.39% in the same order. It could be observed that the salt and salt on moisture content of all cheese treatments gradually increased with the increase of the ripening period to be 3.50, 3.60, 3.53 and 3.57% for different treatments A, B, C and D, in the same sequence at the end of ripening period. While, the corresponding values for salt in moisture content were 9.20, 9.79, 9.31 and 9.37% in the same order. This could be due to the loss of some of moisture content of the cheese during ripening period. The general trend of these results is similar to those reported by **Mostafa *et al.,* (2002) and Mahana *et al.,* (2003) and El-Tawel (2004)** who observed a slight gradual increase in the salt and salt in moisture content of Edam cheese during ripening period. On the other hand, it can be observed from these results that the addition of freeze or heat shocked *Lb. helveticus* did not considerably affect the salt or salt in moisture content. These results are confirmed by **Bartels *et al.,* (1987 a and b)** who reported that the use of heat and freeze shocked starter did not affect the salt content of Gouda cheese**. Spangler *et al.,* (1989)** reported that the using of a liposome entrapped enzyme and freeze shocked lactobacilli did not influence the salt content of UF Gouda cheese. **Kim *et al.,* (1994a)** reported that the addition of fungal proteases and freeze shocked
Lb. helveticus had no effect on salt content of Gouda cheese**.** It can be also observed that cheese treated with
Lb. helveticus (treatment B) had rather increase in salt and salt on moisture content compared with other treatments throughout the ripening period. This may be due to the lowest moisture content of cheese treated with whole cells of *Lb. helveticus* during the ripening period.

The obtained results from the statical analysis for salt content reflect that there were no significant differences between Gouda cheese treatments when fresh or during ripening period. While, there were significant differences ($P \leq 0.05$) in salt/moisture content of Gouda cheese treatments at 90 days of ripening.

Table (7): **The effect of addition of freeze and heat shocked *Lb. helveticus* to cheese milk on salt and salt/moisture content of**

resultant Gouda cheese during ripening period.

Parameters	Ripening period (days)	Treatments				L.S.D (5%)
		A	B	C	D	
Salt %	Fresh	1.57	1.70	1.63	1.63	----*
	30	2.63	2.80	2.60	2.67	----
	60	3.27	3.50	3.30	3.33	----
	90	3.50	3.60	3.53	3.57	----
Salt/moisture %	Fresh	3.26	3.54	3.40	3.39	----
	30	6.27	6.80	6.22	6.30	----
	60	8.17	8.90	8.27	8.37	----
	90	9.20 [B]	9.79 [A]	9.31 [B]	9.37 [B]	0.3460

----* : Not significant
A : Control cheese with commercial starter.
B : Cheese treated with *Lb. helveticus*.
C : Cheese treated with freeze-shocked *Lb. helveticus*.
D : Cheese treated with heat-shocked *Lb. helveticus*.

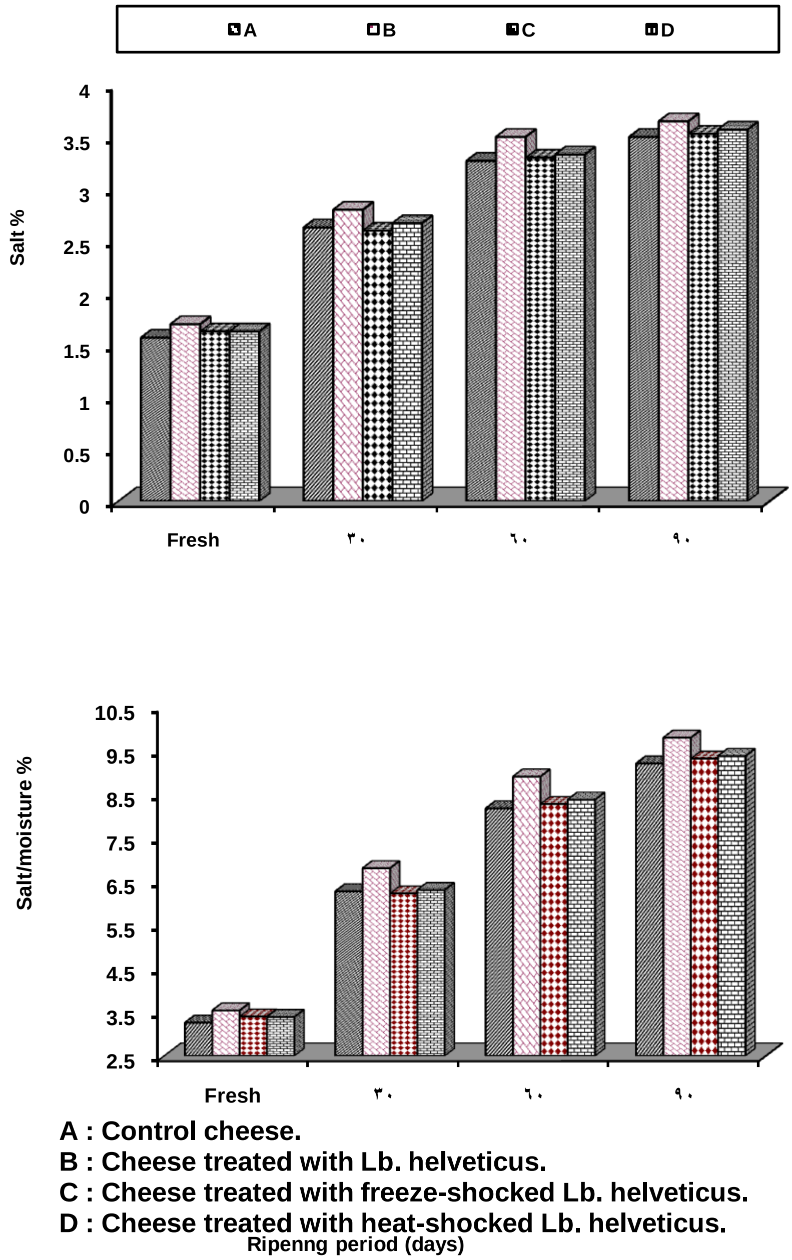


Fig. (13): **The effect of addition of freeze and heat shocked *Lb. helveticus* to cheese on salt and salt/moisture content of resultant Gouda cheese during ripening period.**

2.4. Titratable acidity:

Data presented in Table (8) and Fig (14) show the changes in titratable acidity of all Gouda cheese treatments. The titratable acidity of fresh cheese was 0.99, 1.10, 1.03 and 1.00% for treatments A, B, C and D, successively. The obtained results clearly indicate that the titratable acidity of all cheese treatments gradually increased with the advance of

ripening as it reached to 2.43, 2.61, 2.46 and 2.45% for different treatments in the same order at the end of ripening period. The gradual increase in the titratable acidity of all cheese treatments could be explained by the development of acidity throughout ripening as a result of lactose fermentation and milk constitutes by lactic acid bacteria and liberation of fatty acids. Similar results were obtained by **El-Sonbaty *et al.,* (2002) and El-Tawel (2004)** who stated that the acidity of Edam cheese increase markedly during the first month of ripening then gradually up to the end of ripening. It can be seen from the obtained results that cheese treated with *Lb. helveticus* (treatment B) had the highest titratable acidity throughout all stages of ripening compared with the other treatments. This increase may be due to the addition of whole viable cells of *Lb. helveticus*. On the other side, the titratable acidity of control cheese (treatment A), cheese treated with freeze shocked
Lb. helveticus (treatment C) and cheese treated with heat shocked *Lb. helveticus* (treatment D), were very close to each other. This can be attributed to the acid production by freeze shocked or heat shocked *Lb. helveticus* was sufficiently retarded. These results are in line with those obtained by **Abou Zeid and Mahmoud (1992)** who studied the use of heat shocked *Lb. helveticus* for ripening acceleration of Ras cheese with special reference to slurry and found that the slurry prepared with *Lb. helveticus* gave nearly the same acidity of corresponding control slurry at all incubation interval**. Tungjaroenchai *et al.,* (2001)** reported that the titratable acidity of reduced and full fat Edam cheese increased with aging.

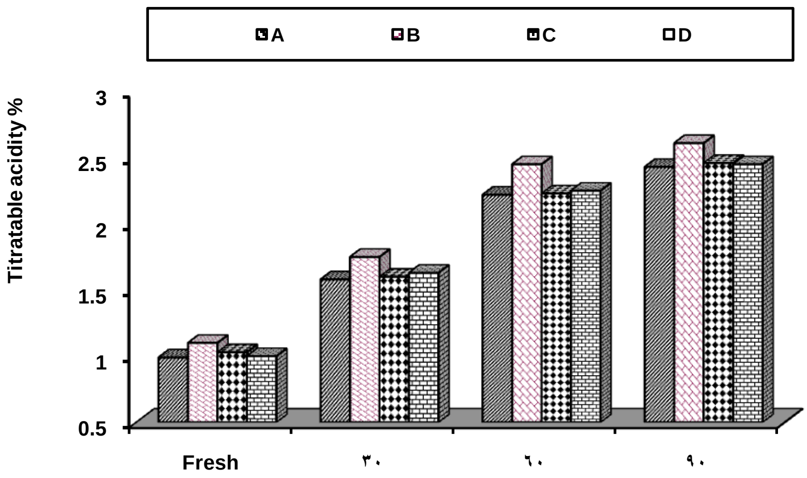
The statical analysis of titratable acidity indicates significant differences ($P \leq 0.05$) between Gouda cheese treatments when fresh, while it was highly significant differences ($P \leq 0.01$) during ripening period (30, 60 and 90 days).

Table (8): **The effect of addition of freeze and heat shocked *Lb. helveticus* to cheese on the titratable acidity (%) and pH values of resultant Gouda cheese during ripening period.**

Parameters	Ripening period (days)	Treatments				L.S.D (5%)
		A	B	C	D	

Titratable acidity %	**Fresh**	0.99^{B}	1.10^{A}	1.03^{AB}	1.00^{AB}	0.1094
	30	1.58^{C}	1.75^{A}	1.60^{BC}	1.63^{B}	0.03460
	60	2.22^{B}	2.45^{A}	2.23^{B}	2.25^{B}	0.06318
	90	2.43^{B}	2.61^{A}	2.46^{B}	2.45^{B}	0.06318
pH values	**Fresh**	5.34^{A}	5.20^{B}	5.30^{A}	5.32^{A}	0.06318
	30	5.24^{A}	5.08^{B}	5.21^{A}	5.20^{A}	0.06318
	60	5.31^{A}	5.18^{B}	5.33^{A}	5.32^{A}	0.03460
	90	5.45^{A}	5.20^{B}	5.41^{A}	5.43^{A}	0.04467

A : Control cheese with commercial starter.
B : Cheese treated with *Lb. helveticus*.
C : Cheese treated with freeze-shocked *Lb. helveticus*.
D : Cheese treated with heat-shocked *Lb. helveticus*.



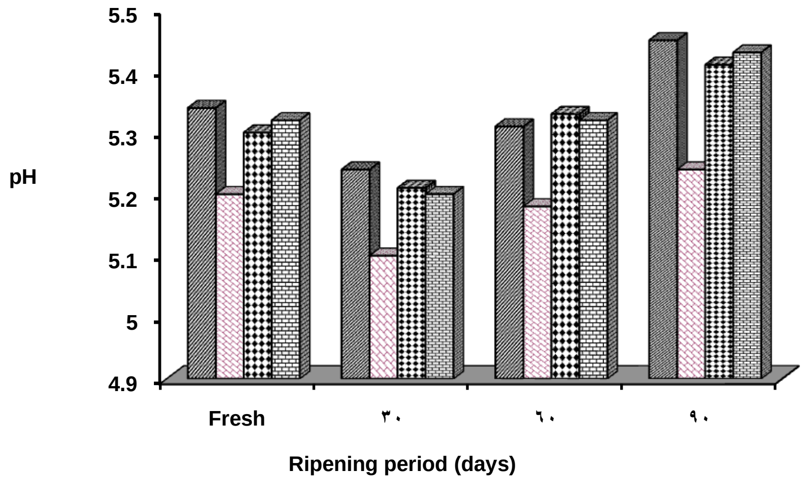


Fig.(14): **The effect of addition of freeze and heat shocked *Lb. helveticus* to cheese on the titratable acidity (%) and pH values of resultant Gouda cheese during ripening period**.

2.5. pH value

Data given in Table (8) and Fig (14) show the changes in the pH values of Gouda cheese made from different treatments during ripening for 90 days. The results indicate that the pH in fresh cheese was 5.34, 5.20, 5.30, and 5.32 for treatments A, B, C and D, respectively. While, it decreased to 5.24, 5.08, 5.21 and 5.20 at 30 days for different treatments in the same order. As shown, there was a general drop in the pH values of all treatments during the first month of ripening, while gradually increased during the second and third month. The pH was 5.45, 5.20, 5.41 and 5.43 at 90 days for different treatments in the same order. Such decrease in the pH of a premature cheese is mainly due to the development of lactic acid from residual lactose fermentation. However, the gradual increase in the pH which was noticed during ripening could be attributed to a further breakdown of lactic acid and forming basic compounds as well as basic amino groups through the protein degradation upon advanced ripening. Similar results were reported by **Rymaszewski and El-Tanboly (1990)** who found that the pH values of Edam cheese made from bovine milk ranged from 5.2 to 5.27 at 6 weeks of ripening. The general trend of results are in agreement with those obtained by **Zaki and Salem (1992); El-Baz (2001) and El-**

Tawel (2004). It is clear from these results that cheese treated with *Lb. helveticus* (treatment B) had the lowest pH values throughout the ripening period. This may be due to the presence of viable whole cells of *Lb. helveticus* which have the ability of producing extra quantities of lactic acid which contribute in decreasing the pH value. Results also reveal that there were no appreciable differences in pH values between treatments A, C and D. This means that neither the addition of freeze shocked nor heat shocked *Lb. helveticus* affected the pH values of resultant Gouda cheese. These results are confirmed by **Kim *et al.,* (1994a)** who reported that the addition of commercial fungal proteases and freeze shocked *Lb. helveticus* to UF and conventional Gouda cheese did not affect on the pH values during ripening period. Consequently, **El-Sisey (2002)** observed that the pH values of low fat Edam cheese were not affected by adding either freeze shocked *Lb. helveticus* or commercial enzymes.

The statical analysis of variance for pH values shows highly significant differences ($P \leq 0.01$) between Gouda cheese treatments when fresh and during ripening period (30, 60 and 90 days).

2.6. Total nitrogen content (T.N %):

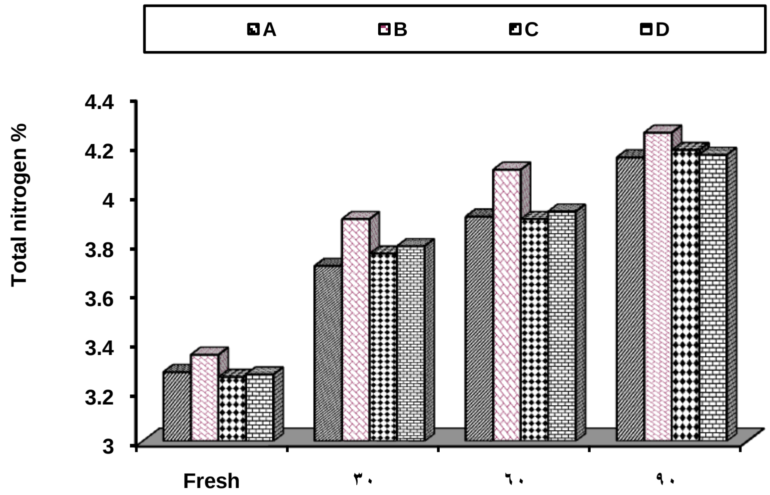
The changes in total nitrogen content and total nitrogen on dry matter basis (T.N/DM%) of Gouda cheese made from different treatments during ripening period for 90 days are presented in Table (9) and Fig (15). The T.N content of fresh cheese was 3.28, 3.35, 3.26 and 3.27% for treatments A, B, C and D, respectively. The T.N content slightly increased during the ripening period to be 4.15, 4.30, 4.18 and 4.16% in the same order at the end of ripening period. On the other hand, the T.N/DM content of fresh cheese was 6.32, 6.44, 6.28 and 6.31% for treatments A, B, C and D, successively. The T.N/DM content also slightly increased throughout the ripening period to be 6.70, 6.86, 6.74 and 6.72% in the same order at the end of ripening period. Generally, it is clear from these data that

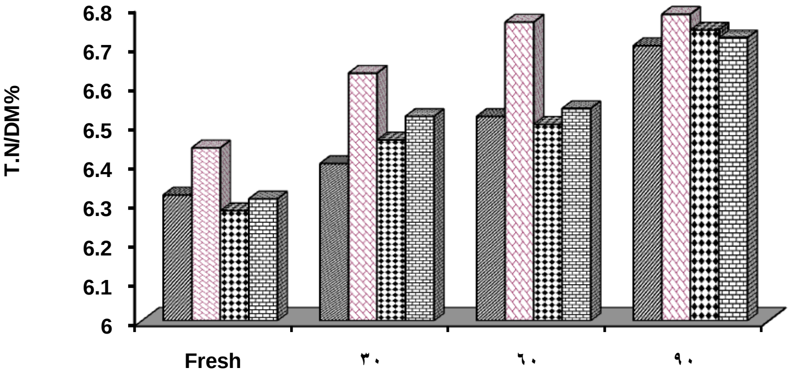
Table (9): The effect of addition of freeze and heat shocked *Lb. helveticus* to cheese on the T.N and T.N/DM content of resultant Gouda cheese during ripening period.

Parameters	Ripening period (days)	Treatments				L.S.D (5%)
		A	B	C	D	
TN%	Fresh	3.28	3.35	3.26	3.27	-----*
	30	3.71^{B}	3.90^{A}	3.76^{B}	3.79^{AB}	0.1094
	60	3.91^{B}	4.10^{A}	3.90^{B}	3.93^{B}	0.06318
	90	4.15^{B}	4.30^{A}	4.18^{B}	4.16^{B}	0.1094
TN/DM%	Fresh	6.32	6.44	6.28	6.31	-----
	30	6.40	6.63	6.46	6.52	-----
	60	6.52^{B}	6.76^{A}	6.50^{B}	6.54^{B}	0.08935
	90	6.70^{B}	6.86^{A}	6.74^{B}	6.72^{B}	0.1094

----* : Not significant
A : Control cheese with commercial starter.
B : Cheese treated with *Lb. helveticus*.
C : Cheese treated with freeze-shocked *Lb. helveticus*.
D : Cheese treated with heat-shocked *Lb. helveticus*.

the T.N and T.N/DM content of all cheese treatments gradually increased as ripening progressed depending on the loss of moisture content. Similar trend of these results were reported by **Omar and Buchheim (1983)** who found that the total nitrogen content of Gouda cheese made from fresh cow's milk (3% fat) increased gradually during ripening to be 4.30% at 8 weeks of ripening. **Hussein (2000)** concluded that neither addition of enzymes nor freeze shocked *Lb. helveticus* significantly affected the total nitrogen content of low fat Ras cheese. These results reveal that the addition of freeze shocked or heat shocked *Lb. helveticus* did not remarkably affect the T.N or T.N/DM content of Gouda cheese. These results are confirmed by





A : Control cheese.
B : Cheese treated with Lb. helveticus.
C : Cheese treated with freeze-shocked Lb. helveticus.
D : Cheese treated with heat-shocked Lb. helveticus.

Fig.(15): **The effect of addition of freeze and heat shocked *Lb. helveticus* to cheese on the TN and TN/DM content of**

resultant Gouda cheese during ripening period.

El-Baz (2001) and Mostafa *et al.,* (2002) who found that the addition of heat or freeze shocked starter to UF Edam cheese did not affect the total nitrogen or TN/DM content**.** Cheese treated with *Lb. helveticus* (treatment B) had the highest T.N and T.N/DM content during the ripening period. This may be attributed to the high loss of moisture content during ripening period.

The statical analysis of TN content clears significant differences ($P \leq 0.05$) between Gouda cheese treatments during ripening period (30, 60 and 90 days). While, the TN/DM content of Gouda cheese treatments shows significant differences at 60 and 90 days of ripening.

2.7. Ripening indices:

It is well known that the process of ripening is the result of a complex of biochemical reactions which emanates from the main components of cheese. Casein being one of the main constituents undergoes several changes during the process with the production of a multitude of simple nitrogenous compounds such as peptones, polypeptides, amino acids and ammonia. The extend of protein degradation represents the main indicator of cheese ripening. Several methods are adopted for the determination of the degree of ripening. Some of these procedures are applied in this work.

2.7.1. Soluble nitrogen content (S.N %):

Data given in Table (10) and Fig (16) show the changes in soluble nitrogen content and soluble nitrogen on total nitrogen basis (S.N/T.N%) of Gouda cheese made from different treatments during ripening for 90 days. The S.N content of fresh cheese was 0.250, 0.287, 0.297 and

Table: (10) **The effect of addition of freeze and heat shocked *Lb. helveticus* to cheese on S.N and S.N/T.N content of resultant Gouda cheese during ripening period.**

Parameters	Ripening period (days)	Treatments				L.S.D (5%)
		A	B	C	D	
SN%	Fresh	0.250	0.287	0.297	0.293	----*
	30	0.403 C	0.500 B	0.600 A	0.593 A	0.02605
	60	0.497 C	0.603 B	0.717 A	0.697 A	0.06318
	90	0.587 C	0.743 B	0.853 A	0.833 A	0.04467
SN/TN%	Fresh	7.932	8.599	9.099	8.957	----
	30	10.872 C	12.821 B	15.960 A	15.641 A	0.8262
	60	12.701 C	14.715 B	18.358 A	17.735 A	0.9726
	90	14.129 C	17.488 B	20.402 A	20.028 A	0.9540

----* : Not significant
A : Control cheese with commercial starter.
B : Cheese treated with *Lb. helveticus*.
C : Cheese treated with freeze-shocked *Lb. helveticus*.
D : Cheese treated with heat-shocked *Lb. helveticus*.

0.293% for treatments A, B, C and D, successively. A gradual increase in S.N content in all cheese treatments was observed all over the ripening period. At the end of ripening period the soluble nitrogen content for all cheese treatments was 0.587, 0.743, 0.853 and 0.833% in the same order. With respect to The SN/TN content for fresh cheese was 7.932, 8.599, 9.099 and 8.957% for treatments A, B, C and D, successively. The SN/TN content gradually increased to be 14.129, 17.488, 20.402 and 20.028% in the same order at the end of ripening period. These increases can be attributed to the protein degradation and the formation of soluble nitrogenous compounds.

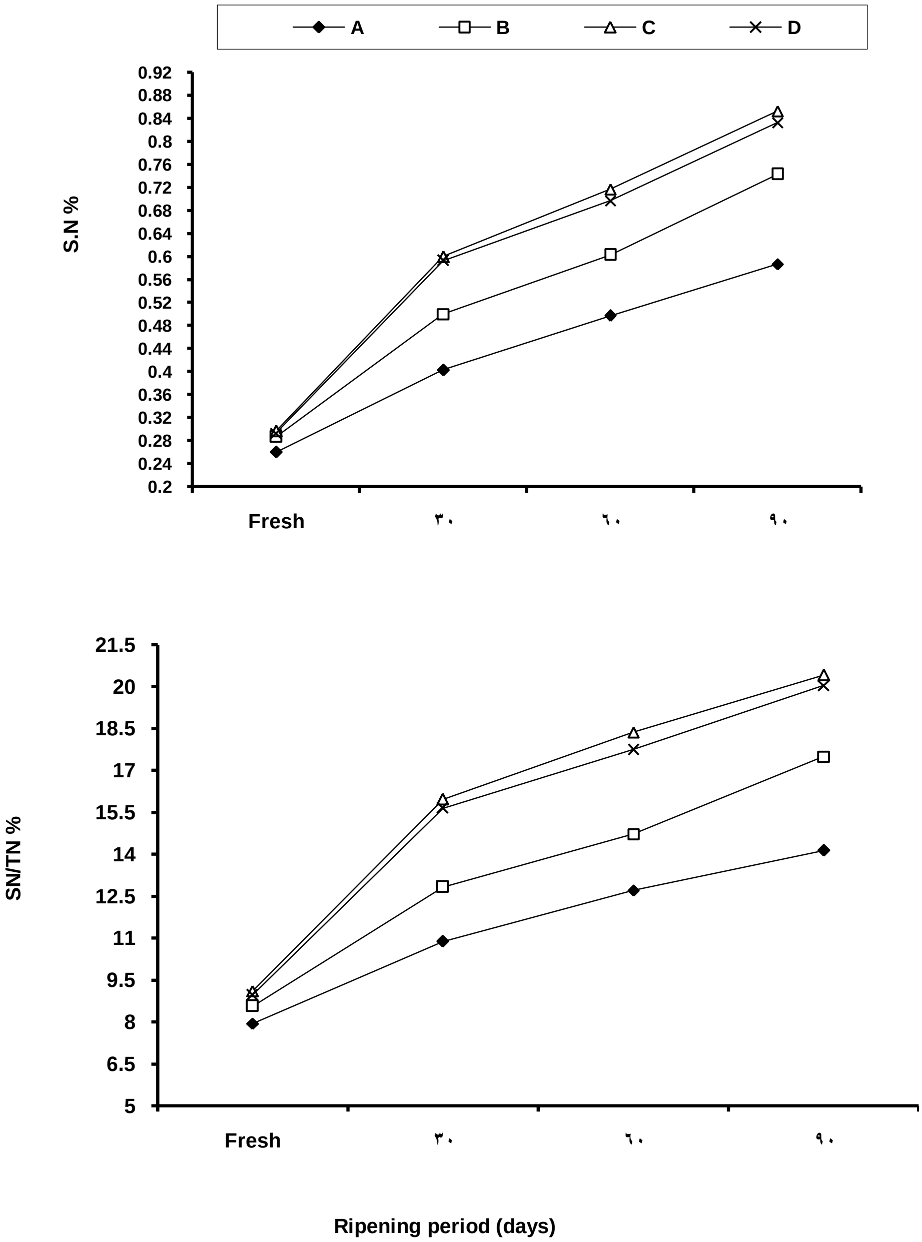


Fig. (16): **The effect of addition of freeze and heat shocked *Lb. helveticus* to cheese on soluble nitrogen and SN/TN content of resultant Gouda cheese during ripening period.**

These results are in agreement with those reported by **El-Etriby *et al.,* (1998**); **El-Tanboly *et al.,* (2003) and El-Shafie *et al.,***

(2003) who found that the soluble nitrogen and SN/TN content of Edam cheese gradually increased with the increase of ripening period. From these results it could be observed that cheese treated with freeze shocked *Lb. helveticus* (treatment C) followed by cheese treated with heat shocked *Lb. helveticus* (treatment D) had the highest S.N and SN/TN content with the increase in ripening period. This may be attributed to the freeze and heat shocked cells of *Lb. helveticus* may have lysed in the cheese to a greater extent than untreated cells of *Lb. helveticus* by freeze or heat shock. Also, freeze and heat shock increase the rate of autolysis in *Lb. helveticus*. This resulted in the greater release of the intracellular enzymes which react with protein and peptides in the cheese. These results are confirmed by **Abd El-Baky *et al.,* (1986)** who reported that the addition of heat shocked lactobacilli to the curd of Ras cheese accelerated the formation of SN. **Vafopoulou *et al.,* (1989)** reported that the addition of heat shocked culture of *Streptococcus thermophilus* and *Lb. delbrueckii* supsp. *bulgaricus* accelerated the production of tri-chloro acetic acid soluble nitrogen. **Kim *et al.,* (1994b)** found that the addition of freeze shocked *Lb. helveticus* cells to Gouda cheese increased tri-chloro acetic acid (TCA) and phosphotungestic acid (PTA) soluble nitrogen values during ripening. **Angelova and Spasova (1999)** developed a method for obtaining heat shocked starter for white–brined cheese and Kashkaval cheese which accelerated proteolysis and reduced the ripening period of these types of cheese by about 2 weeks. On the other hand, Results also reveal that cheese treated with *Lb. helveticus* (treatment B) had higher S.N and SN/TN content than control cheese (treatment A) with the increase in ripening period. This may be due to the presence of *Lb. helveticus* strain which can lyse and release their intracellular proteolytic enzymes in the cheese. These results are confirmed by **Awad *et al.,* (2000)** who concluded that the different lactobacillus strains used as adjunct can lyse and release their intercellular proteolytic enzymes in a cheese-like system. **Valence *et al.,* (2000**) also suggested that the intracellular peptidases of *Lb. helveticus* may play a major role in the proteolysis of Swiss cheese.

The statical analysis of variances clarifies that there were highly significant differences ($P \leq 0.01$) in SN and SN/TN content of all Gouda cheese treatments during ripening period (30, 60 and 90 days).

Consequently, the average of SN and SN/TN content of cheese treated with freeze and heat shocked *Lb. helveticus* after 60 days of ripening was higher than that of the market Gouda cheese samples (local and imported) at an age 90 days of ripening (Table 2).

2.7.2. Soluble tyrosine and tryptophan content:

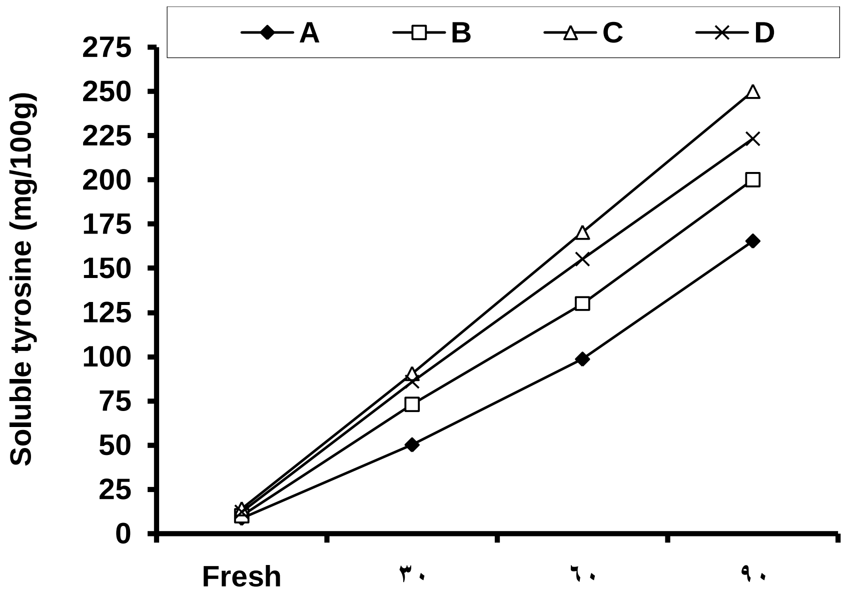
Data of soluble tyrosine and tryptophan of Gouda cheese made from different treatments during ripening for 90 days are presented in Table (11) and Fig (17). The soluble tyrosine content of fresh cheese was 10.0, 11.0, 12.5 and 12.2 mg/100g for treatments A, B, C and D, respectively. On the other hand, the soluble tryptophan content of fresh cheese was 4.1, 5.1, 5.6 and 5.4 mg/100g cheese for different treatments in the same order. These values gradually increased during the ripening period to be 165.3, 200.3, 250.2 and 223.4 mg soluble tyrosine/100g cheese and 90.1, 115, 141 and 130.7 mg soluble tryptophan/100g cheese for different treatments A, B, C and D, respectively. Generally, the increase in soluble tyrosine and tryptophan contents in all cheese treatments during ripening can be deduced to the protein degradation and partially to the decrease in the moisture content. These results are confirmed by **El-Sonbaty *et al.,* (2002)** in low fat Edam cheese **and Ismail *et al.,* (2004)** in Edam cheese**.** From these results, it could be concluded that cheese treated with freeze shocked *Lb. helveticus* (treatment C) followed by cheese treaded with heat shocked *Lb. helveticus* (treatment D) had the highest soluble tyrosine and tryptophan contents throughout the ripening period. This may be due to the freeze and heat shocked cells of *Lb. helveticus* may be have lysed in the cheese to a greater extent than cells were not treated. Also, freeze and heat shock increase the rate of autolysis in *Lb. helveticus*. This resulted in the greater release of the intracellular enzymes which react with protein and peptides in the cheese. These results are confirmed by **Castaneda *et al.*, (1990) who** reported that a variant of Saint-poulin cheese made with an extra inoculum of 1% (v/v) heat-shocked cells suspensions of *Lactobacillus helveticus* CNRZ32 accelerated production of free amino acids during ripening**. El-Soda *et al.,* (2000)** reported that the cheese slurry containing added freeze-shocked cells of *Lb.helveticus* showed considerably higher levels of peptidase activity release and higher rate of proteolysis compared to the

Table: (11) **The effect of addition of freeze and heat shocked *Lb. helveticus* culture to cheese milk on soluble tyrosine and tryptophan content of resultant Gouda cheese during ripening period.**

Parameters	Ripening period (days)	Treatments				L.S.D (5%)
		A	B	C	D	
Soluble tyrosine (mg/100g cheese)	Fresh	10.00 B	11.00 B	12.50 A	12.20 A	1.944
	30	50.02 C	73.10 B	90.06 A	85.90 A	6.216
	60	98.60 D	130.20 C	170.50 A	155.40 B	11.93
	90	165.30 D	200.30 C	250.20 A	223.40 B	11.83
Soluble tryptophan (mg/100g cheese)	Fresh	4.10 C	5.10 B	5.60 A	5.40 A	0.4285
	30	27.50 C	46.70 B	55.20 A	50.10 A B	7.730
	60	53.90 D	75.30 C	98.40 A	88.30 B	5.020
	90	90.10 D	115.00 C	141.00 A	130.70 B	10.05

A : Control cheese with commercial starter.
B : Cheese treated with *Lb. helveticus*.
C : Cheese treated with freeze-shocked *Lb. helveticus*.
D : Cheese treated with heat-shocked *Lb. helveticus*.

Lb. casei strains. The cheese slurry inoculated with freeze-shocked cells of *Lb. helveticus* had the most Cheddar flavor. Results also reveal that cheese treated with *Lb. helveticus* (treatment B) had higher soluble tyrosine and tryptophan contents than control cheese (treatment A) during ripening period. This may be due to the presence of *Lb. helveticus* strain which can lyse and release their intracellular proteolytic enzymes in the cheese. These


A
B
C
D
Soluble tyrosine (mg/100g)
275
250
225
200
175
150
125
100
75
50
25
0
Fresh
٣٠
٦٠
٩٠

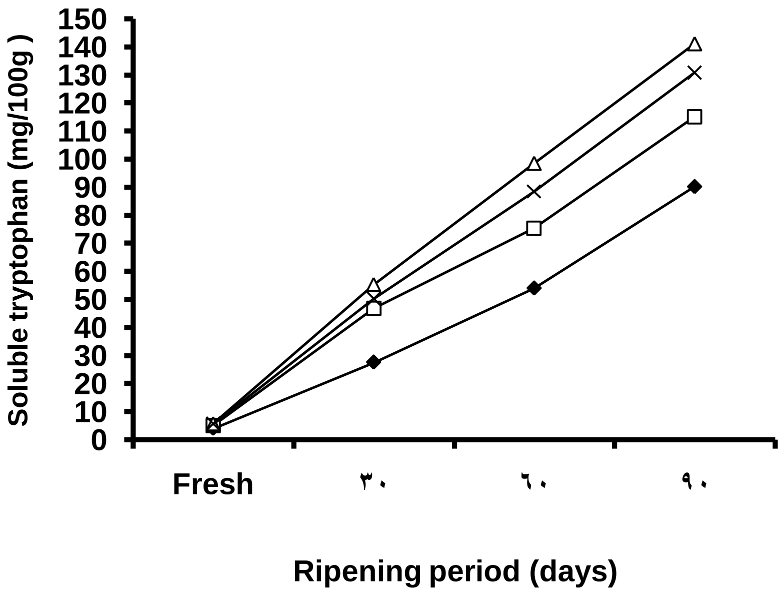

Soluble tryptophan (mg/100g)
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Fresh
٣٠
٦٠
٩٠
Ripening period (days)

Fig. (17): **The effect of addition of freeze and heat shocked *Lb. helveticus* culture to cheese milk soluble tyrosine and tryptophan content of resultant Gouda cheese during ripening period.**

results in agreement with those obtained by **Pahkala *et al.,* (1986)** who reported that one *Lb. casei* and three *Lb. helveticus*. strains showed considerable amino-peptidase activity, concentrated in the cytoplasm and membranes **Skeie *et al.,* (1995)** found that the low fat Gouda cheese with added heat-treated *Lactobacillus helveticus* had increased amount of amino N as well as increased amount of acetaldehyde and other unidentified volatile compounds. **Tungjaroenchai *et al.,* (2001)** found that the reduced fat Edam cheese containing *Lb. helveticus* exhibited the highest free amino acids (FAA) content.

The statical analysis for soluble tyrosine and tryptophan content clears that there were highly significant differences ($P \leq 0.01$) between Gouda cheese treatments when fresh and during ripening period.

Hence, the average of soluble tyrosine and tryptophan content of cheese treated with freeze and heat shocked *Lb. helveticus* at 60 days of ripening were similar to the market Gouda cheese samples (local and imported) at 90 days of ripening (Table 2).

2.7.3. Total volatile fatty acids content (T.V.F.A):

Data in Table (12) and Fig (18) reveal that the changes in total volatile fatty acids content of Gouda cheese made from different treatments during ripening for 90 days. The results clearly indicate that the T.V.F.A content of fresh cheese was 7.1, 8.0, 9.0 and 9.0 ml of 0.1N NaOH/100g cheese for treatments A, B, C and D, successively. These values gradually increased throughout the ripening period to be 33.2, 50.2, 64.3 and 60.0 ml of 0.1 N NaOH/100g cheese in the same order at the end of ripening period. These increases may be due to lipolysis of lipids and the reactions leading to breakdown of fat by microflora activities. These results in agreement with those reported by **Ammar (1995) and El-Tawel (2004)** in Edam cheese**.** It could be seen from these data that cheese treated with freeze shocked *Lb. helveticus* (treatment C) followed by cheese treaded with heat shocked *Lb. helveticus* (treatment D) had the highest T.V.F.A content. These increases may be due to the highest levels of lipolytic enzymes produced by freeze and heat shocked *Lb. helveticus*. Furthermore, the higher rate of protein degradation associated with more accumulation of free amino acids which serve as precurses for volatile fatty acids. In addition, non protein organic material from dead cells may serve as a source of volatile fatty acids upon degradation.

Table:(12) The effect of addition of freeze and heat shocked *Lb. helveticus* culture to cheese milk on total volatile fatty acids content of resultant Gouda cheese during ripening period.

Treatments	T.V.F.A (ml 0.1 N NaOH/100g cheese)			
	Ripening period (days)			
	Fresh	30	60	90
A	7.10^{B}	17.10^{C}	27.00^{D}	33.20^{C}
B	8.00^{B}	23.10^{B}	34.00^{C}	50.20^{B}
C	9.00^{A}	30.00^{A}	45.00^{A}	64.30^{A}
D	9.00^{A}	27.00^{A}	40.30^{B}	60.00^{A}
L.S.D (5%)	0.9808	3.891	2.156	5.419

A : Control cheese with commercial starter.
B : Cheese treated with *Lb. helveticus*.
C : Cheese treated with freeze-shocked *Lb. helveticus*.
D : Cheese treated with heat-shocked *Lb. helveticus*.

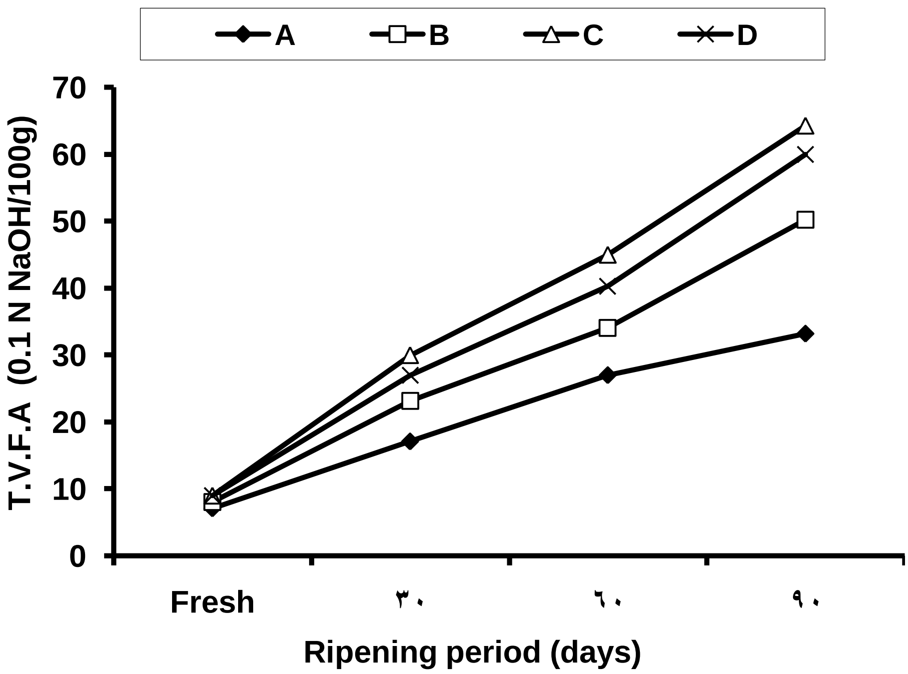


Fig. (18): **The effect of addition of freeze and heat shocked** *Lb. helveticus* **culture to cheese milk on total volatile fatty acids content of resultant Gouda cheese during ripening period.**

These results are in harmony with those obtained by **Smokuti *et al.*, (1981)** who demonstrated that the thermal shocking of lactic acid cultures leaved the bulk of proteolytic and lipolytic enzymes intact and that such cultures might used for developing mature Cheddar flavor in reduced-fat cheese. **El-Baz (2001)** found that the addition of freeze or heat shocked culture of *Lb. helveticus* at a rate of 1, 1.5 and 2% to Edam-like cheese increased the accumulation of the total volatile fatty acids content in ripened Edam-like cheese as compared to the control one. The analysis of variance for T.V.F.A content shows highly significant differences ($P \leq 0.01$) between all Gouda cheese treatments when fresh and during ripening period.

Therefore, the average of T.V.F.A content of cheese treated with freeze and heat shocked *Lb. helveticus* at 60 days of ripening was higher that that of the market Gouda cheese samples (local and imported) at 90 days of ripening (Table 2).

3. Microbiological examinations:

3.1. The effect of freeze and heat shock treatments on the counts of *Lactobacillus delbrueckii* subsp. *helveticus* in cell suspension:

Data illustrated in Table (13) show the changes in viable cell counts of *Lb. helveticus* in cell suspension before and after the freeze and heat shock treatments. It could be observed that the numbers of *Lb. helveticus* before freeze and heat shock were 100 and 90×10^7 cfu/ml, respectively. While, after freeze and heat shock were 1.1 and 2.5×10^7 cfu/ml in the same order. From these results we also observed that the reduction in viable cells of *Lb. helveticus* due to freeze and heat shock were 98.90% and 97.22%, respectively. The general trend of these results is similar to that reported by **Ezzat and El-Shafie (1991)** who found that the cells of *Lb. helveticus* were lost about 97% due to heat–shock and freezing treatment. Also, **Abou-Zeid and Mahmoud (1992)** observed that the reduction of the viable cells of *Lb. helveticus* culture due to heat shocking was about 97.7%.

Table:(13) Total viable cell counts of *Lb. helveticus* in cell suspension as affected by freeze and heat shock treatments.

Treatments	Counts x 10^7 cfu/ml		
	Before shock	After shock	Reduction in viable Cells number %
Freeze shock	100	1.1	98.90
Heat shock	90	2.5	97.22

3.2. Total bacterial counts:

Data presented in Table (14) and Fig (19) illustrated the total bacterial counts of Gouda cheese made from different treatments during the ripening period for 90 days. The total bacterial counts when fresh were 162, 220, 195 and 190x10^6cfu/g for treatments A, B, C and D, respectively. The total bacterial counts of all cheese treatments gradually decreased during the ripening period reaching the lowest counts 41, 49, 56 and 52x10^6 cfu/g for cheese treatments in the same order at the end of ripening period. The decrease in total bacterial counts could be attributed to the decrease of water activity and the increase of salt content and acidity in cheese. These results are in agreement with those obtained by **Mahana *et al.,* (2003) and Ismail *et al.,* (2004)** who found that the total bacterial count of Edam cheese gradually decreased during the ripening period reaching to the lowest count at the end of ripening. It is clear from these results that cheese treated with *Lb. helveticus* (treatment B) had higher value for total bacterial counts than that of all treatments when fresh. This may be due to the presence of viable whole cells of *Lb. helveticus*. While, after the first month of ripening and throughout the ripening period cheese treated with freeze shocked *Lb. helveticus* (treatment C) followed by cheese treated with heat shocked *Lb. helveticus* (treatment D) had higher value for total bacterial counts than that of other treatments. These results could be explained on the basis that the added freeze and heat shocked *Lb. helveticus* contain some simple nitrogenous compounds which stimulate the development of bacterial growth during cheese making and ripening processes. **El-Soda *et al.,* (1999)** found that cheese slurries made with freeze-shocked cells revealed higher enzyme activity and more protein and fat breakdown than cheese slurries inoculated with heat-shocked adjuncts. This was attributed to the higher release of enzyme activity in the case of freeze-shocked adjunct cultures and which in turn stimulated the growth and activity of cheese flora during cheese ripening. **El-Baz (2001)** found that Edam-like cheese treated with freeze or heat shocked *Lb. helveticus* was higher in the total bacterial count than untreated cheese when fresh and throughout ripening.

Table:(14) **The effect of addition of freeze and heat shocked** *Lb. helveticus* **culture to cheese milk on microbiological quality of resultant Gouda cheese during ripening period(count x cfu/g*).**

Microbiological examinations	Ripening period (days)	Treatments			
		A	B	C	D
Total bacterial count x 10^6	Fresh	162	220	195	190
	30	94	125	150	132
	60	63	80	90	85
	90	41	49	56	52
Proteolytic bacterial count x10^4	Fresh	72	110	165	160
	30	120	186	240	225
	60	174	250	300	285
	90	205	290	350	330
Lipolytic bacterial count x10^4	Fresh	47	65	72	70
	30	70	98	124	120
	60	95	126	160	140
	90	119	148	185	170

***Lactobacillus helveticus* count x10^5**	**Fresh**	----**	32	0.2	0.37
	30	----	44	0.6	0.76
	60	----	50	0.92	1.2
	90	----	41	0.75	0.77
Yeasts& Molds count x10^2	**Fresh**	----	----	----	----
	30	----	----	----	----
	60	2.5	3.0	2.2	2.3
	90	4.4	4.9	4.0	4.2

*** cfu** : **Colony forming unit.** ****----** **:** **Not detected.**

Coliform bacteria were not detected in all cheese treatments.

A : Control cheese with commercial starter.
B : Cheese treated with *Lb. helveticus*.
C : Cheese treated with freeze-shocked *Lb. helveticus*.
D : Cheese treated with heat-shocked *Lb. helveticus*.

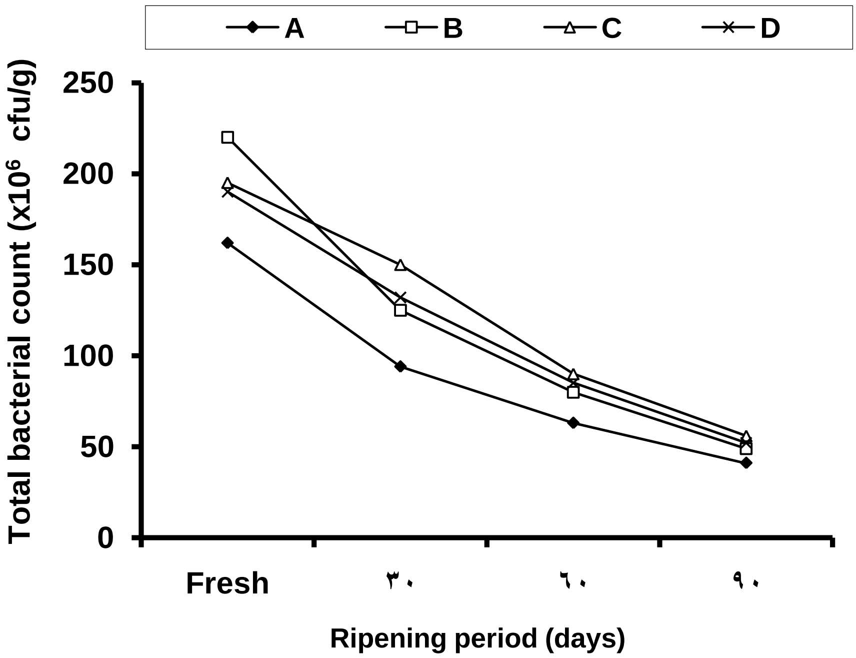
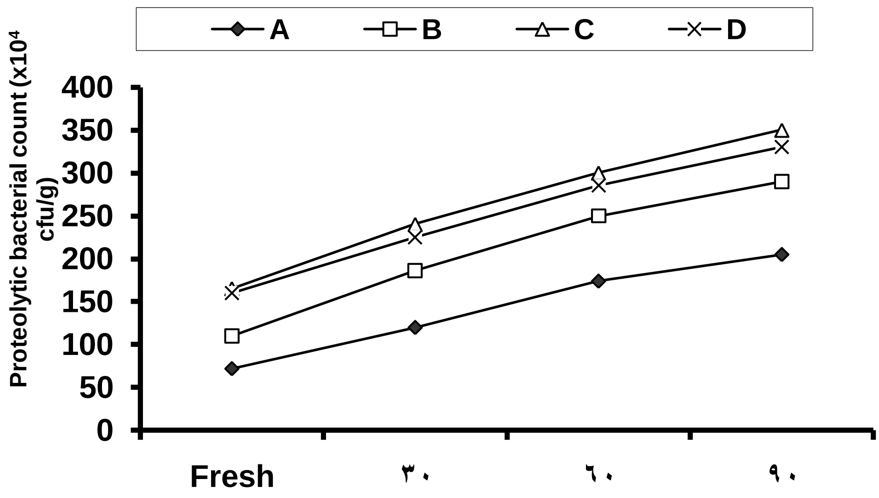


Fig. (19): **The effect of addition of freeze and heat shocked *Lb. helveticus* culture to cheese milk on the total bacterial count of resultant Gouda cheese during ripening period.**



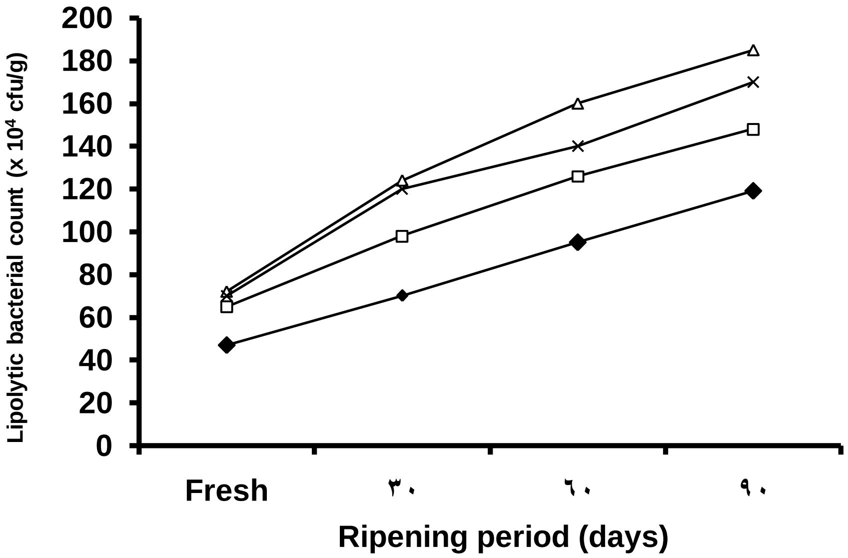
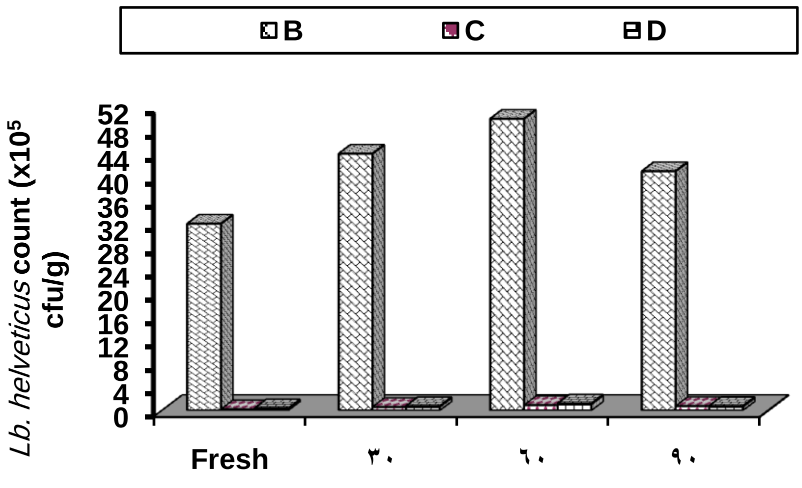


Fig.(20): **The effect of addition of freeze and heat shocked** ***Lb. helveticus*** **culture to cheese milk on the Proteolytic and lipolytic bacterial count of resultant Gouda cheese during ripening period..**



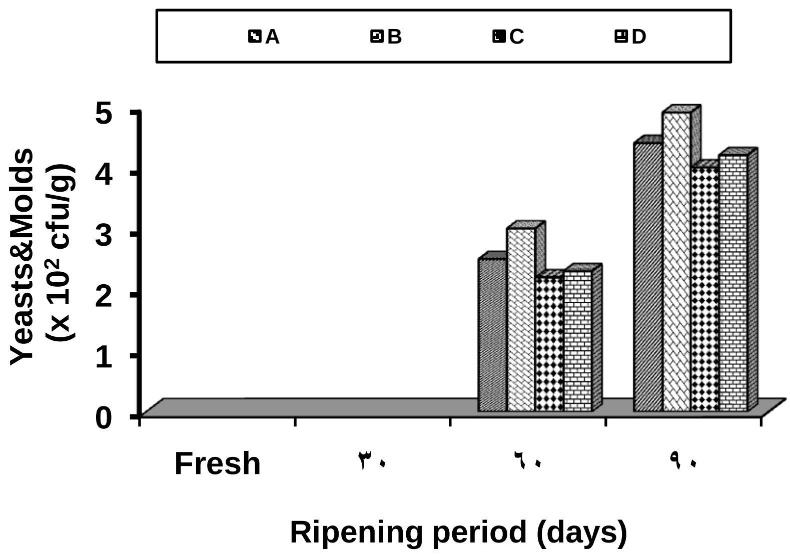


Fig. (21): **The effect of addition of freeze and heat shocked** *Lb. helveticus* **culture to cheese milk on the *Lb. helveticus* and Yeast& Molds count of resultant Gouda cheese during ripening period.**

3.3. Proteolytic bacterial counts:

Table (14) and Fig (20) show the effect of different treatments on the proteolytic bacterial counts of Gouda cheese during ripening. The proteolytic bacterial counts of fresh cheese were 72, 110, 165 and 160 x10^4 cfu/g for different treatments A, B, C and D, respectively. The proteolytic bacterial counts of all cheese treatments gradually increased with increasing of the ripening period to be 205, 290, 350 and 330 $x10^4$ cfu/g in the same order at the end of ripening period. It is clear from the results that cheese treated with freeze shocked *Lb. helveticus* followed by cheese treated with heat shocked *Lb. helveticus* had the highest proteolytic bacterial counts when fresh and throughout ripening period. This could be explained on the basis that the added freeze and heat shocked *Lb. helveticus* stimulate the development of bacterial growth during cheese making and ripening processes. This stimulant effect results some produced simple nitrogenous compounds by the freeze and heat shocked *Lb. helveticus*. Furthermore, during ripening the liberated intracellular enzymes from the freeze or heat shocked *Lb. helveticus* enhanced the proteolysis. Similar results were obtained by **Abd-El-Baky *et al.,* (1986); Aly (1990)** who reported that the proteolytic bacterial count of Ras cheese gradually increased during ripening period and Ras cheese treated with heat and freeze shocked *Lb. helveticus* or *Lb. casei* showed higher counts of proteolytic bacteria than control cheese during ripening period. **El-Baz (2001)** found that Edam-like cheese with added freeze or heat shocked *Lb. helveticus* was higher in the proteolytic bacterial count than untreated cheese when fresh and during ripening period.

3.4. Lipolytic bacterial counts:

Results presented in Table (14) and Fig (20) show the lipolytic bacterial counts of Gouda cheese made from different treatments. Results show that the lipolytic bacterial counts were 47, 65, 72 and 70 $x10^4$ cfu/g for fresh cheese of different treatments A, B, C and D, respectively. The lipolytic bacterial counts followed the same trend of proteolytic bacterial counts as the lipolytic bacterial counts of all cheese treatments gradually increased throughout the ripening period to be 119, 148, 185 and 170 x 10^4cfu/g for different treatments in the same order at the end of ripening period. It could be seen from these results that cheese treated with freeze shocked *Lb. helveticus* followed by cheese treated with heat shocked *Lb. helveticus* had the highest lipolytic bacterial counts when fresh and throughout ripening. This may be due to the same illustrated reasons in the proteolytic bacterial counts. Similar results were obtained by **Abou-Zeid and Mahmoud (1992)** who found that the lipolytic bacterial count of Ras cheese gradually increased by increasing the ripening period and the cheese treaded with heat shocked *Lb. helveticus* gave higher numbers than control. **El-Sisy (2002)** found that the low fat Edam cheese made with the addition of freeze shocked *Lb. helveticus* contained higher counts of lipolytic bacteria than others.

3.5. *Lactobacillus delbrueckii* subsp. *helveticus* counts:

The viable counts of *Lb. helveticus* in Gouda cheese made from different treatments during the ripening period for 90 days are registered in Table (14) and Fig (21). It is clear from these data that the viable counts of *Lb. helveticus* were not detected in control cheese (treatment A) when fresh or throughout ripening period. Because the cheese starter of the control cheese was free from *Lb. helveticus*. While, the viable counts of *Lb. helveticus* in fresh cheese were 32, 0.2 and 0.37 x10^5cfu/g for treatments B, C and D, respectively. The counts of *Lb. helveticus* in treatments B, C and D gradually increased reaching the maximum counts 50, 0.92 and 1.2 x10^5cfu/g, respectively after 60 days of the ripening period. Then, slightly decreased to be 41, 0.75 and 0.77 x 10^5cfu/g in the same order at the end of ripening period. The depression in counts of *Lb. helveticus* may be due to the acid development in different treatments of cheese. Such results in agreement with **Salem (1998)**. It can be observed from these data that treatment B had the highest viable counts of *Lb. helveticus* when fresh and during the ripening period. This may be due to the addition of whole cells of *Lb. helveticus* without freeze or heat shock. On the other hand, the cheese treated with freeze *Lb. helveticus* followed by cheese treated with heat shocked *Lb. helveticus* had the lowest viable counts of *Lb. helveticus* when fresh and during the ripening period. This may be due to 98.90 and 97.22% of the viable cells of *Lb. helveticus* were lost by freeze and heat shock, respectively Table (13). These results are in agreement with those obtained by **Bartels *et al.,* (1987b)** who reported that approximately 96% to 98% of the viable cells of *Lb. helveticus* were killed during the freezing procedures. **Aly (1990)** observed that the reduction in the viable cells of *Lb. helveticus* culture and *Lb. casei* culture due to freeze shocking were 99.22% and 99.09%, respectively.

3.6. Yeasts& molds and coliform bacteria counts:

Table (14) and Fig. (21) show that no colonies of yeasts and molds appeared in all cheese treatments when fresh or after 30 days. But, after the second month of ripening few colonies had been observed (less than 10 x 10^2cfu/g).The counts of yeasts and molds were corresponded with the lowest pH values appropriate for enhancing their growth. These results are in according with those obtained by **Jordano *et al.,* (1991) and Gafour (2005).**With respect to coliform bacteria no colonies were detected in all cheese treatments either when fresh or during the ripening period. This reflects the hygienic standards and sanitary conditions during the cheese making and ripening period. In addition, the role of lactic acid bacteria in preservation of the product which associated with their ability to produce a range of antimicrobial compounds **(Gould, 1991)**.

4. Textural properties

The texture of any food product, in general, and of cheese in particular, is of paramount importance because it is the property which the consumer identifies the variety and judges the quality before its flavor is assessed. The texture changes are one of the major events occurring during ripening. The texture of cheese depends upon the status of composition and the extent of biochemical changes taken place during ripening. In general, proteins are the main structural components in cheese and the rheological properties of the protein matrix depend on the presence of free water, fat particles and salt **(Innocente *et al.,* 2002).** The rate and extent of texture development, thereby, the quality of cheese can be determined by measuring the quantitiable rheological properties of cheese in terms of hardness, springiness, cohesiveness, gumminess and chewiness.

a) Hardness, springiness and cohesiveness:

The texture characteristics of Gouda cheese made from different treatments are presented in Table (15) and illustrated in Fig. (22).

Hardness is described to the panellists as the force required to compress a food between the molars. The average of hardness of fresh Gouda cheese was 11.14, 11.16, 11.02 and 11.12 N for treatments A, B, C and D, consecutively. A gradual increase in hardness of cheese in all treatments was observed during the ripening period to be 13.79, 12.95, 12.14 and 12.21 N in sequence at 60 days of ripening. This may be due to moisture decrease in cheese resulted in a firmer texture due to alterations in the casein matrix **(Tunick *et al.,* 1991).** Also, hardness of cheese increased with the lower levels of proteolysis, the coarser and the stronger protein network **(Fredrick and Dulley, 1984).** But, there was a decrease in hardness cheese at 90 days of ripening in all treatments to be 12.11, 11.64, 11.07 and 11.17 N in the same order. This could be mainly due to the proteolysis of casein produces compounds that are very soluble in water and that do not contribute to the protein network responsible for the cheese rigidity. Similar results were reported by **El-Tawel (2004).** From these results, it is obvious that control cheese followed by cheese treated with *Lb. helveticus* recorded the highest value of hardness throughout the ripening period as they had the lowest level of proteolysis. While, cheese treated with freeze shocked *Lb. helveticus* followed by cheese treated with heat shocked *Lb. helveticus* recorded the lowest value of hardness during all stages of ripening as they had the highest level of proteolysis which contributed to weaken the structure leading to cheese softening through the breakdown of the casein matrix and, especially the hydrolysis of α_{s1}-casein (**Creamer and Olson, 1982).** Similar results in semi-hard cheese (Tybo-Argentino cheese) were reported by **Norac *et al.,* (1992)**, in Cheddar cheese by **Kanawjia and Singh (1996)** and in Edam like-cheese by **El-Batawy *et al.,* (2004).** These results confirmed with the statical analysis of data for hardness clears highly significant differences ($P \leq 0.01$) between treatments of Gouda cheese at 60 and 90 days of ripening.

Springiness is described to the panellists as the rate at which a deformed material goes back to its undeformed condition after the deforming force is removed. The springiness values of Gouda cheese treatments when fresh were 10.33, 10.00, 9.50 and 9.67 m.m which increased to be 12.17, 11.83, 11.50 and 11.67 m.m at 60 days for treatments A, B, C and D, respectively. Then, these values decreased to be 11.35, 10.67, 9.83 and 10.17 m.m at 90 days, in the same order. The observed differences in springiness values of cheese treatments may be attributed to amount of protein matrix present and its strength **(Lawrence *et al.,* 1983 and Innocente *et al.,* 2002).** Springiness took the same trend of hardness as it was the highest in control cheese followed by cheese treated with *Lb. helveticus*, while it was the lowest in cheese treated

Table (15): **The effect of addition of freeze and heat shocked *Lb. helveticus* culture to cheese milk on hardness, springiness, cohesiveness, gumminess and chewiness of resultant Gouda cheese during ripening period.**

Parameters	Ripening period (days)	Treatments				L.S.D (5%)
		A	B	C	D	
Hardness (N)	**Fresh**	11.14	11.16	11.02	11.12	----*
	30	13.00	12.50	12.00	12.10	----
	60	13.79 A	12.95 AB	12.14 B	12.21 B	0.9498
	90	12.11 A	11.64 B	11.07 C	11.17 C	0.4238
Springiness (m.m)	**Fresh**	10.33	10.00	9.50	9.67	----
	30	11.50	11.17	10.83	11.00	----
	60	12.17 A	11.83 AB	11.50 B	11.67 B	0.4728
	90	11.35 A	10.67 B	9.83 C	10.17 BC	0.5544
Cohesiveness	**Fresh**	0.657	0.627	0.617	0.627	-----
	30	0.697	0.667	0.640	0.650	-----
	60	0.750 A	0.703 AB	0.673 B	0.683 B	0.06318
	90	0.693 A	0.650 B	0.643 B	0.647 B	0.03460
Gumminess (N)	**Fresh**	7.32	7.02	6.78	6.96	-----
	30	8.62	8.15	7.69	7.87	-----
	60	9.45 A	8.74 AB	8.17 B	8.35 AB	1.118
	90	8.02 A	7.34 B	7.12 B	7.23 B	0.5994
Chewiness (N.m.m)	**Fresh**	75.32	70.20	64.57	67.36	-----
	30	98.93 A	91.01 AB	83.18 B	86.61 B	10.81
	60	114.99 A	103.59 AB	93.96 B	97.33 B	14.91
	90	88.17 A	78.24 AB	70.07 B	73.46 B	12.48

-----* : Not significant
A : Control cheese with commercial starter.
B : Cheese treated with *Lb. helveticus*.
C : Cheese treated with freeze-shocked *Lb. helveticus*.
D : Cheese treated with heat-shocked *Lb. helveticus*.
N : Newton
m.m Millimeter
N.m. Newton millimeter

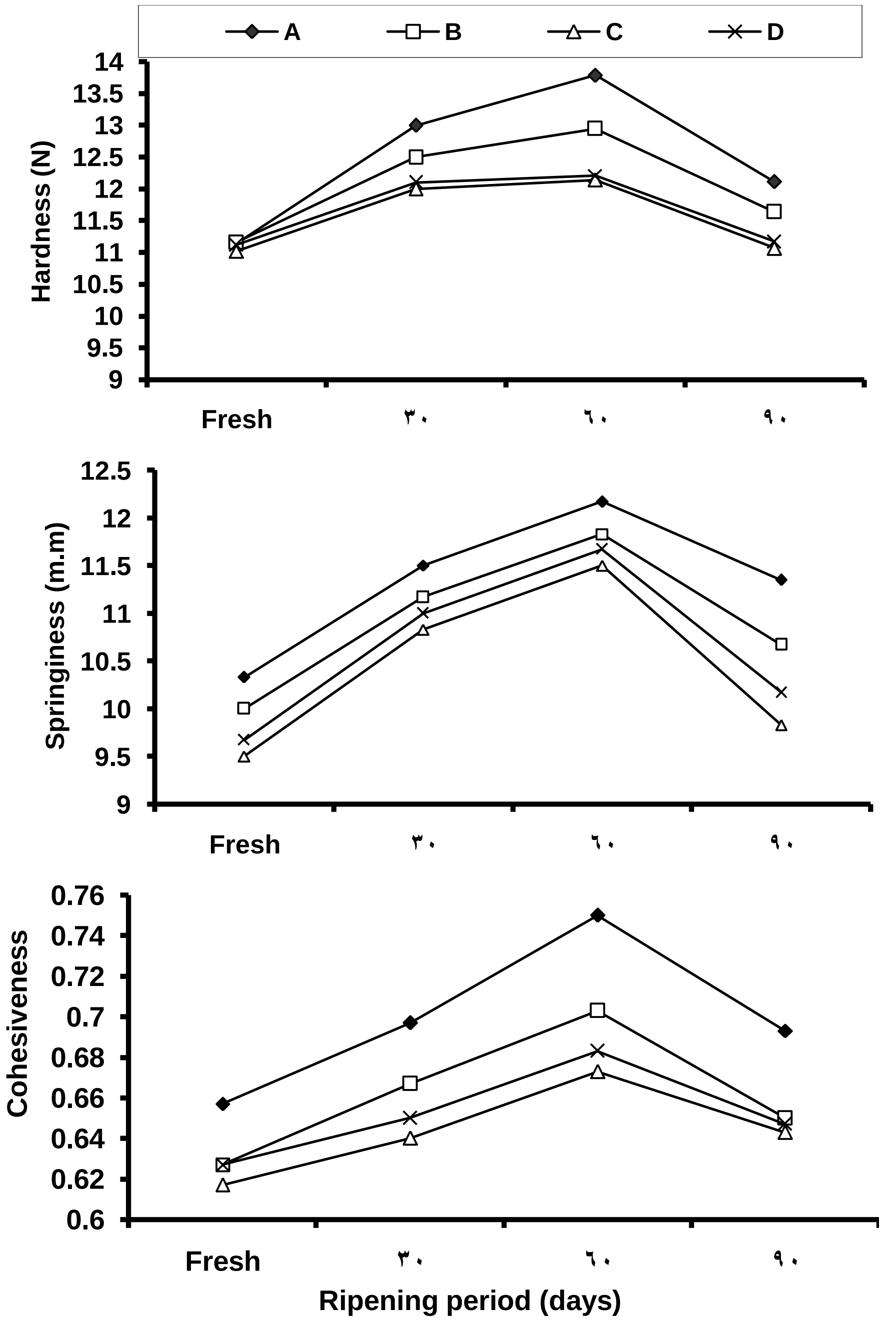


Fig. (22): **The effect of addition of freeze and heat shocked *Lb. helveticus* culture to cheese milk on hardness, springiness and cohesiveness of resultant Gouda cheese during ripening period.**

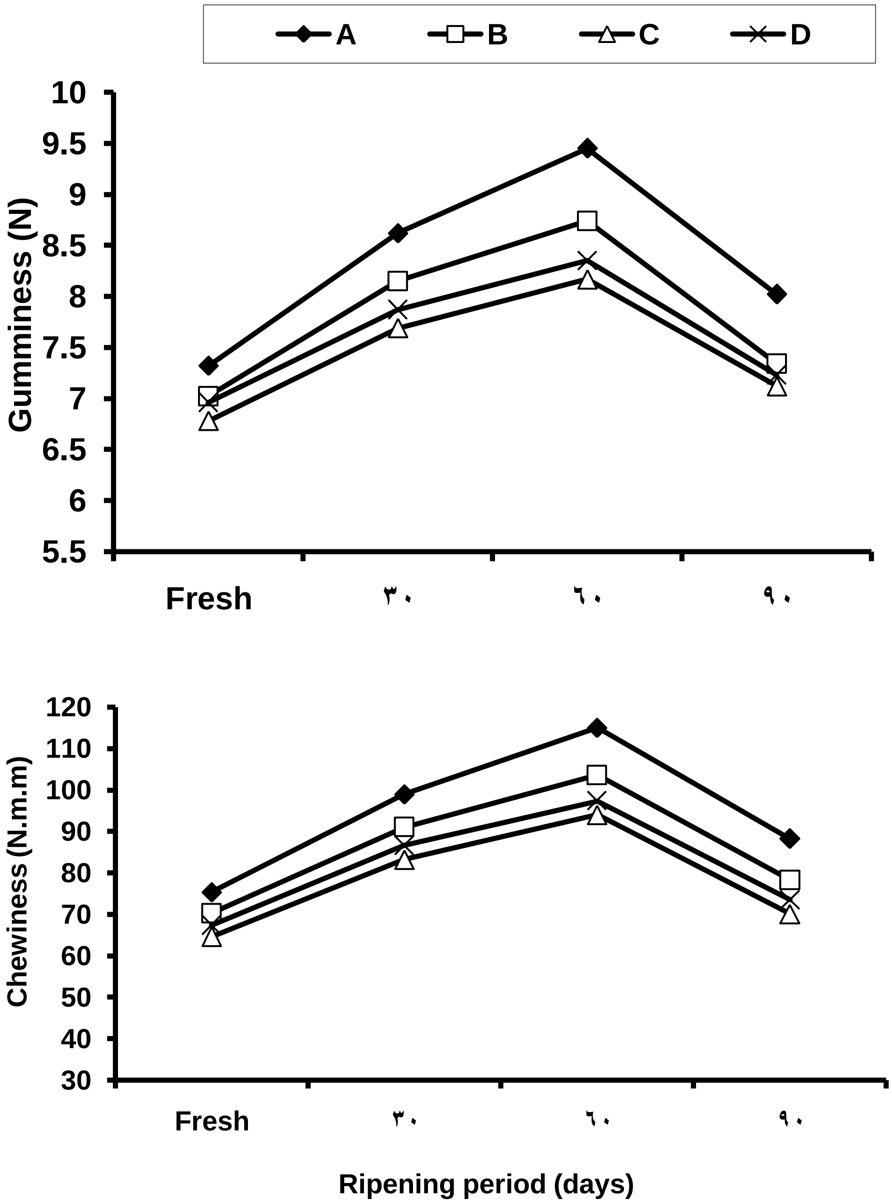


Fig. (23): **The effect of addition of freeze and heat shocked *Lb. helveticus* culture to cheese milk on gumminess and chewiness of resultant Gouda cheese during ripening period.**

with freeze shocked *Lb. helveticus* followed by cheese treated with heat shocked *Lb. helveticus*. The results are in the vicinity of those reported by **Fredrick and Dulley (1984)** who reported that the decrease in springiness of Cheddar cheese related with increase in proteolysis during ripening. The analysis of variance for springiness indicates significant differences (P≤0.05) between Gouda cheese treatments at 60 days, while the differences were highly significant (P≤0.01) at 90 days of ripening.

Cohesiveness known as the extent of deformation before reputure, therefore, cohesiveness values are a direct function of the work needed to overcome the internal bonds of the material. The cohesiveness values of Gouda cheese treatments when fresh were 0.657, 0.627, 0.617 and 0.627 which increased during the ripening to be 0.750, 0.703, 0.673 and 0.683 at 60 days for treatments A, B, C and D, respectively. Then, these values decreased to be 0.693, 0.650, 0.643 and 0.647 at 90 days, in the same order. From these results, it could be observed that control cheese followed by cheese treated with *Lb. helveticus* recorded the highest cohesiveness, while cheese treated with freeze shocked *Lb. helveticus* followed by cheese treated with heat shocked *Lb. helveticus* recorded the lowest cohesiveness. This could be attributed to the highest proteolysis of the cheese treated with freeze and heat shocked *Lb. helveticus*, which could have been responsible for distruption of casein-casein interaction, loosening the protein matrix **(Molina *et al.,* 2000)**.

The statical analysis of variance for Cohesiveness values shows significant differences (P≤0.05) between Gouda cheese treatments at 60 and 90 days of ripening.

b) Gumminess and Chewiness

Gumminess and chewiness of Gouda cheese made from different treatments are presented in Table (15) and illustrated in Fig. (23). Gumminess and chewiness are secondary parameters derived from hardness, cohesiveness and springiness.

Gumminess is expressed as the energy required to disintegrate a semi solid food product to a state ready for swallowing. On the other hand, **chewiness** is expressed as the energy required to chew a solid food product to a state where it is ready for swallowing. The progressive significant increase in gumminess (a product of hardness and cohesiveness) and chewiness (a product of gumminess and springiness) with the increase in ripening period until 60 days could be attributed to the progressive increase in the hardness, cohesiveness and springiness. There was a decrease at 90 days of ripening in both of gumminess and chewiness. This may be due to inversely related between textural profiles like hardness, springiness and chewiness to ripening period **(Fredrick and Dulley, 1984 and Kanawjia and Singh, 1996)**. The highest gumminess and chewiness values were recorded for control cheese followed by cheese treated with *Lb. helveticus*, while the lowest values were recorded for cheese treated with freeze shocked *Lb. helveticus* followed by cheese treated with heat shocked *Lb. helveticus*. It could be concluded that decreasing the moisture in cheese results in a firmer texture due to alterations in the casein matrix. Therefore, increase the hardness and springiness, along with gumminess and chewiness.

The analysis of variance shows significant differences ($P \leq 0.05$) for Gumminess values between treatments of Gouda cheese at 60 days, while the differences were highly significant ($P \leq 0.01$) at 90 days of ripening. On the other side, the chewiness of Gouda cheese treatments clears significant differences during ripening period (30, 60 and 90 days).

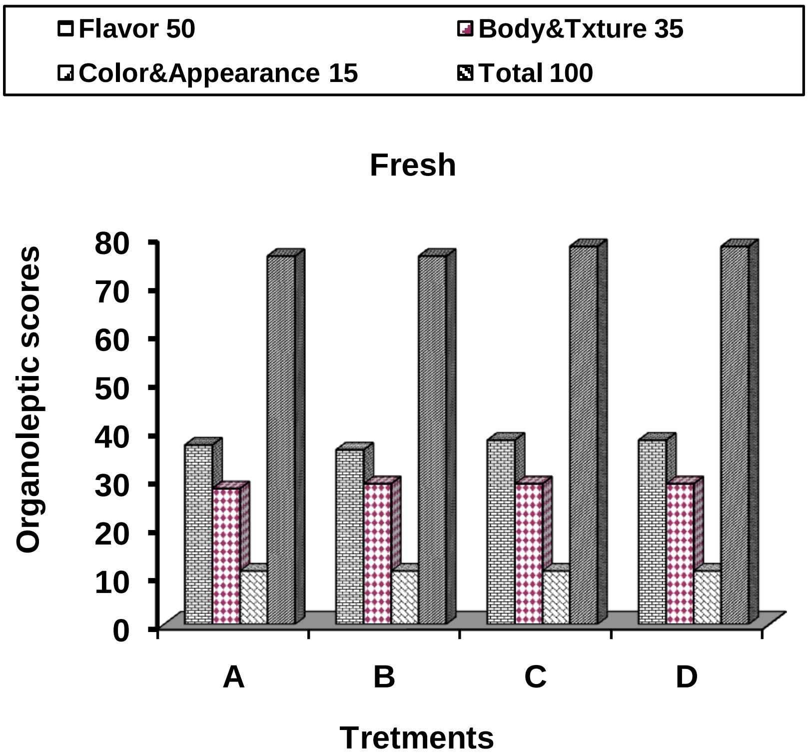
5. Organoleptic properties of cheese:

The organoleptic properties of Gouda cheese made from different treatments and their corresponding scores are presented in Table (16) and Fig. (24). The scoring of cheese was carried out at four different stages namely fresh, 30, 60 and 90 days. The obtained results reveal that the fresh cheese of all treatments had nearly the same score points for flavor and body&texture characteristics. After 30 days of cheese ripening and throughout the ripening period, cheese treated with freeze shocked *Lb. helveticus* followed by cheese treated with heat shocked *Lb. helveticus* gained the highest score points for flavor development and body&texture characteristics. The score points given for cheese treated with freeze shocked *Lb. helveticus* and cheese treated with heat shocked *Lb. helveticus* were 87 and 85 points out of 100 after 30 days of cheese ripening, respectively. These values increased as ripening advanced reaching the maximum score points at the end of ripening period, being 96 and 94 points out of 100 in the same order. It could be attributed to the higher levels of soluble nitrogenous compounds and total volatile fatty acids produced by the enzymes of freeze and heat shocked

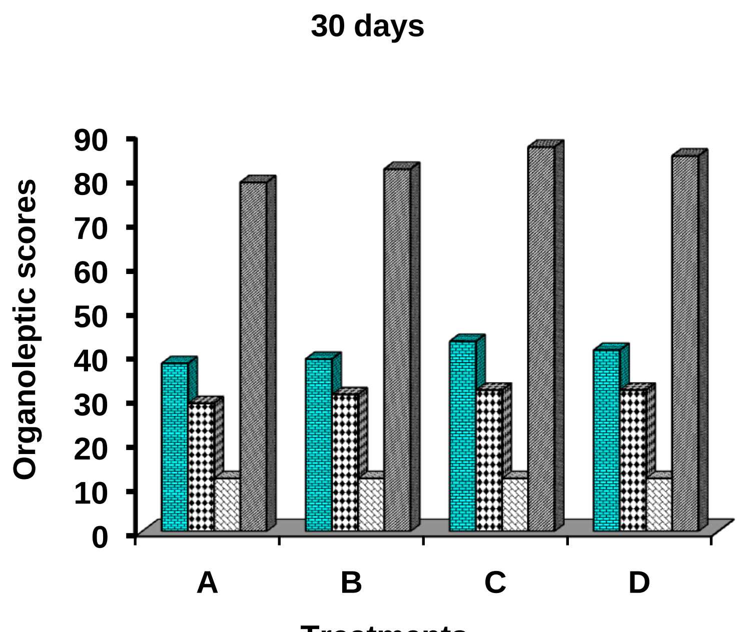
Table:(16) The effect of addition of freeze and heat shocked *Lb. helveticus* culture to cheese milk on the organoleptic properties of resultant Gouda cheese during ripening period.

Parameters	Ripening period (days)	Treatments				L.S.D (5%)
		A	B	C	D	
Flavor (50)	Fresh	37	36	38	38	----*
	30	38 ^{C}	39 ^{BC}	43 ^{A}	41 ^{AB}	2.079
	60	40 ^{B}	42 ^{B}	47 ^{A}	46 ^{A}	2.234
	90	40 ^{C}	43 ^{B}	48 ^{A}	46 ^{A}	2.234
Body& Texture (35)	Fresh	28	29	29	29	----
	30	29 ^{B}	31 ^{AB}	32 ^{A}	32 ^{A}	2.079
	60	30 ^{B}	32 ^{AB}	33 ^{A}	33 ^{A}	2.234
	90	31 ^{B}	32 ^{AB}	34 ^{A}	34 ^{A}	2.234
Color& Appearance (15)	Fresh	11	11	11	11	----
	30	12	12	12	12	----
	60	13	13	14	13	----
	90	13	13	14	14	----
Total (100)	Fresh	76	76	78	78	----
	30	79 ^{C}	82 ^{B}	87 ^{A}	85 ^{A}	2.234
	60	83 ^{C}	87 ^{B}	94 ^{A}	92 ^{A}	2.234
	90	84 ^{C}	88 ^{B}	96 ^{A}	94 ^{A}	2.234

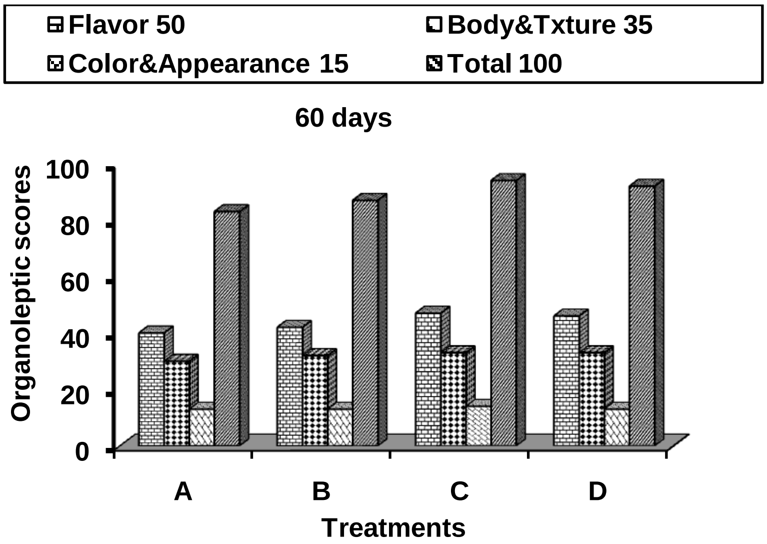
----* : Not significant
A : Control cheese with commercial starter.
B : Cheese treated with *Lb. helveticus*.
C : Cheese treated with freeze-shocked *Lb. helveticus*.
D : Cheese treated with heat-shocked *Lb. helveticus*.



Flavor 50
Body&Txture 35
Color&Appearance 15
Total 100
Fresh
Organoleptic scores
0
10
20
30
40
50
60
70
80
A
B
C
D
Tretments



30 days
Organoleptic scores
0
10
20
30
40
50
60
70
80
90
A
B
C
D


Flavor 50
Body&Txture 35
Color&Appearance 15
Total 100
60 days
Organoleptic scores
100
80
60
40
20
0
A
B
C
D
Treatments

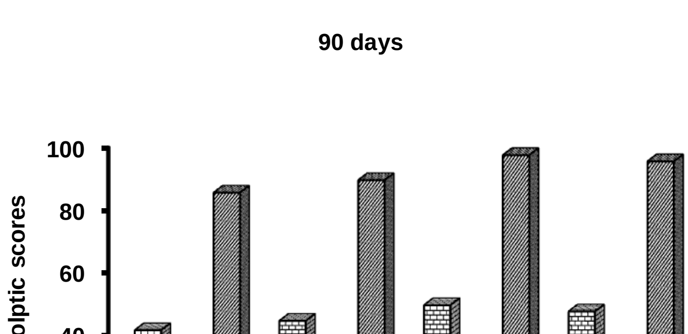

90 days
100
80
60

Fig. (24): The effect of addition of freeze and heat shocked *Lb. helveticus* culture to cheese milk on the organoleptic properties of resultant Gouda cheese during ripening period.

Lb. helveticus. The use of freeze and heat shocked *Lb. helveticus* did not develop bitterness during the entire period of ripening. This could be explained on the basis that the freeze and heat shocked *Lb. helveticus* allowed a complete mixing of amino peptidases with intact bacterial cells. Similar results were obtained by **Nasr *et al.,* (1991); Vafopoulou *et al.,* (1989)**; **Abdeen (2000) and El-Baz (2001)** who studied many different types of cheese and reported that the best organoleptic characteristics were obtained for the cheese treated with freeze and heat shocked culture. **Abd-El-Baky *et al.,*** **(1986)** concluded that cheese containing heat-shocked lactobacilli showed desirable flavor and consistency 1-2 months earlier than control cheese made without additives. **Kebary *et al.,* (1996)** found that addition of either freeze shocked *Lb. casei* or *Lb. helveticus* yielded cheeses having higher ripening indices and organoleptic scores than cheese made with heat shocked lactobacilli. On the other hand, it could be seen from these results that cheese treated with *Lb. helveticus* (treatment B) gained higher score points for flavor and body&texture characteristics than control cheese. This may be due to the whole cells of *Lb. helveticus* had the ability to enhance the proteolysis and lipolysis of Gouda cheese. These results are confirmed by **Bartels *et al.,* (1987b)** who found that addition of whole cells of *Lb. helveticus* to Gouda cheese milk enhanced flavor intensity and reduced bitterness, but off-flavor developed cheese during ripening. **Exterkate *et al.,* (1987)** concluded that cheese made with up to 5% thermo shocked cells had a better taste and moderately increased flavor intensity and the ripening time was reduced by about 25%. **Ardo *et al.,* (1989)** studied that the effect of peptidolysis rate during early cheese ripening in a low-fat (10%) Swedish semi-hard type of cheese and mentioned that in the cheese with heat-.treated lactobacilli compared with the control cheese there was marked increase in cheeses flavor intensity simultaneously with a desired elimination of bitterness

The statical analysis of variance shows highly significant differences ($P \leq 0.01$) between all treatments of Gouda cheese for flavor and total score at 30, 60 and 90 days of ripening. While, there wee significant differences ($P \leq 0.05$) for body&texture during ripening period (30, 60 and 90 days). On the other hand, the color& appearance of Gouda cheese treatments show no significant differences when fresh or during ripening period.

6. Fatty acids and electrophoretic patterns of resultant Gouda cheese:

According to the prior analyses (chemical, rheological and organoleptic properties), the best treatment of cheese (treatment C) with control cheese (treatment A) were analyzed after 90 days of ripening by gas liquid chromatography (GLC) and electrophoresis for determination of fatty acids content and protein breakdown, successively.

6.1. Patterns of free fatty acids (FFA):

Fat hydrolysis is one of the major biochemical reactions occurs during ripening of several types of cheese **(Mabbitt, 1961 and Patton,**

1963). Free fatty acids play a major role in flavor of many cheese varieties **(Anderson and Day, 1965)**. The fatty acids content of resultant Gouda cheese from treatments A and C after 90 days of ripening are given in Table (17). By studying these data it could be

Table: (19) Pattern of free fatty acids (% of total) of Gouda cheese after 90 days of ripening.

	Fatty acids	FFA Group	A	C
Volatile FFA	Caproic	C_6	-----	0.15
	Caprylic	C_8	1.26	2.50
	Capric	C_{10}	4.15	7.42
		Total	5.41	10.07
Non Volatile FFA	Lauric	C_{12}	3.10	2.33
	Tridecylic	C_{13}	0.60	3.01
	Myristic	C_{14}	12.53	10.70
	Myristoleic	$C_{14:1}$	1.19	1.10
	Palmitic	C_{16}	32.96	28.02
	Palmitoleic	$C_{16:1}$	1.91	1.26
	Margaric	C_{17}	0.323	3.57
	Stearic	C_{18}	12.46	10.46
	Oleic	$C_{18:1}$	28.41	23.86
	Linoleic	$C_{18:2}$	1.11	1.13
	linolenic	$C_{18:3}$	----	4.49
		Total	94.59	89.93

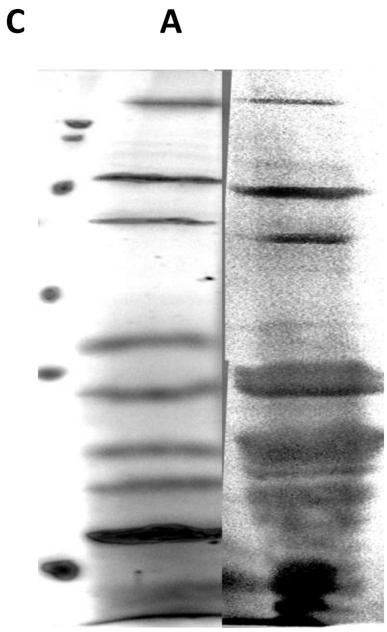
A : Control cheese with commercial starter.
C : Cheese treated with freeze-shocked *Lb. helveticus*.

seen that the percentage of volatile free fatty acids in cheese treated with freeze shocked *Lb. helveticus* (10.07%) was higher than control cheese (5.41%). On the other hand, non volatile free fatty acids showed higher level in control cheese (94.59%) especially **C_{12},C_{14},C_{16},C_{18} and $C_{18:1}$** compared with cheese treated with freeze shocked *Lb. helveticus* (89.93%).While, the percentage of **C_{13},C_{17}, and $C_{18:3}$** in cheese treated with freeze shocked *Lb. helveticus* was higher than that of control cheese. **Kamaly *et al.,* (1989)** studied the characteristics of Cheddar cheese made with mutant strains of lactic streptococci as adjunct sources of enzymes. They found that the greater amount of free fatty acids accumulated in the mutant-containing cheeses at 2 and 6 months of age than in the control cheese and the free fatty acid generally accumulated in the following order: $C_{16:0} > C_{18:1} > C_{18:0} > C_{14:0} > C_{12:0} > C_{10:0}$. These results could be explained on the basis that the addition of freeze shocked *Lb. helveticus* enhanced the formation of volatile free fatty acids ($C_4 - C_{10}$). These results are similar to those reported by **El-Soda *et al.,* (1999)** who found that cheese slurries made with freeze-shocked cells revealed higher enzyme activity as well as more fat breakdown. Also, similar results were found by **Madkor *et al.,* (1999)** who studied seven strains of freeze shocked adjunct lactobacilli for their proteolytic and lipolytic activity of a cheese slurry system. They found that the relative concentration of total free fatty acids (FFA) assessed in cheese slurries revealed high extent of lipolysis in most adjunct treated-slurries. **Madkor *et al.,* (2000b)** found that Cheddar cheese made with freeze shocked or heat shocked *Lb. helveticus* adjunct exhibited significantly greatest rates of free amino group formation and freeze shocked *Lb. helveticus* obtained the highest flavor and aroma scores. **El-Baz (2001)** reported that the addition of freeze shocked *Lb. helveticus* at a rate of 1, 1.5 and 2% to Edam-like cheese increased the percentage of free fatty acids (C_4-C_{10}) and the concentration of free fatty acid was proportional to the level of the added freeze shocked culture of *Lb. helveticus*. **Mostafa *et al.,* (2002)** found that the UF Edam cheese treated with freeze shocked starter showed higher amount of short chain fatty acids compared to untreated UF cheese which play an important role in the formation of cheese test. These previous results confirm the results of the present study.

6.2. Electrophoretic patterns of Gouda cheese

During cheese ripening, the major biochemical changes are fermentation of lactose, degradation of proteins, hydrolysis of fat and production of volatile compounds. Decomposition of proteins is the most important phenomenon as they are partially converted from insoluble to soluble forms and are broken down to proteoses, peptones, polypeptides, amino acids and finally to ammonia **Ling (1963)** and **Schormuller (1968).**

The electrophoretic patterns of Gouda cheese (treatments A and C) after 90 days of ripening are shown in Fig. (25). It is clear that there were more bands of protein in the range of 145 to 94 kDa in cheese treated with freeze shocked *Lb.helveticus* (treatment C) compared with control cheese (treatment A).This may be attributed to the freeze shocked *Lb. helveticus* release intracellular enzymes and a good source of aminopeptidase, dipeptidase and proteinase (**Frey *et al.,* 1986).** The same trend was observed by several investigators namely, **El-Shabrawy (1985); Hassan and El-Deeb (1988). Kim *et al.,* (1994b)** studied the effect of adding commercial fungal proteases and freeze-shocked *Lactobacillus helveticus* CDR 101 on proteolysis of Gouda cheese made from ultrafiltrated milk (UFmilk). They reported that, after 12 weeks β-casein was completely hydrolyzed in fungal protease-treated cheese, whereas approximately 60 and 80% of β-casein were still intact in conventional and UF cheese respectively.**Zedan (1997)** reported that the electrophoretic patterns of low fat Ras cheese at the end of 3 months of ripening showed more bands in cheese made from milk with freeze shocked *Lb. helveticus* as compared with other treatments and that which may be attributed to the proteolytic effect of freeze shocked *Lb. helveticus*. **Madkor *et al.,* (1999)** found that the ripened cheese slurries with freeze shocked adjunct lactobacilli exhibited more degradation of protein on poly



Band No.	M.W	Treatments	
		C	A
1	160.0	1	1
2	145.0	1	0
3	119.0	1	0
4	104.0	1	0
5	94.0	1	0
6	86.0	1	1
7	71.0	1	1
8	59.0	1	1
9	48.0	1	1
10	42.0	1	1
11	37.0	1	1
12	31.0	1	1

13	30.0	1	1
14	28.9	1	1
15	28.8	1	1
16	28.1	1	1
17	25.0	1	1
18	20.0	1	1
Total		18	14

0: Not detected 1: Detected

A : Control cheese with commercial starter.
C : Cheese treated with freeze-shocked *Lb. helveticus*.

Fig. (25): The electrophoretic patterns of Gouda cheese (treatments A and C) after 90 days of ripening.

acrylamide gel electrophoresis (PAGE) indicating greater peptidase activity in the cured compared to control slurry. **Awad *et al.,* (2001a)** found that *Lb. helveticus* LHS was more active against β-casein