RIPENING ACCELERATION AND QUALITY IMPROVEMENT OF GOUDA CHEESE USING FREEZE OR HEAT- SHOCKED *Lactobacillus delbrueckii* subsp*. helveticus* DSMZ 20082

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SUMMARY

The effect of adding freeze or heat-shocked *Lactobacillus delbrueckii subsp. helveticus* DSMZ 20082 + commercial starter (*Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis, Leuconostoc mesenteroides* subsp. *cremoris* and *Lactococcus lactis* subsp. *diacetilactis*) to cheese milk (cow's and buffalo's milk 1:1 standardized to 3.5% fat) to accelerate Gouda cheese ripening and improve the quality compared with controls (cheese treated with commercial starter alone and cheese treated with commercial starter + viable cells of *Lb. helveticus*). Resultant cheese was then kept in the ripening room at 10-12ºC and 85-95% relative humidity for 3 months. Cheese samples were analyzed chemically, microbiologically, rheologically and organoleptically when fresh and after 30, 60 and 90 days of ripening. The obtained results indicated that the ripening indices (SN, SN/TN, soluble tyrosine & tryptophan and T.V.F.A contents); the proteolytic & lipolytic bacterial counts and sensory evaluation scores increased with the increase of ripening period. The cheese treated with freeze-shocked *Lb. helveticus* had the highest values of these parameters followed by cheese treated with heat-shocked *Lb. helveticus* compared with other treatments in all stages of ripening. Cheese treated with freeze or heat-shocked *Lb. helveticus* had more desirable texture along ripening and there was a reduction of 33% in the ripening period compared to other treatments.

The patterns of free fatty acids and electrophoretic patterns in control cheese treated with commercial starter alone and cheese treated with freeze-shocked *Lb. helveticus* at 90 days showed higher levels of volatile fatty acids and higher number of bands of protein respectively in cheese treated with freeze-shocked *Lb. helveticus* than that of control cheese.

Key words: - Gouda cheese - Commercial starter - Freeze –shock
- Heat-shock - *Lactobacillus delbrueckii* subsp. *helveticus*

INTRODUCTION

Gouda cheese is a semi-hard cheese variety originated in Netherlands. Gouda cheese resembles Edam cheese, but has a firmer curd, larger size and flat shape. Gouda cheese has been widely spread in many countries**.** Six varieties of Gouda cheese are manufactured with ripening times ranging from 6 weeks to 24 months (Vanrusselt, 1992). Recently, the demand for this cheese type has been increased in Egypt and some local dairy factories manufactured it in commercial scale. The economic advantage of rapid development of more intense cheese flavour in shorter periods of time would be substantial. Acceleration of cheese ripening can also be a mean for increasing the production of cheese in developing countries where investment in storage facilities. Development of the flavours characteristics can occur partially by bacteria and enzymes through different metabolic pathways, as well as, changes in the texture and body of the cheese matrix (Kamaly *et al*., 1989). Adding lactic acid bacteria to cheese is an effective way to accelerate cheese ripening. The higher numbers of desired lactic acid bacteria in cheese may cause over production of acid in the final cheese. This problem was solved by reducing this acid production capacity of the cells with only a limited reduction of their cellular proteinase/peptidase activity. This was accomplished by using physical methods such as heat shocking and freeze shocking (El-Soda, 1993). Heat and freeze-shocked lactic acid bacteria were used to accelerate ripening by increasing proteolysis and cheese flavour without introducing bitter taste in the resultant cheese. The addition of freeze and heat-shocked *Lb. helveticus* cells to Gouda and Edam-like cheese increased soluble nitrogen, free amino acids, acetaldehyde and total volatile fatty acids content during ripening (Kim *et al*., 1994; Skeie *et al*., 1995; El-Baz, 2001 and Tungjaroenchai *et al*., 2001)

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.

Therefore, this study was carried out to accelerate ripening of Gouda cheese using freeze and heat-shocked *Lactobacillus delbrueckii* subsp. *helveticus* and improve the quality of resultant cheese compared with controls (cheese treated with commercial starter alone and cheese treated with commercial starter + viable cells of *Lb. helveticus*).

MATERIALS AND METHODS

Materials

- Fresh whole buffalo's and cow's milk were obtained from the herds of Faculty of Agriculture, Moshtohor, Benha University. Skim milk powder (SMP) was obtained from ADPI extra grade-Swedish origin Scanasir, Gothenburg, Sweden.

- Commercial starter of mesophilic aromatic culture, type LD**.**Multiple mixed strain culture containing *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis, Leuconostoc mesenteroides* subsp. *cremoris* and *Lactococcus lactis* subsp. *diacetylactis.* was obtained from Chr. Hansen's Lab., Denmark.

- Pure strain of *Lactobacillus delbrueckii* subsp. *helveticus* DSMZ 20082 (*Lb. helveticus*) was obtained from the Egyptian Microbial Culture Collection (EMCC) at Cairo Microbial Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt.

- Rennet powder (Hannilase L2235), Annatto (550) and White plastic 5% Natamycin were obtained from Chr. Hansen's Lab., Denmark.

Methods

Preparation of heat-shocked culture:

Lactobacillus delbrueckii subsp**.** *helveticus* was heat shocked as described by Bartels *et al.,* (1987a)**.** The culture was subcultured for 12 hr. at 37ºC in 11.5% reconstituted skim milk powder (RSM) at least twice before use.

Preparation of freeze-shocked culture**:**

Lactobacillus delbrueckii subsp. *helveticus* was freeze-shocked as described by Bartels *et al*., (1987b) with some modifications by Spangler *et al.,* (1989). The culture was subcultured for 12 hr at 37ºC in 11.5% reconstituted skim milk powder at least twice before use.

Manufacture of Gouda cheese:

Gouda cheese was manufactured as described By Scott (1998), using standardized fresh mixed cow's and buffalo's milk 1:1 (Mixed milk composition was 86.69% moisture, 3.5% fat, 3.41% protein, 0.93% ash, 5.47% lactose, 0.17% TA and 6.78 pH value). Resultant cheese treatments were then carefully coated with plastic coat and kept in the ripening room at 10-12ºC and 85-95% relative humidity for 3 months and analyzed when fresh and after 1, 2 and 3 months of ripening period. The experimental replicated on three occasions and the analysis were duplicated.

Experimental design:

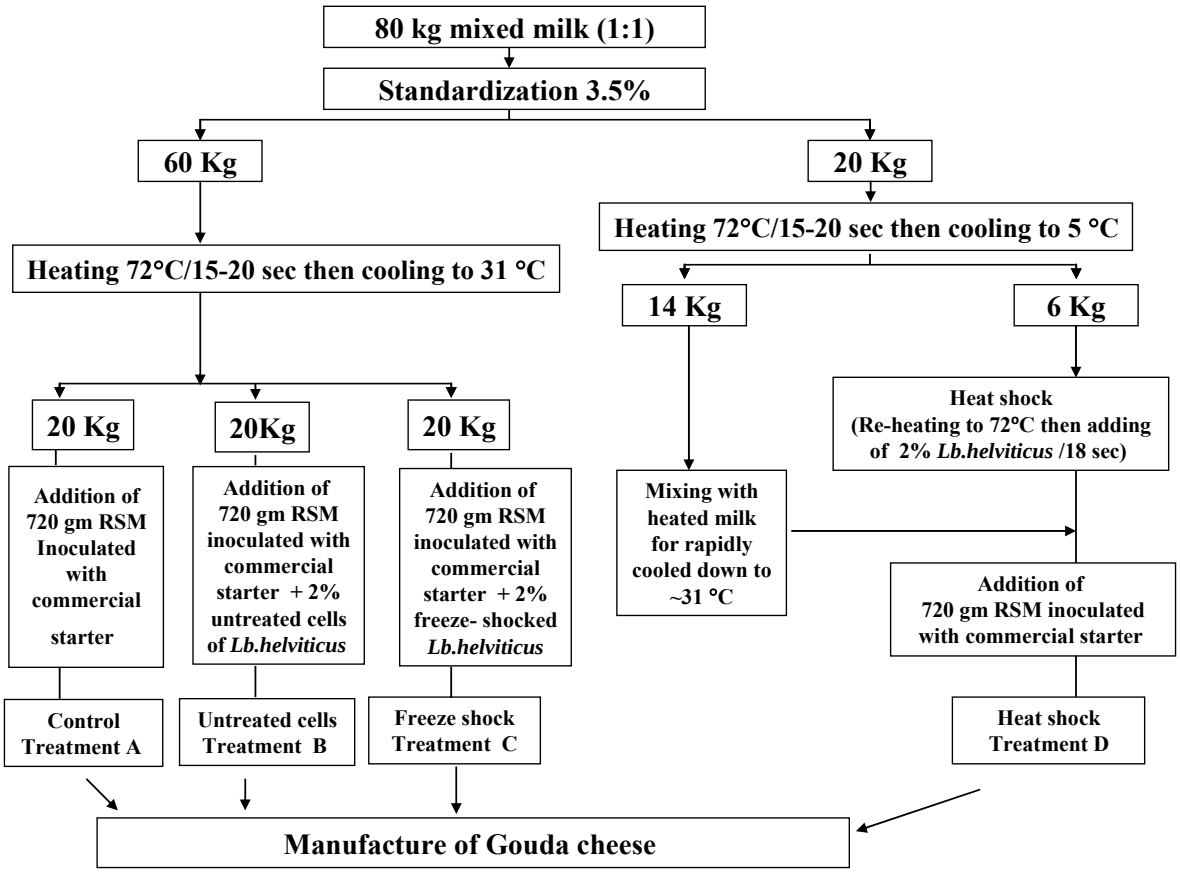


Fig. (1): Flow diagrams of Gouda cheese manufacture by different treatments

Chemical analysis:

The moisture and salt contents of cheese were determined according to British Standards Institution (BSI, 1989). Titratable acidity (TA), fat, lactose and soluble nitrogen (SN) content of milk and cheese were determined by the methods described by Ling (1963). The ash content of milk was determined as described in AOAC (2000). Total nitrogen (TN) was determined by the method of International Dairy Federation (IDF, 1991). Soluble tyrosine & Tryptophan contents (s-tyrosine & s-tryptophane) were determined by the method of Vakaleris and Price (1959); values were expressed as mg/100g cheese. The total volatile fatty aids (T.V.F.A.) of cheese were determined by the distillation method described by Kosikowski (1978); values were expressed as ml (0.1N) NaOH/100g cheese.

The pH values of milk and cheese treatments were measured according to the methodology of BSI (1989) using a glass electrode digital pH meter, type (Orion Research model SA720) USA.

Electrophoretic patterns of Gouda cheese:

SDS-polyacrylamide gel electrophoresis was performed in 12 % acrylamide slab gels following the system of Laemmli (1970) to identify their protein profiles.

Identification and determination of fatty acids:

The method described by Farag et *al*., (1986) was applied for determination of fatty acids by gas liquid chromatograph **(**GLC). The method described by AOAC (2000) and Vogel (1975) were applied for lipid extraction and separation of fatty acids respectively.

Microbiological analysis:

Total bacterial counts (TBC) was determined using plate count agar according to Houghtby *et al.,* (1992). Yeasts & moulds and coliforms were counted according to Marshall (1992). Proteolytic bacterial counts was determined as described by Chalmer (1962). Lipolytic bacterial counts was determined as given by Sharf (1970). Count of *Lb. helveticus* was determined using MRS-agar pH 5.4 according to IDF (1988).

Textural properties:

Textural properties such as hardness, springiness, cohesiveness, gumminess and chewiness of cheese were measured with an Instron Universal Testing Machine (Model 4302, Instron Corporation, Canton M.A, England) according to the procedure of Bourne (1978).The Instron load cell of 100 Newton and strip chart recorder were employed for various tests under the following operating conditions: Load range 40%, Cross-head speed 25mm/min., Chart recorder speed 50mm/min, Cross-head speed: Chart recorder speed 1:2, Depth 10 mm and Test temperature 15ºC± 1ºC.

Organoleptic evaluation:

Organoleptic tests of Gouda cheese treatments were carried out according to scoring sheet proposed by El-Kenawy (1977). The general characteristics of cheese samples were classified into three distinctive properties. The first for flavour was allowed 50 points, the second for body & texture was allowed 35 points and the third for colours and appearance was allowed 15 points. The scoring was carried out simultaneously with chemical analysis of the samples at different ages of cheese ripening (0, 30, 60 and 90 days) by staff members of Food Science Department, Faculty of Agriculture, Moshtohor, Benha University and Dairy Research Department, Food Technology Research Institute, Agricultural research Center, Ministry of Agriculture.

Statistical analysis:

Statical analysis for obtained data was done according to the methods described by Cox and Snell (1981).

Results and Discussion

Chemical composition of Gouda cheese treatments:

The chemical composition of Gouda cheese treated with commercial starter either alone (A) or in association with viable whole cells of *Lb. helveticus* (treatment B), freeze or heat-shocked *Lb. helveticus* (treatment C and D respectively) is presented in Table (1).

The results clear that neither the addition of freeze-shocked *Lb. helveticus* nor heat-shocked *Lb. helveticus* noticeably affected the moisture, Fat/DM and salt in moisture contents of Gouda cheese. The cheese with viable whole cells of *Lb. helveticus* (treatment B) had the lowest moisture and slight increase in Fat/DM and salt in moisture content compared to other treatments during the increase in ripening period. This can be explained on the basis that viable whole cells of *Lb. helveticus* with starter culture increased lactic acid developments which result in increasing of curd contraction and expulsion of extra aqueous phase of cheese. These results are accordance with Bartels *et al.,* (1987, a and b); Spangler *et al.,* (1989) and El-Baz (2001). Also, the moisture content of all cheese treatments decreased during ripening due to the biochemical changes and lactic acid development which result in curd contraction and expulsion of aqueous phase of cheese. These results are in agreement with those obtained by El-Sonbaty *et al*., (2002).

The Fat/DM and salt in moisture content of different treatments markedly increased during the first month of ripening, and then slightly increased up to the end of ripening period. These increases could be attributed to evaporation of water during ripening. Similar results were obtained by El-Tawel (2004) and Ismail *et al*., (2004).

There is significant differences ($P \leq 0.05$) at 90 days of ripening between Gouda cheese treatments, while no significant differences between the treatments of Gouda cheese in Fat/DM content when fresh or during ripening period.

Also, the cheese treated with whole viable cells of *Lb. helveticus* (treatment B) had the highest titratable acidity throughout all stages of ripening ($P \leq 0.01$) compared with the other treatments. The acid production of Gouda cheese by freeze or heat-shocked *Lb. helveticus* was sufficiently retarded. These results are in line with those obtained by Abou Zeid and Mahmoud (1992). On the other hand, the TA of all cheese treatments gradually increased with the advancing of ripening. This increase in the TA could be explained by the development 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).

Table (1): Effect of adding freeze- and heat-shocked *Lb. helveticus* culture to cheese milk on the chemical composition and pH value of resultant Gouda cheese.

Contents	Ripening period (days)	Treatments				LSD (5%)
		A	B	C	D	
Moisture %	Fresh	48.14	47.97	48.10	48.12	----*
	30	42.03^{A}	41.16^{B}	41.79^{A}	41.78^{A}	0.3996
	60	40.02^{A}	38.62^{B}	39.92^{A}	39.85^{A}	0.8616
	90	38.06^{A}	37.27^{B}	37.96^{A}	38.06^{A}	0.5994
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	----
Salt/moisture %	Fresh	3.26	3.54	3.40	3.40	----
	30	6.27	6.80	6.22	6.22	----
	60	8.17	8.90	8.27	8.27	----
	90	9.20^{B}	9.79^{A}	9.31^{B}	9.31^{B}	0.3460
TA %	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 value	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
TN/DM%	Fresh	6.32	6.44	6.44	6.31	-----
	30	6.40	6.63	6.63	6.52	-----
	60	6.52^{B}	6.76^{A}	6.76^{A}	6.54^{B}	0.08935
	90	6.70^{B}	6.86^{A}	6.86^{A}	6.72^{B}	0.1094

----* : Not significant

A : Control cheese with commercial starter.

B : Cheese treated with commercial starter + *Lb. helveticus*.

C : Cheese treated with commercial starter + freeze-shocked *Lb. helveticus*.

D : Cheese treated with commercial starter + heat-shocked *Lb. helveticus*.

The opposite trend of acidity results was observed with respect to pH values ($P \leq 0.01$). A continuous decrease in pH values of all treatments during storage was noticed.

The addition of freeze or heat-shocked *Lb. helveticus* did not remarkably affect the T.N/DM content of Gouda cheese (Table 1). These results are confirmed by El-Baz (2001) and Mostafa *et al*., (2002). Cheese treated with *Lb. helveticus* (treatment B) had the highest T.N/DM content ($P \leq 0.05$) at 60 and 90 days of ripening period. This may be attributed to the high loss of moisture content during ripening period. The 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 El-Baz (2001).

Ripening indices of Gouda cheese treatments:

The changes in soluble nitrogen, soluble nitrogen on total nitrogen (S.N/T.N), soluble tyrosine, soluble tryptophan (s-tyrosine & s-tryptophan) and total volatile fatty acids (T.V.F.A) contents of Gouda cheese made from different treatments during ripening period for 90 days are presented in Table (2). It could be observed that the cheese treated with freeze-shocked *Lb. helveticus* (treatment C) had the higher followed by cheese treated with heat-shocked *Lb. helveticus* (treatment D) and lastly cheese treated with whole viable cells of *Lb. helveticus* (treatment B) in S.N, SN/TN, s-tyrosine & s-tryptophan and T.V.F.A. contents with the increase in ripening period ($P \leq 0.01$) compared with control cheese (treatment A). This may be attributed to the freeze or heat-shocked cells of *Lb. helveticus* which were lysed and release their intracellular proteolytic and lipoytic enzymes in the cheese to a greater extent and also, increase the rate of autolysis than untreated viable cells of *Lb. helveticus*. These results are confirmed by El-Soda *et al.,* (2000); El-Sonbaty *et al.,* (2002) and Ismail *et al.,* (2004). Furthermore, the higher rate of protein degradation associated with more accumulation of free amino acids serve as precurses for volatile fatty acids. In addition, non protein organic materials from dead cells may serve as a source of volatile fatty acids upon degradation. These results are in harmony with those obtained by El-Baz (2001) who 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.

A gradual increase of the ripening indices in all cheese treatments were observed all over the ripening period. These increases can be attributed to the protein degradation, fat hydrolysis and the formation of soluble nitrogenous and volatile fatty acid compounds. These results are in agreement with those reported by Al-Tanboly *et al.,* (2003).

Table (2): Effect of adding freeze- and heat-shocked *Lb. helveticus* culture to cheese milk on ripening indices of resultant Gouda cheese.

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

----* : Not significant

A : Control cheese with commercial starter.

B : Cheese treated with commercial starter + *Lb. helveticus*.

C : Cheese treated with commercial starter + freeze-shocked *Lb. helveticus*.

D : Cheese treated with commercial starter + heat-shocked *Lb. helveticus*.

Microbiological examinations:

Total bacterial counts:

The changes of total bacterial count (TBC), proteolytic and lipolytic bacterial counts, viable count of *Lb. helveticus*, yeasts and molds and coliform count of Gouda cheese made from different treatments during the ripening period for 90 days are shown in Fig 2 a, b, c, d and e respectively). It could be observed that cheese treated with *Lb. helveticus* (treatment B) had higher value for TBC than that of all treatments when fresh (Fig 2 a). 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 values for TBC than that of other treatments. These results could be explained on the basis that the added freeze and heat- shocked *Lb. helveticus* revealed higher enzyme activity and more protein breakdown and fat hydrolysis which stimulate the development of bacterial growth during ripening processes (El-Soda *et al.,* 1999 and El-Baz 2001). On the other hand, the TBC of all cheese treatments gradually decreased during the 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 Ismail *et al.,* (2004).

Fig (2 b and c) show that cheese treated with freeze-shocked *Lb. helveticus* followed by cheese treated with heat-shocked *Lb. helveticus* had higher proteolytic and lipolytic 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 from 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 and lipolysis. Similar results were obtained by Abou-Zeid and Mahmoud (1992) and El-Baz (2001)**.** On the other hand, the proteolytic and lipolytic bacterial counts of all cheese treatments gradually increased with increasing of the ripening period.

The viable counts of *Lb. helveticus* in Gouda cheese made from different treatments during the ripening period for 90 days are registered in Fig (2 d). It is clear from these data that the viable counts of *Lb. helveticus* in fresh cheese were 32, 0.2 and 0.37 $x10^5$cfu/g for treatments B, C and D, respectively. It can be observed from these data that treatment B

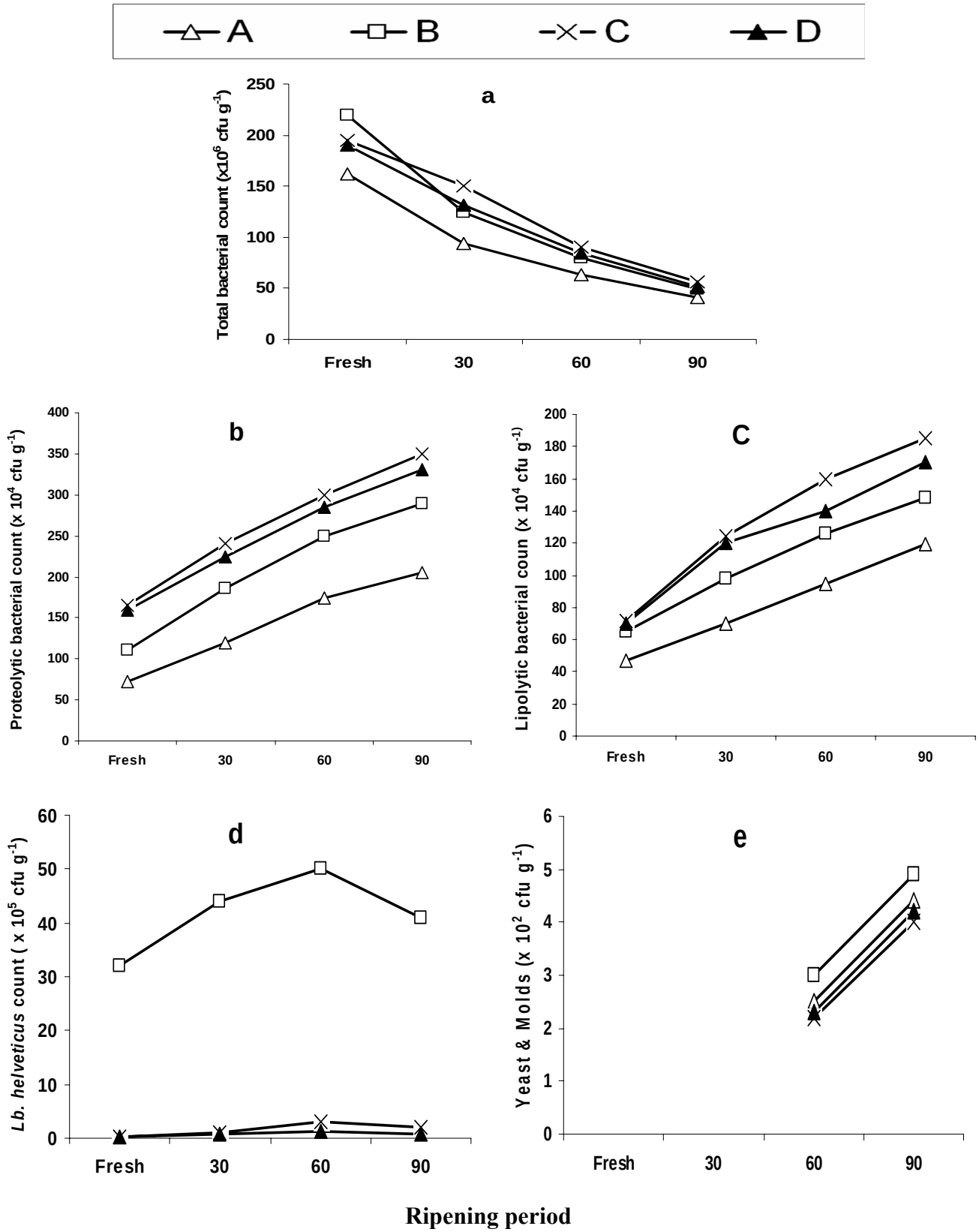


Fig (٢ a,b,c,d and e): Effect of adding freeze- and heat-shocked *Lb. helveticus* culture to cheese milk on microbiological quality of resultant Gouda cheese. (A): Control cheese with commercial starter, (B): Cheese treated with commercial starter + *Lb. helveticus*, (C): Cheese treated with commercial starter + freeze-shocked *Lb. helveticus* and (D): Cheese treated with commercial starter + heat-shocked *Lb. helveticus*.

had the highest viable counts of *Lb. helveticus* when fresh and during the ripening period compared with treatments C and D. This may be due to that the viable cells of *Lb. helveticus* were lost by freeze and heat-shock; while, commercial starter of the control cheese (treatment A) was free from *Lb. helveticus*. These results are in agreement with those obtained by Bartels *et al.,* (1987b). The counts of *Lb. helveticus* in treatments B, C and D gradually increased reaching the maximum counts 50, 0.92 and 1.2 $x10^5$ cfu/g, respectively after 60 days of the ripening period and then slightly decreased 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.

Fig. (2 e) show that, no colonies of yeasts & 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).

Textural properties:

Data in Table (3), clear that, the control cheese (treatment A) followed by cheese treated with viable cells of *Lb. helveticus* (treatment B) recorded higher values of hardness and another texture characteristics throughout the ripening period as they had a lower level of proteolysis. While, cheese treated with freeze-shocked *Lb. helveticus* (treatment C) followed by cheese treated with heat-shocked *Lb. helveticus* (treatment D) had more desirable texture along ripening as they had a higher level of proteolysis which attributed to weaken the structure leading to cheese softening through the breakdown of the casein matrix. Similar results were reported by Fredrick and Dulley (1984).

Generally, the values of texture characteristics were increased in all treatments during 60 days of ripening. This may be due to the moisture decrease in cheese resulted in a firmer texture due to alterations in the casein matrix (Fredrick and Dulley, 1984). But, their was a decrease in the values of texture characteristics of cheese after 90 days of ripening in all treatments. This could be mainly due to the proteolysis of casein producing

Table (3): Effect of adding freeze- and heat-shocked *Lb. helveticus* culture to cheese milk on textural properties of resultant Gouda cheese.

Parameters	Ripening period (days)	Treatments[1]				L.S.D (5%)
		A	B	C	D	
Hardness (Newton)	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 (Milimeter)	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 (Newton)	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 (Newton . Milimeter)	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 commercial starter + *Lb. helveticus*.

C : Cheese treated with commercial starter + freeze-shocked *Lb. helveticus*.

D : Cheese treated with commercial starter + heat-shocked *Lb. helveticus*.

very soluble compounds and that do not contribute to the protein network responsible for the cheese rigidity. Similar results were reported by El-Tawel (2004).

The analysis of variance shows significant differences (P≤0.05) for springiness, cohesiveness, gumminess and chewiness values between treatments of Gouda cheese at 60 days and 90 days of ripening. On the other side, the hardness of Gouda cheese treatments clears highly significant (P≤0.01) differences during ripening period at 60 and 90 days.

Organoleptic properties of cheese:

The organoleptic properties of Gouda cheese made from different treatments during ripening period and their corresponding scores are presented in Fig (3). The obtained results reveal that the fresh cheese of all treatments had nearly the same score points for flavour 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 a higher score points for flavour and body & texture characteristics. 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 *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 Vafopoulou *et al.,* (1989) and El-Baz (2001). On the other hand, it could be seen from these results that cheese treated with viable whole cells of *Lb. helveticus* (treatment B) gained higher score points for flavour and body & texture characteristics than control cheese (treatment A). This may be due to the whole cells of *Lb. helveticus* which had the ability to enhance the proteolysis and lipolysis of Gouda cheese. These results are confirmed by Bartels *et al.,* (1987b), Exterkate *et al.,* (1987) and Ardo *et al.,* (1989).

The statical analysis of variance shows highly significant differences (P≤0.01) between all treatments of Gouda cheese for flavor and total score at 30, 60 and 90 days of ripening. While, there were significant differences (P≤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.

In conclusion, it was clear from the ripening indices, microbiological quality, rheological and organoleptic characteristics that ripening of Gouda cheese treated with freeze-shocked *Lb. helveticus* (treatment C) followed by cheese treated with heat-shocked

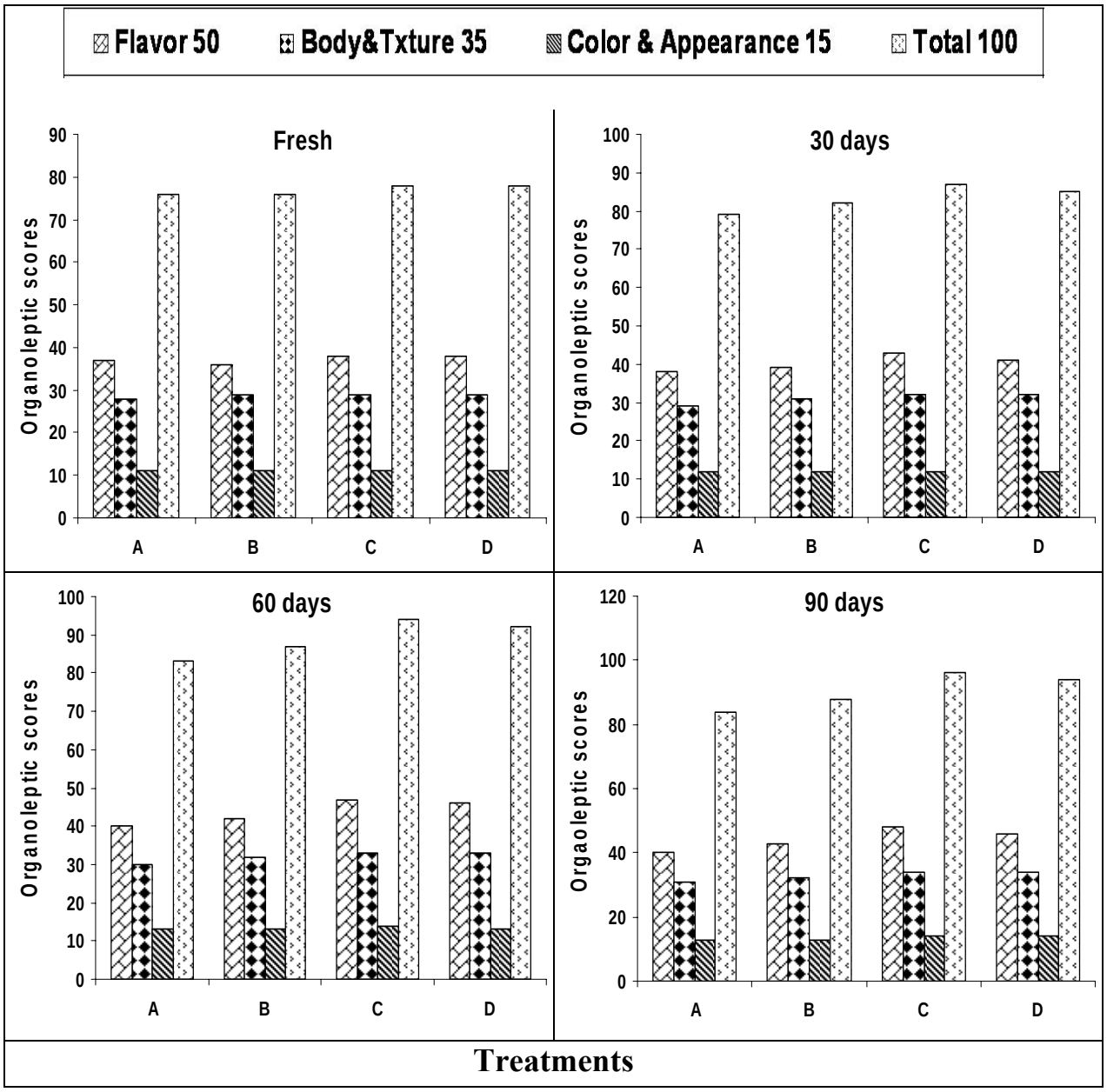


Fig. (3): Effect of adding of freeze- and heat- shocked *Lb. helveticus* culture to cheese milk on the organoleptic properties of resultant Gouda cheese. (A**):** Control cheese with commercial starter, (B): Cheese treated with commercial starter + *Lb. helveticus*, (C): Cheese treated with commercial starter + freeze-shocked *Lb. helveticus* and (D): Cheese treated with commercial starter + heat-shocked *Lb. helveticus*

Lb. helveticus (treatment D) were accelerated and the cheese quality was improved within 60 days as there were a reduction of 33 % in the ripening period in comparing with other treatments.

According to the prior analyses (ripening indices, rheological and organoleptic characteristics) the best treatment of Gouda cheese (treatment C) and 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.

Patterns of free fatty acids (FFA):

The fatty acids content of resultant Gouda cheese from treatments A and C after 90 days of ripening are given in Table (4). By studying these data it could be 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. These results are similar to those reported by Kamaly *et al.,* (1989); El-Soda *et al.,* (1999); El-Baz (2001) and Mostafa *et al.,* (2002)

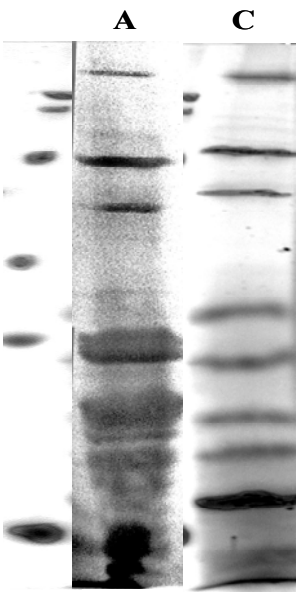
Electrophoretic patterns of Gouda cheese:

The electrophoretic patterns of Gouda cheese (treatments A and C) after 90 days of ripening are shown in Fig. (4). 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 that the freeze-shocked *Lb. helveticus* release intracellular enzymes which are a good source of aminopeptidase, dipeptidase and proteinase (Frey *et al.,* 1986). The same trend was observed by several investigators namely, Kim *et al.,* (1994) and Awad *et al.,* (2001) who found that the ripened cheese slurries with freeze-shocked adjunct lactobacilli exhibited more degradation of protein on poly acrylamide gel electrophoresis (PAGE) indicating greater peptidase activity in the curd compared to control slurry.

Table: (4): Pattern of free fatty acids (% of total) of Gouda cheese after 90 days of ripening.

Parameters	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

Band No.	M.W KDa	Treatments	
		A	C
1	160.0	1	1
2	145.0	0	0
3	119.0	0	0
4	104.0	0	0
5	94.0	0	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		14	18



1: Detected - 0: Not detected

A : Control cheese with commercial starter.

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

Fig. (4): The electrophoretic patterns of Gouda cheese (treatments A and C) after 90 days of ripening.

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إسراع تسوية وتحسين جودة جبن الجودا باستخدام

Lactobacillus delbrueckii subsp. *helveticus* DSMZ 20082

المعامل بالصدمة التجميدية والحرارية

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درس تأثير إضافة *Lb. helveticus* المعامل بالصدمة التجميدية أوالحرارية مع البادئ التجاري (*Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis, Leuconostoc mesenteroides* subsp. *cremoris* and *Lactococcus lactis* subsp. *diacetilactis*) للبن الخليط (لبن بقري وجاموسي ١:١ المعدل به نسبة الدهن إلي ٣,٥ %) لتصنيع جبن الجودا بغرض إسراع تسوية وتحسين جودة الجبن الناتج بالمقارنة بجبن الكنترول (الجبن المصنع بالبادئ التجاري فقط والجبن المصنع بالبادئ التجاري والمضاف إلية *Lb. helveticus* بدون معاملة). تم تخزين الجبن الناتج بغرفة التسوية علي ١٠- ١٢°م ورطوبة نسبية ٨٥-٩٥٪ لمدة ثلاثة شهور مع إجراء التحليلات الكميائية والميكروبيولوجية والريولوجية وكذلك التقييم الحسي للجبن وهو طازج وبعد ٣٠ ، ٦٠، ٩٠ يوم من التسوية. وقد أظهرت النتائج انه بتقدم فترة التسوية حدثت زيادة في مؤشرات التسوية (النيتروجين الذائب – التربتوفان والتيروسين – الأحماض الدهنية الكلية الطيارة) وأيضا حدثت زيادة في أعداد البكتريا المحللة للبروتين والمحللة للدهون. وكانت أعلي درجات التحكيم الحسي و تحسن الخصائص الريولوجية نتيجة إضافة *Lb. helveticus* المعامل بالصدمة التجميدية يلية *Lb. helveticus* المعامل بالصدمة الحرارية مع انخفاض ~ ٣٣ ٪ من فترة التسوية عن مثيلتها بدون هذه الإضافات (جبن المقارنة).

أظهرت نتائج التحليل بجهاز الفصل الكروماتوجرافى الغازي (GLC) وبجهاز الفصل الكهربي للبروتين (Electrophoresis) زيادة ملحوظة في نسبة الأحماض الدهنية الحرة الطيارة وارتفاعاً ملحوظاً في تحلل البروتين علي الترتيب في الجبن المضاف الية *Lb.helveticus* المعامل بالصدمة التجميدية مقارنة بالجبن المصنع بالبادئ التجاري فقط وذلك عند عمر ٩٠ يوم من التسوية.