

## PRODUCTION OF DOMIATI CHEESE WITH TYPICAL CHARACTERISTICS FROM PASTEURIZED MILK USING *Lactobacillus helveticus* AND *Lactobacillus casei* CULTURES

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### ABSTRACT

Domiat cheese was made from pasteurized buffalo's milk with 2 different levels of salt (5 & 10%) inoculated with *Lactobacillus helveticus* and *L. casei* as single or mixed cultures. The cheese was pickled in salted whey for 8 weeks. Cultures increased the production of water-soluble nitrogen, total volatile fatty acids and free tyrosine and tryptophane amino acids and enhanced cheese ripening rate. Starter formed of *L. helveticus* + *L. casei* (2:1, v/v) produced Domiat cheese with typical characteristics at 4 weeks of ripening, half the period required for the control. The cheese was superior to the control and the other experimental cheeses. *Lactobacillus helveticus* and *L. casei* when used as single cultures were not satisfactory.

### INTRODUCTION

Pickled Domiat cheese, which is processed mainly from raw milk, has a characteristic flavour and texture. These characteristics take long period to develop and if the cheese was made from pasteurized milk, the typical characteristics were not easily attained even with longer pickling periods. Therefore, experts worked hard to overcome this problem using dairy cultures but following different approaches. Aly (1996) found that inoculating milk with *L. delbrueckii* subsp. *helveticus* resulted in Domiat cheese of typical flavour, body and texture after 4-6 weeks of ripening at room temperature (20-25°C). El-Alfy (1998) hydrolyzed mature Ras cheese with *L. helveticus* for 3 days, and the hydrolyzate (3-5%, v/v) was added to milk. The hydrolyzate stimulated cheese proteolysis and microbial growth giving a typical Domiat cheese in one month. Darwish, et al. (1969) obtained Domiat cheese with typical flavour, body and texture after 30 days of pickling at 10°C by adding cell free extract from *L. helveticus* to cheese curd containing 5 or 8% salt directly after ladling. Abbas, et al. (1993) added full aged pre-ripened goat slurry into ultrafiltered milk. They obtained smooth Domiat cheese with favorite body and texture. El-Sissi (2002) added 2% of blue cheese slurry to pasteurized milk salted with 7% NaCl to enhance Domiat ripening at room temperature.

As can be seen, *L. helveticus* was the major microorganism proposed to be used to enhance Domiat cheese ripening. However, our experience (Metwally, et al., 1984) of dealing with *L. helveticus* in ripening Ras cheese pointed out that *L. helveticus* behaved better and in controlled way when was in combination of other microorganisms such as *S. thermophilus* or *L. casei*. On the other hand, *L. casei* was found to speed up

Ras cheese ripening whether used as a single culture or in combination of *L. helveticus*.

Therefore, this research was set to test the feasibility of using *L. helveticus* and *L. casei* cultures either as single or mixed starters for ripening Domiati cheese made from pasteurized salted milk.

## **MATERIALS AND METHODS**

Fresh buffalo's milk of the Faculty of Agriculture, Cairo University herd, *Lactobacillus delbrueckii subsp. helveticus* (*L. helveticus*) and *Lactobacillus casei* pure cultures from Chr. Hansen Laboratory, Copenhagen, Denmark and Chemicals of highest purity were used.

Domiati cheese was manufactured from pasteurized buffalo's milk (75°C/1 min.) according to the method of Fahmi and Sharara, (1950). Milk was standardized into 6.5% fat and 8.85% S.N.F., salted with 5% and 10% NaCl and then inoculated with 0.5% of the following single and mixed starters: T<sub>1</sub> = single: *L. helveticus*, T<sub>2</sub> = single *L. casei*, T<sub>3</sub> = mixed: *L. helveticus* + *L. casei* (1:2 v/v) and T<sub>4</sub> = mixed: *L. helveticus* + *L. casei* (2:1 v/v). While control cheese was made without culture.

The cheese of each treatment was pickled in its whey after adjusting the salt content into 13% and was stored for 8 weeks at 15±1°C. The cheese was sampled every 2 weeks for analysis.

Cheese was analyzed for moisture, fat, salt and acidity using the method of Ling (1963), total nitrogen (TN) and water soluble nitrogen (WSN) according to Polychroniadon, et al. (1994), total volatile fatty acids (TVFA) according to the method of Kosikowski (1982) and tyrosine and tryptophane according to the method of Vakaleris and Price (1959).

Sensory evaluation of the cheese was determined by the Dairy Department Staff members, giving a score of 60 for flavour and 40 for body and texture.

A 3-way ANOVA analysis was carried out by SPSS for Window's Version 11 software, 2001.

## **RESULTS AND DISCUSSION**

Domiati cheese was made with 2 different levels of salt inoculated with *L. helveticus* and *L. casei* as single or mixed cultures. Table (1) shows the total solids of the cheese at different pickling periods. Total solids (T.S.) decreased by salt content but increased by storage. The increase in T.S. with salt content was not significant, but the increase by pickling period was significant (P<0.05 and P<0.01). The great portion of the increase was obtained after 2 weeks of pickling, it was between 38 to 59% of the total increase. The increase in T.S. was more in cheese with 5% salt than with 10%.

There was no obvious effect for culture presence on the changes of T.S., though the changes were slightly greater in *L. casei* cheeses than other cultures. The increase in T.S. was due to the withdrawal of moisture by the

salted pickling whey. Maximum water removal was achieved within the first 2 weeks, after which salt contents of cheese was increased and the rate declined. The 10% cheese contained more salt from the beginning, therefore, removal of water and in turn the increase in T.S. was less than the 5% cheese.

**Table (1): Total solids of Domiati cheese made with different cultures and sodium chloride.**

Pickling period, (wk)	Cultures									
	None, (control)		<i>L. helveticus</i>		<i>L. casei</i>		<i>L. helv. + L. casei</i> (1:2)		<i>L. helv. + L. casei</i> (2:1)	
	Salt, %									
	5	10	5	10	5	10	5	10	5	10
	<b>Total solids, %</b>									
0	40.1	37.7	41.2	39.2	39.3	37.4	40.8	38.6	41.4	38.3
2	44.1	42.2	45.1	43.1	44.8	42.5	45.0	42.8	45.6	43.9
4	46.6	44.4	47.6	45.0	46.8	45.0	46.4	44.7	47.1	45.2
6	48.7	45.7	50.0	46.0	49.6	47.2	48.1	45.9	49.1	46.9
8	50.6	46.8	51.9	47.2	51.0	48.2	50.7	46.9	51.6	47.7

LSD (0.05) = 1.62  
(0.01) = 2.14

Table (2) shows the fat contents of the experimental cheeses during pickling. Fat/dry matter significantly ( $P < 0.5$ ) increased during storage up to the 4<sup>th</sup> week, then declined slightly. The trend followed cheese T.S. trend.

**Table (2): Fat contents of Domiati cheese made with different cultures and sodium chloride.**

Pickling period, (wk)	Cultures									
	None, (control)		<i>L. helveticus</i>		<i>L. casei</i>		<i>L. helv. + L. casei</i> (1:2)		<i>L. helv. + L. casei</i> (2:1)	
	Salt, %									
	5	10	5	10	5	10	5	10	5	10
	<b>Fat/dry matter, %</b>									
0	46.2	47.8	47.1	47.7	46.1	47.6	47.1	47.9	46.9	48.0
2	48.3	49.8	49.2	49.8	48.0	49.7	49.1	50.0	49.3	50.4
4	56.8	57.5	56.9	57.8	56.5	57.5	57.2	58.0	57.7	58.2
6	55.4	56.5	55.8	56.9	55.4	56.6	56.5	57.1	56.6	57.3
8	52.6	54.5	52.8	54.7	52.8	54.5	53.2	55.0	53.3	55.4

LSD (0.05) = 1.39  
(0.01) = 1.85

Table (3) shows the titratable acidity development in the experimental cheeses during pickling. The acidity increased during storage. Cheeses with less salt and with added cultures developed more acid, but with a reasonable rate, so as not to give acid cheese.

Table (3): Titratable acidity of Domiati cheese made with different cultures and sodium chloride.

Pickling period, (wk)	Cultures									
	None, (control)		<i>L. helveticus</i>		<i>L. casei</i>		<i>L. helv. + L. casei (1:2)</i>		<i>L. helv. + L. casei (2:1)</i>	
	Salt, %									
	5	10	5	10	5	10	5	10	5	10
	Titratable acidity / dry matter, %									
0	1.0	0.9	1.1	0.9	1.1	0.9	1.1	1.0	1.2	1.0
2	1.5	1.2	1.7	1.4	1.5	1.3	1.8	1.6	1.9	1.5
4	2.2	1.9	2.3	1.9	2.3	1.9	2.4	2.1	2.5	2.1
6	2.3	2.3	2.6	2.4	2.6	2.2	2.8	2.5	2.8	2.5
8	2.9	2.7	3.1	2.9	2.9	2.6	3.2	3.0	3.3	3.0

LSD (0.05) = 0.30  
(0.01) = 0.48

Total nitrogen decreased during storage, of course due to casein micelle solubilization by its change from ca-caseinate into the more soluble sodium caseinate. Total nitrogen started by ~6% and ended by 4.1% after 8 weeks of storage (unreported data). On the other hand, water soluble nitrogen as reported in Table (4) significantly increased by storage. The change was greater in low salt cheeses to reach 4 times the original amounts. The presence of cultures effectively produced more soluble nitrogen. Cheese with *L. helveticus* and *L. casei* (2:1) produced more of soluble nitrogen and at a faster rate than any other culture.

Table (5) presents total volatile fatty acids produced in the cheeses. Their production followed the same pattern of soluble nitrogen and again *L. helveticus + L. casei* (2:1) culture being the most effective.

Table (4): Water soluble nitrogen contents of Domiati cheese made with different cultures and sodium chloride.

Pickling period, (wk)	Cultures									
	None, (control)		<i>L. helveticus</i>		<i>L. casei</i>		<i>L. helv. + L. casei (1:2)</i>		<i>L. helv. + L. casei (2:1)</i>	
	Salt, %									
	5	10	5	10	5	10	5	10	5	10
	Soluble nitrogen/TN, %									
0	5.9	3.6	6.9	4.8	6.6	4.4	6.9	5.2	7.5	5.3
2	9.5	5.1	12.1	6.8	11.4	6.2	13.3	7.9	14.8	8.7
4	13.5	8.6	15.14	10.2	14.8	9.7	16.9	11.7	19.8	12.7
6	16.1	11.6	19.0	15.1	17.3	13.1	23.3	15.9	24.7	16.5
8	21.2	15.7	27.0	20.0	25.1	18.7	30.1	20.8	32.4	21.5

LSD (0.05) = 0.36  
(0.01) = 0.47

Table (5): Total volatile fatty acids contents\* of Domiati cheese made with different cultures and sodium chloride.

Pickling period, (wk)	Cultures									
	None, (control)		<i>L. helveticus</i>		<i>L. casei</i>		<i>L. helv. + L. casei (1:2)</i>		<i>L. helv. + L. casei (2:1)</i>	
	5	10	5	10	5	10	5	10	5	10
	<b>Total volatile fatty acids, %</b>									
0	8.1	5.0	8.8	6.0	8.6	5.6	9.2	6.5	9.7	7.8
2	15.4	9.2	17.4	10.8	16.9	10.6	17.9	11.1	18.9	13.8
4	21.7	12.3	26.1	17.7	25.7	16.4	26.8	18.7	27.2	19.6
6	26.6	16.3	32.8	22.1	31.1	20.9	33.1	24.6	33.7	26.3
8	30.9	22.6	34.2	28.3	33.3	26.4	36.3	30.2	38.2	31.3

LSD (0.05) = 1.62  
(0.01) = 2.15

\* TVFA expressed as ml of 0.1 N NaOH/100 g cheese.

Table (6) points out the release of tyrosine and tryptophane amino acids. The amounts released once again emphasized the importance of the presence of culture and in particular *L. helveticus + L. casei (2:1)* which produced significantly more free acids and with a faster rate than other cultures.

Table (6): Free tyrosine and tryptophane amino acids contents of Domiati cheese made with different cultures and sodium chloride.

Pickling period, (wk)	Cultures									
	None, (control)		<i>L. helveticus</i>		<i>L. casei</i>		<i>L. helv. + L. casei (1:2)</i>		<i>L. helv. + L. casei (2:1)</i>	
	5	10	5	10	5	10	5	10	5	10
	<b>Tyrosine plus tryptophane, mg/100g</b>									
0	52.7	51.4	55.2	51.6	54.50	50.3	57.2	53.8	59.3	55.9
2	69.7	66.7	74.6	70.1	72.8	67.8	78.0	73.3	80.9	74.9
4	87.1	83.8	99.3	90.8	95.8	86.3	104.1	96.9	108.7	100.0
6	103.1	101.1	122.3	113.3	115.9	105.9	132.2	121.8	136.8	128.6
8	115.2	113.5	135.8	127.3	120.8	116.4	145.1	136.3	148.6	142.9

LSD (0.05) = 0.27  
(0.01) = 0.37

Table (7) presents the sensory evaluation of the cheeses. The values proved the importance of using cultures in speeding up the ripening and in obtaining a good typical, smooth Domiati cheese. Cheese made with *L. helveticus + L. casei (2:1)* possessed the typical well ripened Domiati cheese flavour and texture obtaining the highest score. The cheese reached its full flavour after 4 weeks of storage and to obtain a score of 81 which was similar to the 8<sup>th</sup> week control cheese score. On the 8<sup>th</sup> week, the *L. helveticus + L.*

*casei* (2:1) cheese scored 95, the highest score of all cheeses. Ripening indices, such as free tryptophane and tyrosine, TVFA and soluble nitrogen coincided with sensory evaluation. The *L. helveticus* + *L. casei* (1:2) cheese was the second in order either in sensory scoring or in ripening indices.

**Table (7): Sensory evaluation of Domiati cheese made with different cultures and salt levels.**

Culture used	Salt, %					
	5			10		
	Cheese score					
	Flavour (60)	Body & Texture (40)	Total (100)	Flavour (60)	Body & Texture (40)	Total (100)
<b>Cheese age, week</b>						
<b>Fresh</b>						
Control	33	25	58	28	23	51
T <sub>1</sub>	35	28	63	29	24	53
T <sub>2</sub>	34	27	61	29	23	52
T <sub>3</sub>	37	29	66	32	27	59
T <sub>4</sub>	38	31	69	34	28	62
<b>2 weeks of Pickling</b>						
Control	34	26	60	30	24	54
T <sub>1</sub>	36	30	66	32	25	57
T <sub>2</sub>	35	30	65	31	25	56
T <sub>3</sub>	38	31	69	34	30	64
T <sub>4</sub>	42	32	74	35	31	66
<b>4- Weeks of pickling</b>						
Control	37	28	65	32	26	58
T <sub>1</sub>	41	31	72	34	29	63
T <sub>2</sub>	39	31	70	31	29	60
T <sub>3</sub>	45	32	77	37	32	69
T <sub>4</sub>	47	34	81	38	33	71
<b>6 Weeks of pickling</b>						
Control	41	31	72	38	27	65
T <sub>1</sub>	45	34	79	40	32	72
T <sub>2</sub>	44	33	77	39	30	69
T <sub>3</sub>	49	35	84	43	33	76
T <sub>4</sub>	52	36	88	45	33	78
<b>8 weeks of pickling</b>						
Control	47	34	81	42	32	74
T <sub>1</sub>	52	36	88	45	34	79
T <sub>2</sub>	51	35	86	43	33	76
T <sub>3</sub>	55	37	92	46	35	81
T <sub>4</sub>	57	38	95	48	35	83

\* Cultures used:

C = Control cheese (without cultures),

T<sub>1</sub> = *L. helveticus*,

T<sub>2</sub> = *L. casei*,

T<sub>3</sub> = *L. helveticus* + *L. casei* (1:2),

T<sub>4</sub> = *L. helveticus* + *L. casei* (2:1).

Results statistical analysis:

Texture: LSD (0.05) = 5.15

Flavour: LSD (0.05) = 5.63

Total score: LSD (0.05) = 8.54

As for salt content, the 5% salt was the proper ratio that gave best results. The 10% salt showed slower ripening and the cheese developed unpleasant flavour. *L. helveticus* was more active than *L. casei* in the ripening process, however using each, as single culture was not recommended, because they developed unacceptable flavour.

In conclusion, in making Domiati cheese from pasteurized salted milk, the use of culture is important. *L. helveticus* + *L. casei* (2:1) starter was the culture that speeded up the ripening, produced well ripened smooth Domiati cheese with the desired typical flavour after 4 weeks of ripening and the improvement was continued up till the end of 8<sup>th</sup> week of pickling.

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إنتاج جبن دمياطي نموذجي من لبن مبستر باستخدام مزارع *L. casei*,  
*L. helveticus*

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صنعت الجبن الدمياطي من اللبن الجاموسي الطازج المبستر المملح بنسبة ٥%، ١٠%+ باستخدام مزارع *L. casei*, *L. helveticus* كبادئات فردية أو مختلطة ، ثم خزنت الجبن الناتج في الشرش المملح (١٣%) لمدة ٨ أسابيع. أشارت النتائج إلى أن استخدام هذه المزارع البكتيرية كبادئ أدى إلى زيادة واضحة في النيتروجين الذائب في الماء (WSN) والأحماض الدهنية الطيارة (TVFA)، والأحماض الأمينية: تيروسين وتربتوفان في الجبن الناتج بالإضافة إلى تحسين معدل التسوية فيها. وكان الجبن الدمياطي الناتج من اللبن المبستر المملح باستخدام البادئ المكون من *L. casei* + *L. helveticus* (١:٢) نموذجياً في خواصه بعد ٤ أسابيع من التخزين في المحلول الملحي، وهي مدة تعادل نصف المدة اللازمة لجبن المقارنة. وتميز هذا الجبن أيضاً بتفوقه في خواصه عن جبن المقارنة والجبن المصنع باستخدام البادئات الأخرى الفردية أو المختلطة.