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Heat-Shocked Lactobacilli for Accelerating Flavour Development of Ras Cheese

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ABSTRACT

An attempt has been made to shorten the ripening period of Ras cheese. Cheese was made from curd incorporated with a heat-shocked culture of either Lactobacillus casei *or* Lactobacillus helveticus *at levels of 1% and 2% each. These treatments did not considerably affect the gross chemical composition of the cheese but influenced flavour intensity, body characteristics, the formation of soluble nitrogen compounds and free volatile fatty acids. Meanwhile, total proteolytic and lipolytic bacterial counts were also stimulated. Cheese with added heat-shocked lactobacilli showed desirable flavour and consistency 1–2 months earlier than control cheese made without additives.*

INTRODUCTION

It is generally recognised that the mechanism of the ripening process of cheese is due mostly to microbial enzymatic transposition. Lactic acid bacteria are known to contain a wide range of proteinases and peptidases. These enzymes supply a pool of free amino acids which yield a number of substances found in ripened cheese through oxidative, reductive or hydrolytic deamination, along with decarboxylation and some other processes (Visser, 1977; Law, 1980). Lowrie *et al.* (1974) showed that increasing the population of lactic acid bacteria in cheese milk influenced the manufacturing process and produced an atypical flavour whereas a high viable population in Cheddar curd resulted in a high incidence of

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bitterness in cheese. Law & Sharpe (1975) tried to avoid the acid production problem by adding cell-free starter enzyme extracts to cheese milk. They reported that about 40–45% of the intracellular dipeptidase activity was lost in the whey and that the distribution of the enzymes added in this form was unlikely to have been the same as if they had been released in discrete loci form lysing cells entrapped mechanically in the curd matrix.

Eriksson & Sjostrom (1967) tried to increase the concentration of starter enzymes in cheese by augmentation of normal starter inoculi with starter preparations which have been subjected to sub-lethal heat treatment to prevent them from producing acid during cheese making, yet have their important degradative enzyme intact. The effect on proteolysis and ripening was negligible because the number of starter Streptococci was not much greater than in a normal cheese. Petterson & Sjostrom (1975) showed that the rate of proteolysis was increased in Swedish household cheese containing heat-shocked cells equivalent to 4–5 times the normal starter population. They reported that the experimental cheeses contained up to 60% more TCA-soluble nitrogen than the control cheese made with normal starter alone and, even though the lactobacilli, as *L. helveticus*, were added in lower numbers than the mesophilic starters, they had a greater effect both on proteolysis and on flavour scores.

Thompson *et al.* (1979) reported preliminary findings that low fat (20%) Cheddar cheese had more flavour if heat-shocked starters were added. However, the numbers involved were extremely low (10^6 cell/ml) and the results will require careful evaluation to ensure that the effects on proteolysis and lipolysis were not due to other variables induced by the process.

The present work was undertaken to assess information on the quality and ripening changes of Ras cheese (common hard type in Egypt) as they are affected by incorporation into the curd of a heat-shocked culture of *L. casei* or *L. helveticus*.

MATERIALS AND METHODS

Milk

Cow's milk was obtained from the herd of the Faculty of Agriculture, Zagazig University, Egypt.

Rennet

A rennet powder (1:100 000) was obtained from L. C. Glad Company A/S, Copenhagen, Denmark.

Normal starter for cheese making

Single cultures of *S. lactis* and *S. thermophilus* were obtained from Chr. Hansen Laboratory, Copenhagen, Denmark. These cultures were activated before being used and mixed at the ratio of 1:1.

Heat-shocked cultures

Cultures of *Lactobacillus casei* or *Lactobacillus helveticus* obtained from Hansen Laboratory were first activated as described by Limsowtin *et al.* (1982) and then heated to 69°C for 15 s as recommended by Pettersson & Sjostrom (1975).

Cheese making

Ras cheese was made as described by Abdel Tawab (1963). Cheese milk was heated to 72°C for 15 s, rapidly cooled to a setting temperature of 33°C and inoculated with an active culture of *S. lactis* and *S. thermophilus* (1:1) at a level of 1·0%. Ras cheese curd was prepared and divided into five equal parts. The first part was moulded without additives as a control. The remaining four parts were mixed with a heat-shocked culture of *Lactobacillus casei* or *Lactobacillus helveticus* at levels of 1% and 2% of curd weight for each type of heat-shocked culture. The cheese making process was completed and the resultant cheeses were ripened at 12 ± 2°C for 4 months. Trials were conducted in triplicate.

Chemical analysis

Cheese samples were analysed when fresh, then after 2 and 4 months, for moisture, fat, salt, total N, titratable acidity, soluble N and non-protein N as described by Ling (1963). Both peptide N and amino acid N were estimated according to Stadhouders (1959).

Volatile fatty acids with carbon chain of C_2–C_8

The method of Kuzdzal & Kuzdzal-Savoie (1966) was adopted for the extraction of free fatty acids and the preparation of its soap. Volatile fatty acids with chain lengths of C_2–C_8 were determined as described by Ross *et al.* (1963). The separation of volatile fatty acids was carried out by gas–liquid chromatography using a Pye Unicam Series 104 Chromatograph with ionization detector under the following conditions. Glass column, 3 m long and 2 mm internal diameter with 10% polyethylene glycol adipate as a stationary phase. Carrier gas flow (He) was adjusted to 35 ml/min. Chart speed was 5 mm/min, temperature of the column was 150°C and that of the detector, 250°C.

Bacteriological examination

Cheese samples were bacteriologically examined for total, proteolytic and lipolytic bacterial counts as described by Marth (1978).

Organoleptic properties

Cheese samples were organoleptically examined for appearance, body characteristics and flavour according to Abdou *et al.* (1977).

Statistical analysis

The effects on gross chemical composition, nitrogen fractions and scores of Ras cheese of the addition of heat-shocked cultures to cheese curd were statistically analysed by the *F* test. In cases of significant *F*, differences between treatments were examined by Duncan's multiple range test (Snedecor, 1961).

RESULTS

Gross chemical composition

Table 1 shows that the addition of heat-shocked cultures of either *L. casei* or *L. helveticus* to cheese curd at levels of 1% and 2% had an insignificant effect on the moisture, fat and salt contents of the resultant

TABLE 1
Gross Chemical Composition of Ras Cheese as Affected by Heat-shocked Cultures (HSC)

Property (%)	*Ripening period* (*months*)	*Control cheese*		*Cheese from curd with HSC*							
				L. casei				L. helveticus			
				1%		*2%*		*1%*		*2%*	
		$\bar{X}$	*SE*	$\bar{X}$	*SE*	$\bar{X}$	*SE*	$\bar{X}$	*SE*	$\bar{X}$	*SE*
Moisture	Fresh	38·96	0·17	39·41	0·15	40·01	0·13	40·06	0·24	40·90	0·25
	2	36·50	0·18	36·73	0·13	36·45	0·13	37·02	0·27	37·92	0·24
	4	34·26	0·20	34·77	0·59	34·07	0·22	35·11	0·37	35·60	0·27
Fat (dry matter)	Fresh	48·50	0·32	48·42	0·22	48·42	0·20	48·50	0·18	47·91	0·06
	2	49·40	0·19	49·31	0·23	49·38	0·21	49·41	0·15	48·42	0·37
	4	50·21	0·13	50·11	0·16	50·28	0·17	50·41	0·11	49·72	0·44
Salt (dry matter)	Fresh	3·02	0·03	3·00	0·05	2·98	0·07	2·99	0·02	3·96	0·02
	2	3·42	0·11	3·36	0·05	3·31	0·06	3·28	0·03	3·30	0·03
	4	3·81	0·04	3·70	0·13	3·62	0·07	3·68	0·07	3·58	0·12
Titratable acidity (as lactic acid)	Fresh	0·30	$0·003^{c}$	0·32	$0·003^{ab}$	0·32	$0·004^{cb}$	0·33	$0·004^{ab}$	0·34	$0·008^{a}$
	2	1·18	$0·004^{d}$	1·20	$0·004^{c}$	1·21	$0·004^{cb}$	1·23	$0·004^{b}$	1·29	$0·004^{a}$
	4	1·72	$0·01^{e}$	1·80	$0·01^{d}$	1·88	$0·01^{c}$	1·92	$0·01^{b}$	2·08	$0·02^{a}$

^{a,b,c,d,e}, Values in the same row with different letters differ significantly.
$\bar{X}$, Averages of three replicates. SE, Standard error.

TABLE 2
Distribution of Soluble Nitrogen Compounds in Ras Cheese as Affected by Heat-shocked Cultures (HSC)

Nitrogen fraction (% of TN)	*Ripening period (months)*	*Control cheese*		*Cheese from curd with HSC*							
				L. casei				L. helveticus			
				1%		*2%*		*1%*		*2%*	
		$\bar{X}$	*SE*	$\bar{X}$	*SE*	$\bar{X}$	*SE*	$\bar{X}$	*SE*	$\bar{X}$	*SE*
Soluble N	Fresh	8·98	$0·15^{c}$	9·76	$0·10^{b}$	10·1	$0·11^{b}$	10·7	$0·41^{a}$	10·7	$0·14^{a}$
	2	17·0	$0·21^{e}$	20·6	$0·20^{d}$	23·7	$0·08^{c}$	26·6	$0·42^{b}$	29·4	$0·17^{a}$
	4	22·2	$0·21^{d}$	25·7	$0·53^{c}$	28·4	$0·58^{b}$	29·2	$0·24^{b}$	39·4	$0·35^{a}$
Non-protein N	Fresh	1·72	$0·02^{d}$	1·90	$0·03^{c}$	2·06	$0·02^{b}$	2·0	$0·03^{b}$	2·36	$0·03^{a}$
	2	4·48	$0·18^{d}$	4·95	$0·13^{d}$	5·42	$0·16^{c}$	8·4	$0·21^{b}$	9·62	$0·17^{a}$
	4	6·00	$0·16^{d}$	7·02	$0·19^{cd}$	8·16	$0·71^{c}$	10·4	$0·20^{b}$	12·8	$0·15^{a}$
Peptide N	Fresh	0·68	$0·02^{c}$	0·90	$0·01^{b}$	1·00	$0·04^{a}$	0·98	$0·03^{a}$	1·02	$0·02^{a}$
	2	1·44	$0·05^{e}$	1·68	$0·03^{d}$	1·82	$0·03^{c}$	2·40	$0·03^{b}$	2·96	$0·03^{a}$
	4	1·62	$0·02^{e}$	2·22	$0·06^{d}$	2·96	$0·02^{c}$	3·94	$0·04^{b}$	4·60	$0·15^{a}$
Amino acid N	Fresh	1·00	$0·04^{e}$	1·42	$0·02^{d}$	1·56	$0·02^{c}$	1·70	$0·02^{b}$	2·16	$0·05^{a}$
	2	2·31	$0·05^{e}$	3·00	$0·04^{d}$	3·96	$0·09^{c}$	4·96	$0·03^{b}$	5·92	$0·03^{a}$
	4	3·96	$0·07^{d}$	4·02	$0·02^{d}$	5·00	$0·37^{c}$	7·32	$0·07^{b}$	8·40	$0·14^{a}$

^{a,b,c,d,e}, Values in the same row with different letters differ significantly. $\bar{X}$, Averages of three replicates. SE, Standard error.

TABLE 3

Free Volatile Fatty Acids (C_2–C_8) of Ras Cheese as Affected by Heat-shocked Cultures (HSC). Averages of Three Replicates

Free fatty acids (mg/100 g)	*Ripening period (months)*									
	2					*4*				
	Control	L. casei		L. helveticus		*Control*	L. casei		L. helveticus	
		1%	*2%*	*1%*	*2%*		*1%*	*2%*	*1%*	*2%*
C_2	1·00	2·00	2·16	2·20	2·40	1·40	2·60	2·33	2·41	2·60
C_3	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
C_4	0·40	0·90	1·11	1·26	1·40	1·20	2·06	1·41	1·16	2·40
C_5 iso	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
C_5	Trace	Trace	Trace	Trace	0·50	Trace	Trace	0·50	0·90	1·00
C_6	0·40	0·68	0·90	0·90	1·10	1·20	1·60	1·76	2·20	2·44
C_8	0·80	1·22	1·42	1·42	1·70	1·30	2·16	2·46	2·30	2·62
Total	$2·60^{a}$	$4·80^{ab}$	$5·59^{cb}$	$5·78^{cb}$	$7·10^{e}$	$4·10^{a}$	$8·42^{b}$	$8·46^{cb}$	$9·97^{cbd}$	$11·06^{ee}$

^{a,b,c,d,e}, Means in the same row for each period of ripening with different letters differ significantly.

cheese. However, this treatment showed a significant stimulating effect on the development of cheese acidity.

Soluble nitrogenous compounds

From the results given in Table 2, it can be seen that the addition of heat-shocked cultures containing *L. casei* or *L. helveticus* had a significant effect on the formation of SN, NPN, peptide N and amino acid N. The differences in the levels of soluble nitrogenous compounds of cheeses in the different treatments were significant.

Thus, the levels of all nitrogen fractions in 2–3 months old cheese with added heat-shocked cultures (2%) were similar to, or even more than, those of control cheese after 4 months of ripening. However, addition of cultures containing *L. helveticus* was most effective in this respect.

Free volatile fatty acids

Table 3 shows that the patterns of free volatile fatty acids (C_2–C_8) extracted from cheese made with and without the addition of heat-shocked cultures were similar. However, Ras cheese made from curd

TABLE 4

Bacteriological Contents* of Ras Cheese as Affected by Heat-shocked Cultures (HSC)

	Ripening period (months)	*(Million per one gram of cheese)*				
		Control	*Ras cheese with HSC*			
			L. casei		L. helveticus	
			1%	*2%*	*1%*	*2%*
Total bacterial count	Fresh	183·4	204·6	236·2	211·8	248·2
	2	78·6	132·4	153·7	161·5	168·4
	4	42·1	51·6	62·3	68·2	72·3
Proteolytic bacterial count	Fresh	7·2	11·8	13·6	12·7	15·6
	2	15·3	17·2	21·2	19·1	24·8
	4	19·9	22·4	26·8	24·3	29·2
Lipolytic bacterial count	Fresh	4·6	6·2	7·3	6·9	8·2
	2	9·3	11·4	13·1	12·8	14·6
	4	11·8	13·7	16·2	14·6	19·8

* Averages of three replicates.

TABLE 5
Organoleptic Properties* of Ras Cheese as Affected by Heat-shocked Cultures (HSC)

Property	*Ripening period (months)*	*Control cheese*		*Cheese from curd with HSC*							
				L. casei				L. helveticus			
				1%		*2%*		*1%*		*2%*	
		$\bar{X}$	*SE*	$\bar{X}$	*SE*	$\bar{X}$	*SE*	$\bar{X}$	*SE*	$\bar{X}$	*SE*
Flavour (50)	2	32	$0{\cdot}67^{d}$	33	$0{\cdot}33^{c}$	41	$0{\cdot}31^{ab}$	40	$0{\cdot}50^{b}$	42	$0{\cdot}48^{a}$
	4	36	$0{\cdot}58^{d}$	39	$0{\cdot}31^{c}$	42	$0{\cdot}73^{b}$	43	$0{\cdot}57^{b}$	45	$0{\cdot}31^{a}$
Body and	2	32	$0{\cdot}42^{b}$	35	$0{\cdot}40^{a}$	35	$0{\cdot}25^{a}$	35	$0{\cdot}58^{a}$	35	$0{\cdot}26^{a}$
texture (40)	4	34	$0{\cdot}25^{c}$	37	$0{\cdot}01^{b}$	37	$0{\cdot}36^{b}$	37	$0{\cdot}17^{b}$	38	$0{\cdot}42^{a}$

$\bar{X}$, Averages of three replicates. SE, Standard error. * Score for appearance for all treatments during ripening is 8 out of 10.
^{a,b,c,d,e}, Values in the same row with different letters differ significantly.

with added heat-shocked cultures of both types contained slightly higher concentrations of volatile fatty acids. The effect of heat-shocked cultures was proportional to the level of added cultures and was more remarkable with the heat-shocked culture of *L. helveticus*.

Bacterial examination

Table 4 shows that the addition of both types of heat-shocked culture to Ras cheese curd had some stimulating effect on bacterial growth. The total number of bacteria in the cheese of different treatments reached a maximum after a ripening period of 2 months and decreased as ripening advanced. However, the proteolytic and lipolytic bacterial counts in all treatments showed gradual increases during ripening. On the other hand, the increase in the level of added heat-shocked culture did not greatly affect the bacterial numbers.

Organoleptic properties

Table 5 shows that cheese made from curd with added heat-shocked cultures of both types had better consistency and more pronounced flavour intensity than the control at each stage of ripening. The score points given for flavour and body characteristics of cheese made with each type of heat-shocked culture at levels of 1% and 2% were comparable. However, cheese containing heat-shocked culture of *L. helveticus* was superior to all treatments and acquired desirable flavour, as well as good body characteristics, 1–2 months earlier than control cheese.

DISCUSSION

In this investigation, a trial has been carried out to accelerate the ripening of Ras cheese using certain types of heat-shocked lactobacilli. The trial is based on two fundamental assumptions—first, that cheese ripening depends on enzymes from lactic starter bacteria and, secondly, that an increase in the total amount of bacterial enzymes in the final cheese will increase the reaction rate for the formation of important flavour compounds. Introduction of a heat-shocked culture of *L. casei* or *L. helveticus* influenced cheese flavour and body characteristics (especially with *L. helveticus*) whereas cheese with added heat-shocked

culture acquired strong flavour intensity and good consistency after 2–3 months of ripening. Hofi *et al.* (1970) showed that the development of desirable flavour and consistency of Ras cheese made from pasteurized milk required about 6 months. In contrast to methods for accelerating cheese flavour by increasing viable starter population, cheese made from curd with added heat-shocked starters did not develop bitterness during the entire period of ripening. This could be because heat-shocked lactobacilli allowed complete mixing of cell enzymes with the intact bacterial cells and the heat treatment used for shocking the bacterial cells (to reduce lactic acid production) could have inactivated cheese ripening enzymes other than protease, thus causing abnormal ripening (Pettersson & Sjostrom, 1975).

The intensive protein breakdown observed in cheese with added heat-shocked Lactobacilli could be due to the increased concentration of starter proteinases and peptidases. Several investigators have shown that Lactobacilli play a rôle in protein breakdown through their enzymes which are released after the death and lysis of the cells (Nath & Ledford, 1973; Sorhaug & Solberg, 1973; Somkuti *et al.*, 1981). The increased level of simple nitrogenous compounds in cheeses with added heat-shocked cultures might stimulate the growth and activity of cheese flora. Meanwhile, these simple nitrogenous compounds serve as a pool of flavour precursors. Nakae & Elliot (1965) showed that certain lactic acid bacteria were able to form volatile fatty acids from amino acids through specific metabolic pathways. Also, Stadhouders & Veringa (1973) showed that starter culture bacteria would contribute to fat hydrolysis during cheese ripening and that their lipases attack mainly mono- and diglycerides, rather than triglycerides. Free fatty acids play a major rôle in the flavour of many cheese varieties. They have been considered the backbone of Cheddar cheese flavour by Patton (1963) and generally have been acknowledged to contribute cheesiness in Cheddar flavour (Forss, 1979).

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