NOTE TECHNIQUE

Acceleration of Domiatti cheese ripening using extracts from several lactobacilli

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Résumé

*Accélération de l'affinage du fromage Domiatti par l'addition
d'extrait cellulaire de lactobacilles*

L'addition d'extrait cellulaire brut de *L. lactis*, *L. brevis* et *L. plantarum* à un caillé de Domiatti a été effectuée afin d'accélérer l'affinage de ce fromage qui représente une des ressources principales de protéines animales en Egypte. Les résultats obtenus montrent qu'il est possible d'accélérer l'affinage de ce fromage par l'incorporation d'extraits bruts obtenus après le broyage de ces bactéries.

Parmi les extraits testés, *L. plantarum* montre les indices d'affinage les plus élevés.

Les fromages, contenant les extraits des lactobacilles, ne montrent aucun défaut de texture ou de goût.

Mots clés : *L. brevis*, *L. plantarum*, *L. lactis* - Fromage Domiatti - Accélération de l'affinage.

Introduction

White pickled cheeses (which include the egyptian Domiatti) are the most popular varieties in the mediterranean area. Cheese can be consumed either fresh or after a ripening period varying from 2 to 6 months.

The only work previously accomplished to accelerate Domiatti cheese ripening showed that although the flavour intensity of a 4 week old cheese made from lipase treated milk was more pronounced than that of an 8 week

old control cheese, but, a rancid flavour rapidly developed in the cheese after 8 weeks of pickling (EL NESHAUWY *et al.*, 1982).

The present work is another attempt for accelerating flavour formation in Domiatti cheese using extracts of microorganisms normally isolated from this type of cheese in order to minimize the production costs of one of the main protein resources in Egypt without impairment of flavour balance.

I. Materials and methods

Preparation of the crude extract of the *Lactobacilli* :

L. lactis CNRZ 250, *L. plantarum* CNRZ 73 and *L. brevis* CNRZ 215 strains known for their active intracellular peptidase (EL SODA and DESMAZEAUD, 1982 ; EL SODA *et al.*, 1982 a, 1983) and esterase (EL SODA *et al.*, 1984 a, b) systems were used for this study. The method used for culturing the bacteria and preparation of the crude cell free extract, were accomplished as previously described by EL SODA *et al.* (1978).

A. Domiatti cheese manufacture

Domiatti cheese was manufactured from standardized pasteurized cows milk (3 % fat and 8.5 % solids not fat). A 1 % mixed culture of *Streptococcus thermophilus* and *S. cremoris* 15 was added to the cheese milk 1 hr. before renneting.

The rennet coagulated curd was then cutted into cubes and, after removal of half of the whey, 5 % sodium chloride was added. The curd was then divided into four equal parts A, B, C and D which were individually ladled into wooden frames. After transferring the different cheeses to the wooden frames, 6 mg protein per kg cheese of the crude cell free extract of *L. plantarum*, *L. lactis* and *L. brevis* were added to curd A, B and C respectively while curd D was considered as a control. Aliquots of the four cheeses were analysed fresh and at 15 days intervals over a period of 60 days.

B. Analysis

1. Control analysis

pH was measured by probing directly the cheese homogenate with a glass electrode. Determination of total solids was made by oven drying at 105° C. Fat was determined according to the butyrometer Gerber method, while the conventional Kjeldahl procedure was used to measure the total and soluble nitrogen content of the cheese.

2. Ripening indexes

In addition to soluble protein determination, the procedure described by RAKSHY *et al.* (1974) for the analysis of the total volatile acidity was undertaken to follow the ripening of the cheese.

3. Flavour evaluation

Flavour intensity, bitterness and rancidity were assessed using as a general guide the flavour scores and criticism proposed by KOSIKOWSKI and IWASAKI, 1975. With 0 indicating flavour defects or no flavour and 5 indicating strong flavour.

II. Results and discussion

The general composition of the cheese and the obtained yield (table 1) show that only little differences could be detected between the treatments. As far as the ripening indexes are concerned, fig. 1 A and B show higher values for protein breakdown and volatile fatty acid formation in the cheeses to which *Lactobacillus* extracts were added. From the data illustrated in figure 1 (A and B) it is also possible to notice that the highest values for soluble protein and total volatile acidity were obtained in Domiatti cheese samples to which the crude extract of *L. plantarum* CNRZ 73 was added.

The *L. plantarum* samples also showed the highest scores in flavour and texture evaluation (table 2).

In order to determine the optimum concentration of *L. plantarum* extract to be used for accelerating Domiatti cheese ripening, a similar experiment was carried out using 3, 6, 18 and 40 mg *L. plantarum* extract/kg of cheese.

The general composition of the cheese and the yield were not significantly affected by the concentration of the *L. plantarum* extract and, the values for % fat, protein, total solids and for the yield were very closed to those reported in table 1. On the other hand, the increase in enzyme concentration was followed by an increase in volatile acids formation (fig. 2 A) and protein breakdown (fig. 2 B) in the cheese as well as an increase in the scores given by the members of the taste panel.

These findings are comparable to previous work on accelerated ripening with starter enzymes. GRIPON *et al.* (1977) showed that the addition of proteolytic enzymes obtained from *S. lactis*, *Penicillium roqueforti* and *P. caseicolum*

TABLE 1 — TABLEAU 1
Technological characteristics of Domiatti cheese

A = Soft cheese + cell free extract from *L. plantarum* (concentration, 6 mg enzyme/kg cheese).

B = Soft cheese + cell free extract from *L. lactis* (concentration, 6 mg enzyme/kg cheese).

C = Soft cheese + cell free extract from *L. brevis* (concentration, 6 mg enzyme/kg cheese).

D = Control.

* The yield is defined as the number of kg cheese obtained from 100 kg of milk.

Caractéristiques technologiques du fromage Domiatti

A = Fromage frais + extrait enzymatique obtenu à partir de *L. plantarum* (concentration 6 mg/kg de fromage).

B = Fromage frais + extrait enzymatique obtenu à partir de *L. lactis* (concentration 6 mg/kg de fromage).

C = Fromage frais + extrait enzymatique obtenu à partir de *L. brevis* (concentration 6 mg/kg de fromage).

D = Témoin.

* Le rendement fromager est défini comme étant le nombre de kg de fromage obtenus à partir de 100 kg de lait.

Ripening time (days)	Total protein				Fat				pH			
	A	B	C	D	A	B	C	D	A	B	C	D
0	15.84	15.95	16.19	15.84	15.0	14.6	14.6	14.5	6.05	6.15	6.05	6.10
15	15.72	15.72	15.84	15.60	15.3	15.0	15.1	14.9	4.99	4.95	4.95	5.00
30	15.60	15.48	15.72	15.48	15.7	15.5	15.6	15.4	4.60	4.50	4.65	4.65
45	15.48	15.24	15.60	15.36	16.3	16.0	16.2	15.9	4.40	4.35	4.45	4.50
60	15.36	15.12	15.48	15.24	16.9	16.5	16.8	16.6	4.35	4.20	4.30	4.40

Ripening time (days)	Dry matter				Yield %			
	A	B	C	D	A	B	C	D
0	36.82	36.05	36.05	36.46	18.18	18.18	18.18	18.18
15	37.13	36.28	36.52	36.81	18.11	18.12	18.05	18.06
30	37.55	36.89	37.00	37.56	17.99	17.94	17.91	17.86
45	38.07	37.31	37.55	38.21	17.84	17.82	17.75	17.67
60	38.79	37.99	38.27	39.03	17.63	17.63	17.55	17.45

Fig. 1

Effect of adding crude extracts from three different *Lactobacilli* on the (A) total volatile acidity and (B) soluble proteins p. cent.

- A ——— Soft cheese + cell free extract from *L. plantarum*.
 B - - - - - Soft cheese + cell free extract from *L. lactis*.
 C - - - - - Soft cheese + cell free extract from *L. brevis*.
 D ——— Control cheese (without *Lactobacillus* extract).

Influence de l'addition au fromage Domiatti de l'extrait cellulaire obtenu à partir de trois espèces de lactobacilles sur (A) l'acidité volatile totale et (B) le pourcentage en protéines solubles.

- A ——— Fromage frais + extrait enzymatique de *L. plantarum*.
 B - - - - - Fromage frais + extrait enzymatique de *L. lactis*.
 C - - - - - Fromage frais + extrait enzymatique de *L. brevis*.
 D ——— Fromage témoin (sans addition d'extrait enzymatique).

TABLE 2 — TABLEAU 2

Organoleptic properties of Domiatti cheese

- A = Soft cheese + cell* free extract from *L. plantarum*.
 B = Soft cheese + cell free extract from *L. lactis*.
 C = Soft cheese + cell free extract from *L. brevis*.
 D = Control.

* Extract concentration was 6 mg/kg cheese.

Propriétés organoleptiques du fromage Domiatti.

- A = Fromage frais + extrait* enzymatique obtenu à partir de *L. plantarum*.
 B = Fromage frais + extrait enzymatique obtenu à partir de *L. lactis*.
 C = Fromage frais + extrait enzymatique obtenu à partir de *L. brevis*.
 D = Témoin.

* La concentration de l'extrait enzymatique était de 6 mg/kg de fromage.

Cheese age days	Intensity				Rancidity and Bitterness			
	A	B	C	D	A	B	C	D
0	3.60	3.75	3.70	3.70	—	—	—	—
15	4.50	4.25	3.75	3.75	—	—	—	—
30	4.65	4.10	3.75	3.75	—	—	—	—
45	4.60	4.05	4.15	3.55	—	—	—	—
60	4.65	4.00	4.10	3.50	—	—	—	—

Fig. 2

Effect of concentration of added crude extract from *L. plantarum* on the (A) total volatile acidity and (B) soluble proteins p. cent.

- A Concentration 3 mg enzyme/kg cheese of added crude extract from *L. plantarum*.
 B ----- Concentration 6 mg enzyme/kg cheese of added crude extract from *L. plantarum*.
 C -.-.-.-.- Concentration 18 mg enzyme/kg cheese of added crude extract from *L. plantarum*.
 D -.-.-.-.- Concentration 40 mg enzyme/kg cheese of added crude extract from *L. plantarum*.
 E ————— Control.

Influence de la concentration en extrait cellulaire obtenu à partir de *L. plantarum* et additionné au fromage Domiatti sur (A) l'acidité volatile totale et (B.) le pourcentage en protéines solubles.

- A Concentration 3 mg d'extrait enzymatique de *L. plantarum*/kg de fromage.
 B ----- Concentration 6 mg d'extrait enzymatique de *L. plantarum*/kg de fromage.
 C -.-.-.-.- Concentration 18 mg d'extrait enzymatique de *L. plantarum*/kg de fromage.
 D -.-.-.-.- Concentration 40 mg d'extrait enzymatique de *L. plantarum*/kg de fromage.
 E ————— Témoin.

in a cheese curd is a potential method to improve quality and accelerate ripening.

Similar observations were also reported by TATEMATSU *et al.* (1982) when they evaluated combinations of *Penicillium* proteases with either *S. lactis* or *L. casei* autolysates. More recently, LAW and WIGMORE (1983) demonstrated that intracellular cell free extracts of cheese starter bacteria significantly enhanced the flavour accelerating effect of Neutrase.

From the previously mentioned data, it is possible to conclude that acceleration of Domiatti cheese ripening can be accomplished using extracts from the *Lactobacilli* and more particularly *L. plantarum* without impairment of flavour balance. Soft cheese is different in that respect from Cheddar cheese

where a pronounced bitter flavour was detected in the *Lactobacillus* extract treated cheese after 2 weeks of ripening (EL SODA *et al.*, 1982 b). This might be due to the presence of a high concentration of salt in the cheese and brine which probably regulated some enzymatic activities in the cheese and delayed bitter flavour formation.

These speculations are however confirmed by the work of previous authors on enzyme and starter inhibition in the presence of sodium chloride. FAHMI and SHARARA (1950) noticed an increase in the coagulation time of salted milk by rennet.

More recently, DE JONG and DE GROOT-MOSTERT (1977) showed that rennet action on β casein was totally inhibited in the presence of 11 % sodium chloride. Elastolytic activity was also found to be appreciably affected by sodium chloride (HARTLEY and SHOTTON, 1971). Work on the influence of sodium chloride on the metabolic activities of microorganisms also showed that the rate of acid production by starter bacteria is significantly low in the presence of high concentration of sodium chloride (IRVINE and PRICE, 1961 and RASIC, 1965).

Bitter flavour might also have been relatively masked by the salty flavour of the cheese.

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References

- EL NESHAUWY A., ABDEL BAKY A., FARAHAT S., 1982. Enhancement of Domiatti cheese flavour with animal lipase preparation. *Food Chem.*, 14, 201-243.
- EL SODA M., BERGÈRE J.L., DESMAZEAUD M.J., 1978. Detection and localization of peptide hydrolases in *Lactobacillus casei*. *J. Dairy Res.*, 45, 519-524.
- EL SODA M., DESMAZEAUD M.J., 1982. Les peptide-hydrolases de lactobacilles du groupe *thermobacterium*. I. Mise en évidence de ces activités chez *Lactobacillus helveticus*, *L. lactis* et *L. bulgaricus*. *Can. J. Microbiol.*, 28, 1181-1188.
- EL SODA M., ZEYADA N., DESMAZEAUD M.J., MASHALY R., ISMAIL A., 1982 a. Les peptide-hydrolases des lactobacilles du groupe *betabacterium*. Mise en évidence chez *Lactobacillus brevis*, *L. fermentum*, *L. buchneri* et *L. cellobiosus*. *Sci. Aliments*, 2, 261-273.
- EL SODA M., DESMAZEAUD M.J., ABOU DONIA S., BADRAN A., 1982 b. Acceleration of cheese ripening by the addition of extracts from *L. helveticus*, *L. bulgaricus* and *L. lactis* to the cheese curd. *Milchwissenschaft*, 37, 325-327.
- EL SODA M., SAID H., DESMAZEAUD M.J., MASHALY R., ISMAIL A., 1983. The intracellular peptide-hydrolases of *Lactobacillus plantarum* comparison with *Lactobacillus casei*. *Lait*, 63, 1-14.
- EL SODA M., ABDEL WAHAB H., EZZAT N., ISMAIL A., 1984 a. The esterolytic and lipolytic activities of *Lactobacilli* from the *thermobacterium* group. *J. Dairy Sci.*, 66, Supplement 1, 68.

- EL SODA M., FATHALLAH S., EZZAT N., ABOU DONIA S., 1984 b. The esterolytic and lipolytic activities of *Lactobacilli* from the *streptobacterium* group. *Proc. 2nd Egyptian Conf. for Dairy Sci. and Technol.*, 7.
- FAHMI A.H., SHARARA H.A., 1950. Studies on Egyptian Domiatti cheese. *J. Dairy Res.*, 17, 312-328.
- GRIPON J.C., DESMAZEAUD M.J., LE BARS D., BERGÈRE J.C., 1977. Role of proteolytic enzymes of *Streptococcus lactis*, *Penicillium roqueforti* and *Penicillium caseicolum* during cheese ripening. *J. Dairy Sci.*, 60, 1532-1538.
- HARTLEY B.S., SHOTTON D.M., 1971. *Pancreatic elastase C.F. The enzymes*. Vol. III, Academic Press.
- IRVINE D.M., PRICE W.V., 1961. Influence of salt on the development of acid by lactic starter in skim milk and in curd submerged in brine. *J. Dairy Sci.*, 44, 243-248.
- DE JONG L., DE GROOT-MOSTERT A.E., 1977. The proteolytic action of rennet on different casein substrates under various conditions. *Neth. Milk Dairy J.*, 31, 296-313.
- KOSIKOWSKI F., IWASAKI T. 1975. Changes in Cheddar cheese by commercial enzyme preparation. *J. Dairy Sci.*, 58, 963-970.
- LAW B., WIGMORE A., 1983. Accelerated ripening of Cheddar cheese with a commercial proteinase and intracellular enzymes from starter *Streptococci*. *J. Dairy Res.*, 50, 519-525.
- RAKSHY S.E., EL SODA M., TAHOON M., 1974. Detection of straight chain (C₂-C₈) carboxylic acids in cheese by thin layer chromatography. *Ind. J. Dairy Sci.*, 27, 74-76.
- RASIC J., 1965. L'effet de l'augmentation graduelle des concentrations de chlorure de sodium sur la résistance des bactéries lactiques. *Lait*, 441-442, 27-34.
- TATEMATSU T., YAMAMOTO H., KAWASHIMA T., KUBOYAMA M., 1982. Acceleration of cheese ripening with mold protease and the cell concentrate of lactic acid bacteria. 21st Int. Dairy Congress, Moscow, vol. 1, Book 1, 453-454.