LIPOLYTIC ACTIVITY OF BROCHOTHRIX THERMOSPHACTA AND LACTOBACILLUS CURVATUS

M. Papon and R. Talon, INRA - Station de Recherches sur la Viande Theix 63122 Ceyrat, FRANCE.

SUMMARY

Intracellular lipases of *B.thermosphacta* and *L.curvatus* hydrolysed tributyrin with an optimal temperature of 37°C and were still active at low temperatures. Among the triglycerides tested, they degraded preferentially triglycerides with short chain fatty acids. Natural fats of pork, beef and lamb were weakly hydrolysed at 87°C.

INTRODUCTION

Lactobacilli and *Brochothrix thermosphacta* have frequently been reported as dominant microflora on meat and meat products and may spoil them by producing souring or a pungent cheesy flavour (Reuter 1975; Gardner 1981; Egan 1983). Off-flavour development and definite spoilage of meat can be attributed to microbial lipases. Previous studies have shown that some strains of meat lactobacilli and *B.thermosphacta* are able to hydrolyse tributyrin (Collins-Thompson et al. 1971; Reuter 1975). Lipases of *B.thermosphacta* and *Lactobacillus curvatus* were intracellular and were synthesised during the logarithmic phase of growth (Papon and Talon 1988). For these two strains, lipases have been partially characterized, they have an optimum activity at pH around 7.0. ; they are stable between 4°C and 80°C and at freezing temperatures (-20°C, -60°C). To date despite the possible participation of these enzymes in the flavour changes in meat, little information is available on their ability to hydrolyse natural fats. In this study, intracellular lipases of *B.thermosphacta* and *L.curvatus* were assayed at various temperatures, against synthetic triglycerides with different fatty acids and natural fats.

MATERIALS AND METHODS

Organisms and growth conditions

The bacteria used in this study were *Lactobacillus curvatus* A'8 R126 supplied by Dr Reuter, and *Brochothrix thermosphacta* ATCC 11509. Cultures were grown in APT broth (Merck) at 24°C for *B.thermosphacta* and 30°C for *L.curvatus*. Cells in logarithmic phase were harvested by centrifugation at 6000 g for 10 min at 4°C.

Cell-free extract preparation

Cells resuspended in 20 mM/l Tris HCl buffer pH 7.0 (0.25 g of cells wet weight/ml} were ultrasonically disrupted at 0°C under a nitrogen atmosphere with a Banson Sonifier at full power, for 8 to 10, 30 seconds periods with 1 minute cooling intervals in ice. Unbroken cells and cells debris were removed in the pellet by centrifugation at 30,000 g for 30 min at 1°C. The supernatant was now designated cell-free extract and will be used as enzymatic source.

Lipase activity assay

Lipolytic activity was assayed according to the Castberg et al. (1976) method, modified as follows:

- Synthetic triglycerides emulsions were realised as described in the original paper (Castberg et al. 1976).

- Natural lipids (lamb, beef, pork) were melted by heating at 110°C for ten minutes and were added at a concentration of 5 % (w/v) to an aqueous gum arabic solution. Ultrasonication was then performed as described in the original paper.

- Reaction mixture contained 1 ml of triglyceride emulsion, 0.5 ml of NaCl 1 M, 0.5 ml of 1 M Tris HCl buffer pH 7.0 containing 0.08 M $CaCl_2$, 2 ml of distilled water and finally 1 ml of cell-free extract.

Table 1 - Substrate specificity of *B.thermosphacta* and *L.curvatus* lipases

Substrate	*B. thermosphacta*		*L. curvatus*	
	Lipase activity (µeq/h/mg protein)	Relative lipase activity (%)	Lipase activity (µeq/h/mg protein)	Relative lipase activity (%)
Tributyrin	22.0	100.0	10.5	100.0
Tricaproin	7.0	32.0	ND	ND
Tricaprylin	2.1	9.5	3.2	30.0
Tricaprin	2.5	11.4	1.1	10.5
Trilaurin	1.7	7.7	1.5	14.0
Tripalmitin	1.2	5.4	1.2	11.4
Triolein	1.0	4.5	1.2	11.4

ND : not done

Table 2 - Lipolysis of natural fats at 37°C by *B.thermosphacta* and *L.curvatus*

Lipolytic activity (µeqC4/18h/mg protein)	Pork fat	Lamb fat	Beef fat
B. thermosphacta	1.40	1.24	1.65
L. curvatus	0.51	0.38	0.44

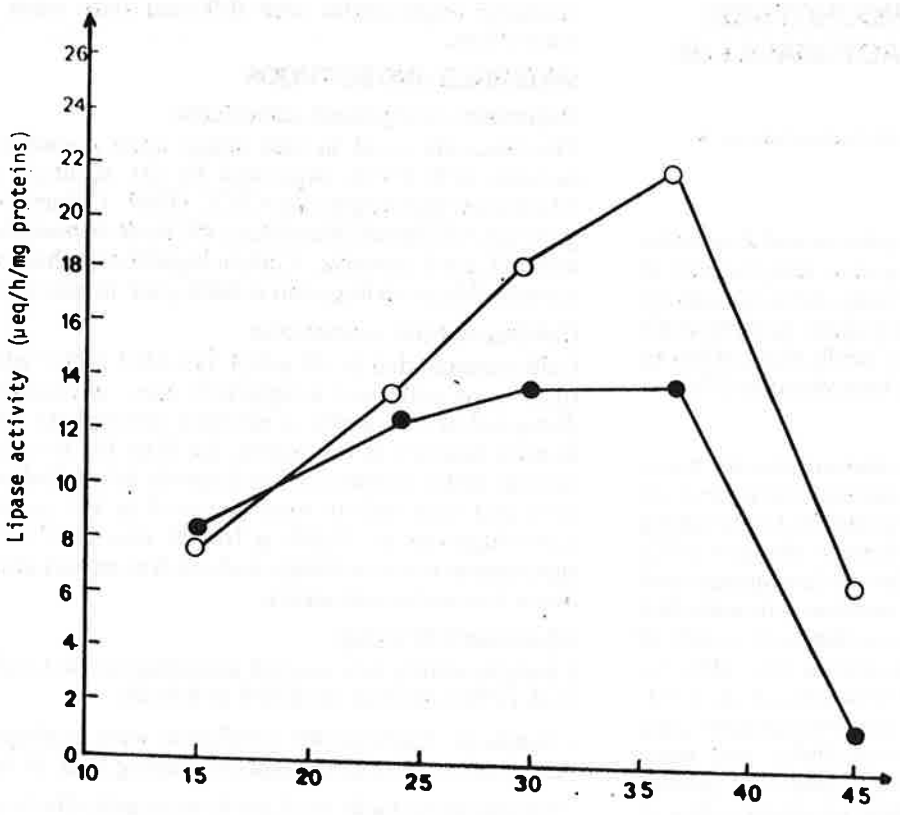

Figure 1 - EFFECT OF TEMPERATURE ON TRIBUTYRIN HYDROLYSIS

○ *B.thermosphacta* ; ● *L.curvatus*

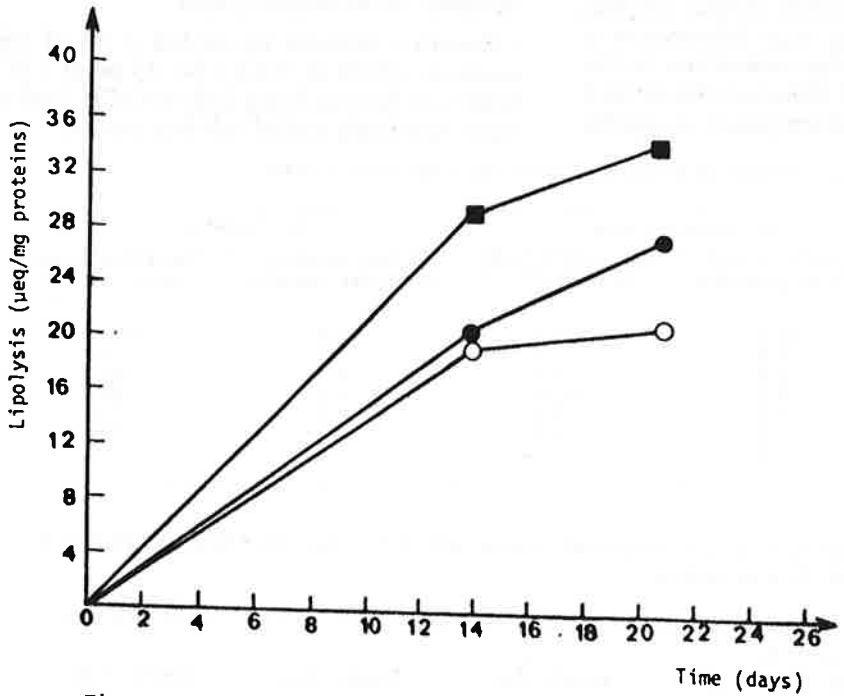

Figure 2 - LIPOLYSIS OF TRIBUTYRIN by *B.thermosphacta*

○ +1°C ; ● +4°C ; ■ +8°C

- Incubations were realised at various temperatures, extraction and titration were then realised according to the original paper.

- Blank containing boiled cell-free extract and substrate without enzyme were similarly titrated.

- Lipase activity was given in microequivalent of fatty acid liberated per hour and per mg of proteins (µeq/h/mg proteins).

Protein concentrations were determined by the method of Lowry et al. (1951) with the use of Bovine Serum Albumin as a standard.

Lipase activity at different temperatures

To determine the optimal temperature for lipase activity of *B.thermosphacta* and *L.curvatus*, incubations were realised at pH 7.0 and at 15, 24, 30, 37, 45°C.

To measure lipase activity at low temperatures, incubations were realised at pH 7.0 at 1, 4, 8°C for several days. In these experiments 0.01% formaldehyde was added to the reaction mixture to prevent microbial growth and 2.5 ‰ agar-agar was added instead of arabic gum to stabilize emulsion.

Hydrolysis of different substrates

Ability of the lipase to hydrolyse synthetic and natural triglycerides was investigated at 87°C. Synthetic triglycerides tested were: tributyrin, tricaproin, trilaurin, tripalmitin and triolein whereas natural fats were pork, lamb and beef. All the lipids were in 1% (w/v) final concentration in the reaction mixture.

RESULTS

Lipase activity at different temperatures

For the two strains studied, hydrolysis of tributyrin was maximal at 37°C (fig.1). At temperatures superior to 37°C, lipase activity decreased quickly. At 45°C lipase of *L.curvatus* was completely inhibited and that of *B.thermosphacta* kept only 30% of it's activity. Below 37°C, lipase of *L.curvatus* was slightly influenced: at 15°C, 65% of the maximum activity was still observed. For *B.thermosphacta*, only 35% of the activity was detected at 15°C.

However, the two lipases were still active at low temperatures (+1, +4, +8°C). For *B.thermosphacta*,

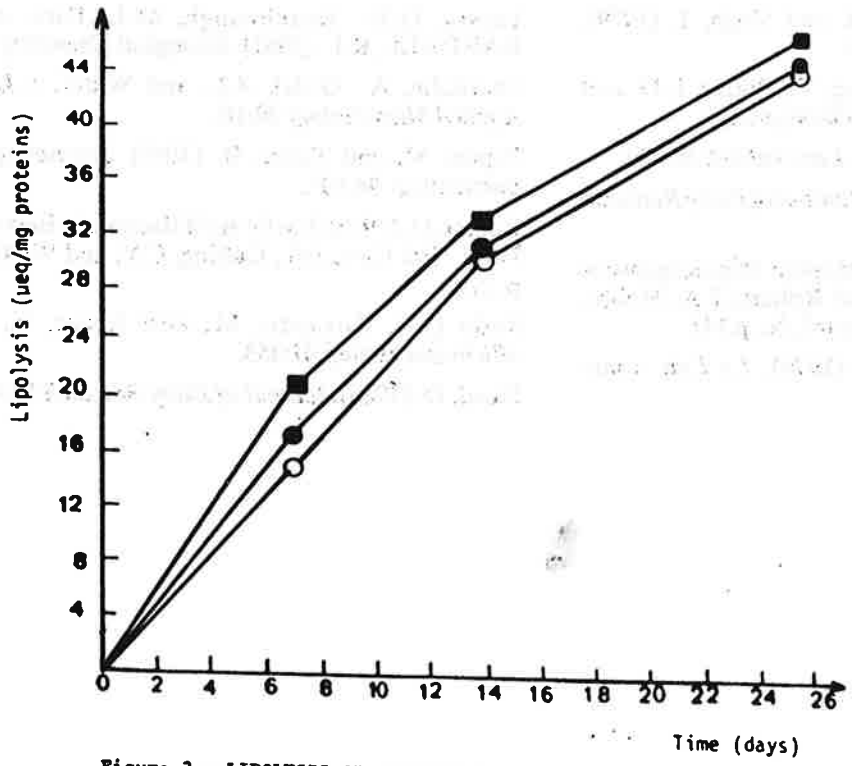

Figure 3 - LIPOLYSIS OF TRIBUTYRIN BY *L.curvatus*
○+1°C ; ●+4°C ; ■+8°C

after an initially rapid lipolysis, the reactions retarded at different levels depending on storage temperature (fig.2). At +1°C, lipolysis remained constant after 14 days whereas it continued at +4°C and +8°C. For *L.curvatus*, lipolysis was similar for the three temperatures and it increased during all the storage time (fig.3).

Hydrolysis of synthetic triglycerides

For the two strains, tributyrin was preferentially hydrolysed as compared to the other triglycerides (table 1). The lipase activity of *B.thermosphacta* decreased quickly as the length of the fatty acid increased. The relative activities were respectively 32% for tricaproin (C6), 10% for tricaprylin (C8) and only 4.5% for triolein (C18:1). For *L.curvatus*, tricaprylin (C8) and trilaurin (C14) were hydrolysed with relative activities of 30% and 14% while the others were identically hydrolysed with a relative activity around 10% (table 1).

Hydrolysis of natural fats

After three hours incubation at 37°C, hydrolysis of natural fats was negligible while in the same time tributyrin was greatly hydrolysed. After 18 hours at 37°C, natural fats were weakly hydrolysed by the lipases of the two bacteria (table 2}. *B.thermosphacta* was more lipolytic than *L.curvatus* and degraded preferentially beef fat, relative activities were respectively 100, 85 and 75% for beef, pork and lamb. Lipase of *L.curvatus* hydrolysed preferentially pork fat, with relative activities of 100, 86, 74% respectively for pork, beef, lamb.

DISCUSSION

The majority of bacterial lipases are most active within a temperature range of 30°C to 40°C (Fox and Stepaniak 1982; Stead 1986), like the lipase of *L.curvatus* and *B.thermosphacta* that have an optimal temperature of 37°C. Above 37°C, the loss of activity was due to enzyme denaturation by heating (Papon and Talon, unpublished data). These results are comparable to those reported for *Propionibacterium shermanii* and *Streptococcus faecalis* which lipases are also inactivated by heating (Oterholm et al. 1970; Chander et al. 1979). Below 37°C, the lipases of *L.curvatus* and *B.thermosphacta* were stable (unpublished data), so the decline in activity may be due to a decreasing affinity of the enzymes for their substrates. It is noteworthy that these enzymes are still active at temperature used for cold storage of foods, as observed for *Pseudomonas fluorescens* (Anderson 1980).

Among synthetic triglycerides, *B.thermosphacta* and *L.curvatus* lipases hydrolysed preferentially tributyrin, and their activity decreased as the length of the fatty acid increased. Similar results were found for some *Lactobacillus* species (El Soda et al. 1986), *Streptococcus faecalis* (Chander et al. 1979), *Penicillium caseicolum* (Lamberet and Lenoir 1976), *Pseudomonas fluorescens* (Fox and Stepaniak 1983). It is not surprising that lipases of *B.thermosphacta* and *L.curvatus* hydrolysed weakly natural fats. Indeed they hydrolysed preferentially triglycerides with short chain fatty acids when natural fats are principally composed with long chain fatty acids (C16 and C18). Other bacterial lipases are able to hydrolyse natural fat at 87°C (Alford et al. 1984; Cantoni et al. 1967), but these activities are not comparable with those obtained for *B.thermosphacta* and *L.curvatus* because many experimental factors including incubation time, substrate concentration, enzyme concentration and procedure to assay lipase vary from study to study.

In conclusion, lipases of *B.thermosphacta* and *L.curvatus* were active at low temperatures, hydrolysed preferentially triglycerides with short chain fatty acids. Their role in the spoilage of fatty foods would be limited because of their substrate specificity.

ACKNOWLEDGMENTS

We are grateful to G. Reuter for supplying *Lactobacillus curvatus* strain.

REFERENCES

Alford, J.A., Pierce, D.A. and Suggs, F.G. (1984). *Journal of Lipid Research* **5**:390.

Anderson, R.E. (1980). *Applied and Environmental Microbiology* **39**:36.

Cantoni, C., Molnar, M.R., Renon, P. and Gioletti, G. (1967). *Journal of Applied Bacteriology* **30**:190.

Castberg, H.B., Solberg, P. and Egelrud, T. (1975). *Journal of Dairy Research* **42**:247.

Chander, H., Rangamathan, B. and Singh, J. (1979). *Journal of Food Sciences* **44**:1747.

Collins-thompson, D.L., Sorhaug, T., Witter, L.D. and Ordal, Z.J. (1971). *Applied Microbiology* **21**:9.

Egan, A.F. (1983). *Antonie Von Leuwenhoek* **49**:327.

Fox, P.F., Stepanlak, L. (1983). *Journal of Dairy Research* **50**:77.

Gardner, G.A. (1981). Psychrotrophic Microorganisms in Spoilage and Pathogenicity. Ed. Roberts,T.A., Hobbs, G., Christian, J.H.B. and Skovgaard, N., p.141.

Lamberet, G. and Lenoir, J. (1976). *Le Lait*, tome LV1:119.

Lowry, O.H., Rosebrough, M.J., Farr, A.L. and RANDALL, R.J. (1961). *Biological Chemistry* **193**:286.

Oterholm, A., Ordal, Z.J., and Witter, L.D. (1970). *Applied Microbiology* **20**:18.

Papon, M. and Talon, R. (1988). *Journal of Applied Bacteriology* **84**:107.

Reuter, G. (1976). Lactic Acid Bacteria in Beverages and Food. Ed. Carr, J.G., Cutting, C.V. and Whiting, G.C., p. 221.

Soda (el), Korayen, M. and Ezzat, N. (1986). *Milchwissenschaft* **41**:353.

Stead, D. (1986). *Journal of Dairy Research* **53**:481.