ACCELERATION OF CHEESE RIPENING WITH LIPOSOME-ENTRAPPED PROTEINASE

J-C. Piard1, M. El Soda2, W. Alkhalaf3, M. Rousseau1, M. Desmazeaud1, L. Vassal3 and J-C. Grippon3

1. Laboratoire de Microbiologie Laitière, INRA, CNRZ
78350 Jouy-en-Josas - France
2. Department of Agricultural Industries, Faculty of Agriculture, Alexandria University, Egypt.
3. Laboratoire de Biochimie et Technologie Laitières, INRA, CNRZ
78350 Jouy-en-Josas - France.

SUMMARY

Rulactine, a proteinase used for the acceleration of cheese ripening, was entrapped in three types of liposomes and these were added to Saint-Paulin cheese type manufacturing milk. Enzyme entrapment rates ranged from 3 to 9% according to the type of liposomes and liposome retention rates in cheese curd from 35 to 65%. An electrophoretic study of protein breakdown in the cheeses gave correlative data.

INTRODUCTION

The economic advantage of rapid development of more intense cheese flavour in shorter period of time would be substantial. Several methods were proposed for accelerating the ripening of cheese: ripening at elevated temperature, addition of free enzymes to the cheese milk or curd, increasing of the microbial population and, use of slurry system. More recently, some workers looked to the application of new scientific findings or to the adaptation of technologies used in other fields i.e. the medical field to cheese ripening.

The adaptation of liposome technology for accelerating cheese ripening was therefore considered for the following reasons:
- the addition of free proteinase in manufacturing cheese milk shows low enzyme retention rate in the cheese curd. That value could be increased by use of liposome-entrapped proteinase.
- phospholipid vesicles can protect potential substrate in the milk during the cheesemaking process and could therefore avoid losses in yield which result from the direct addition of exogenous proteinase to cheese milk (Law and King, 1985).
- the liposomes, offering sizes close to those of bacterial cells are expected to distribute in the curd in a similar way.
- the gradual delivery of proteinase in the cheese would avoid bitterness noticed by Alkhalaf (unpublished results) when free enzyme was used.

In the present communication, we describe the encapsulation efficiency of Rulactine, a metalloproteinasme from Micrococcus caseolyticus (Desmazeaud and Hermier, 1968 a) in three types of liposomes: multilamellar vesicles (MLV), small unilamellar vesicles (SUV) and reverse phase evaporation vesicles (REV). Liposome retention in a model cheese and the rate of protein degradation by the liposome-entrapped enzyme were considered.

**MATERIALS AND METHODS**

**Preparation of C14 labelled Rulactine.** Rulactine was obtained from Roussel Uclaf – France. The enzyme was labelled using (14C) formaldehyde according to the method of Donnelly et al. (1980).

**Preparation of liposomes.** 8.5 ml of 125 mg mixture of labelled and unlabelled Rulactine preparation representing $27.10^6$ DPM and $30.10^3$ units of protease activity were entrapped in three types of liposomes. The lipids used in a molar ratio of 5:5:1 were: 150 mg lecithin (Merck) purified according to Singleton et al. (1965), 82.5 mg cholesterol and 22.5 mg dicetyl phosphate.

Multilamellar vesicles (MLV) were made according to Gregoriadis (1976). Small unilamellar vesicles were obtained after sonication of an MLV preparation for 6x30 sec while the method described by Whilschut (1982) was adopted for the preparation of the reverse phase evaporation vesicles (REV).

Separation of the liposomes from the unentrapped enzyme was realized by centrifugation at 15 000 g for 30 min for MLV and 100 000 g for 1 h for SUV and REV. The liposome pellet was washed 4 times with Tris HCl buffer 0.05 M, pH 7.5 in order to obtain less than 400 DPM in 100 μl of the supernatant fluid.

**Encapsulation efficiency of the liposomes.** The rate of enzyme entrapment in the three types of liposomes was determined by subjecting the liposome preparations to radioactivity determination with a liquid scintillation counter and to proteolytic activity measurement according to Desmazeaud and Hermier (1968 b). Prior to that last assay, liposome membranes were disrupted with Triton X-100 (2 % v/v) (Weissman et al., 1966).

**Determination of the liposomes size.** A liposome size distribution estimation was performed by thin-section of liposomes included in epon. About 50 vesicle diameters were determined on micrographs at the following magnifications: x 60,000 for MLV, x 80,000 for SUV and x 160,000 for REV.