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Influence of Starters on Chemical, Biochemical, and Sensory Changes in Turkish White-Brined Cheese During Ripening

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ABSTRACT

Turkish White-brined cheese was manufactured using *Lactococcus* strains (*Lactococcus lactis* ssp. *lactis* NCDO763 plus *L. lactis* ssp. *cremoris* SK11 and *L. lactis* ssp. *lactis* UC317 plus *L. lactis* ssp. *cremoris* HP) or without a starter culture, and ripened for 90 d. It was found that the use of starters significantly influenced the physical, chemical, biochemical, and sensory properties of the cheeses. Chemical composition, pH, and sensory properties of cheeses made with starter were not affected by the different starter bacteria. The levels of soluble nitrogen fractions and urea-PAGE of the pH 4.6-insoluble fractions were found to be significantly different at various stages of ripening. Urea-PAGE patterns of the pH 4.6-insoluble fractions of the cheeses showed that considerable degradation of α_{s1}-casein occurred and that β-casein was more resistant to hydrolysis. The use of a starter culture significantly influenced the levels of 12% trichloroacetic acid-soluble nitrogen, 5% phosphotungstic acid-soluble nitrogen, free amino acids, total free fatty acids, and the peptide profiles (reverse phase-HPLC) of 70% (vol/vol) ethanol-soluble and insoluble fractions of the pH 4.6-soluble fraction of the cheeses. The levels of peptides in the cheeses increased during the ripening period. Principal component and hierarchical cluster analyses of electrophoretic and chromatographic results indicated that the cheeses were significantly different in terms of their peptide profiles and they were grouped based on the use and type of starter and stage of ripening. Levels of free amino acid in the cheeses differed; Leu, Glu, Phe, Lys, and Val were the most abundant amino acids. Nitrogen fractions, total free amino acids, total free fatty acids, and the levels of peptides resolved by reverse phase-HPLC increased during ripening. No significant differences were found between the sensory properties of cheeses made using a starter, but the cheese made without starter received lower scores than the cheeses made using a starter. It was found that the cheese made with strains NCDO763 plus SK11 had the best quality during ripening. It was concluded that the use of different starter bacteria caused significant differences in the quality of the cheese, and that each starter culture contributed to proteolysis to a different degree.
(**Key words:** White cheese, starter, *Lactococcus,* ripening)

Abbreviation key: EtOH-i = ethanol-insoluble, **EtOH-s** = ethanol-soluble, **FAA** = free amino acids, **HCA** = hierarchical cluster analysis, **PCA** = principal component analysis, **PTA-SN** = 5% phosphotungstic acid-soluble nitrogen, **RP-HPLC** = reverse phase-HPLC, **TCA-SN** = 12% TCA-soluble nitrogen, **WSN** = water-soluble nitrogen.

INTRODUCTION

Cheese ripening is a complex and dynamic biochemical process that includes protein breakdown, fat hydrolysis, and lactose metabolism (El Soda et al., 1995; McSweeney and Sousa, 2000). Proteolysis is catalyzed by proteolytic enzymes from the coagulant, milk, and bacteria (starter, nonstarter, or secondary starter; Fox, 1989). The coagulant is mainly responsible for hydrolyzing the caseins to large and intermediate-size peptides (Law et al., 1992; Lane and Fox, 1997). The enzymes originating from starter (i.e., proteinases, peptidases) play a major role in formation of small peptides and the amino acids, which serve as precursors of flavor compounds in cheese (Urbach, 1997; Broome and Limsowtin, 1998).

The primary role of starter bacteria is to produce lactic acid at a controlled rate; in addition, the bacteria affect the nonstarter microflora. Starter cultures are not used for many cheeses made in Turkey including Turkish White-brined cheese. In artisanal cheese production, the cheese is made without the deliberate addition of a starter culture; the indigenous flora of the milk contribute to ripening. Recently, the use of mixed-strain, mesophilic starter cultures, containing un-

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known genera of lactic acid bacteria commenced in the manufacture of Turkish White-brined cheese. The quality of cheese manufactured with mixed starter cultures varies widely in terms of texture and flavor.

Cheeses made using a starter containing one or more defined *Lactococcus* strains usually have a uniform texture due to the known biochemical activities of their microflora during cheese manufacture and ripening (Powell et al., 2002). The organisms in mixed-strain starters used in the manufacture of the cheeses belong mainly to the genera *Lactococcus* or *Lactobacillus* (Hayaloglu et al., 2002). *Lactococcus* species, of which *L. lactis* ssp. *lactis* or *L. lactis* ssp. *cremoris* are the best known, are used in cheese manufacture – these species were also found to be the predominant flora in Turkish White-brined cheese by Karakus et al. (1992).

The objective of this study was to evaluate the influence of defined starter cultures on the gross composition, proteolytic profile, and other ripening characteristics of Turkish White-brined cheese during ripening. In addition, we attempted to standardize the manufacture and quality of the cheese using a defined-strain starter.

MATERIALS AND METHODS

Lactococcal Strains

Lactococcus lactis ssp. *lactis* UC317, *L. lactis* ssp. *lactis* NCDO763, *L. lactis* ssp. *cremoris* HP, and *L. lactis* ssp. *cremoris* SK11 were obtained from the culture collections of University College, Cork, Ireland; Fonterra Research Centre, Palmerston North, New Zealand; and Groningen Biomolecular Science and Biotechnology Institute, Department of Genetics, University of Groningen, Haren, The Netherlands, respectively. Before use, each strain was cultivated in M17 broth (Merck, Darmstadt, Germany) at 30°C for 24 h with 2 consecutive transfers (1%, vol/vol, inoculum). For starter propagation, the cultures were grown in reconstituted skim milk (10%, wt/vol, heated at 90°C for 30 min) as described by Fenelon et al. (2000).

Cheese Making

Turkish White-brined cheese was made in triplicate. In each trial, pasteurized (68°C for 10 min) milk was cooled to 32°C, divided into 3 equal parts and inoculated with a lactococcal culture at a level of 1% (wt/vol) as follows: 1) *L. lactis* ssp. *lactis* UC317 plus *L. lactis* ssp. *cremoris* HP (**317HP**); 2) *L. lactis* ssp. *lactis* NCDO763 plus *L. lactis* ssp. *cremoris* SK11 (**763SK11**); and 3) Starter-free (**SF**) cheese. Calcium chloride was added to the milk at a level of 0.2 g/L. During all stages of cheese making, precautions were taken to avoid cross-contamination. The inoculated milk (32°C) was held for about 30 min (until pH 6.30), and liquid calf rennet (Chr. Hansen, Copenhagen, Denmark) was added at a level of 1 g per 10 L of cheese milk (sufficient to coagulate the milk in 90 min). Following coagulation, the coagulum was cut into cubes (2 to 3 cm sides) and allowed to rest for 10 min. The curds were carefully transferred from the cheese vat into the molds. After 1 h of draining (without pressing), pressure was applied at room temperature (21°C) for 3 h or until whey drainage had stopped. Then, the weights were removed and the block of cheese cut into cubes of about $7 \times 7 \times 7$ cm with a knife; the pieces, weighing 350 to 400 g each, were placed in brine (14% NaCl) for about 12 h at 21°C. After salting, the cheese blocks were packed in cans ($16 \times 8 \times 8.6$ cm) and covered with 14% NaCl brine. The cans, which contained about 1 kg of cheese, were closed hermetically and the cheese samples ripened at 6 to 8°C for 90 d.

Analysis of Cheese Samples

Gross composition. Cheeses were analyzed at 1 d for moisture by the oven drying method at 102°C (IDF, 1982), salt by titration with $AgNO_3$ (Bradley et al., 1993), fat by the Van Gulik method (Ardo and Polychroniadou, 1999), and total protein by the Kjeldahl method (IDF, 1993). The pH of cheeses was measured in a slurry prepared by macerating 10 g of grated cheese in 10 mL of deionized water. Titratable acidity was determined as grams per 100 grams of lactic acid using the method described in AOAC (1995).

Nitrogen fractions. Water-soluble nitrogen (**WSN**), 12% TCA-soluble nitrogen (**TCA-SN**), and 5% phosphotungstic acid-soluble nitrogen (**PTA-SN**) fractions were prepared by the methods of Kuchroo and Fox (1982), Polychroniadou et al. (1999), and Jarrett et al. (1982), respectively. The nitrogen content of the fractions was determined by the Kjeldahl method (IDF, 1993) and expressed as a percentage of total cheese nitrogen. All determinations were made in duplicate.

Total free amino acid content. The total concentration of free amino acids (**FAA**) in the cheeses was determined in triplicate by the method of Folkertsma and Fox (1992). Results were expressed as milligrams of Leu/gram of cheese. The standard Leu solutions were analyzed in triplicate and a standard curve was prepared.

Total FFA. The total concentrations of FFA in the cheeses was determined by titrating the acidity in the cheese fat with 0.05 *N* ethanolic KOH using the method of described by Nunez et al. (1986); results were expressed as the percentage of oleic acid in cheese fat (IDF, 1989).