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Suitability of a new mixed-strain starter for manufacturing uncooked raw ewe's milk cheeses

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ABSTRACT

Most raw milk Ossau-Iraty cheeses are currently manufactured on-farm using the same commercial streptococcal-lactococcal starter (S1). One way to enhance the microbial diversity that gives raw milk its advantages for cheese-making is to formulate new starters combining diverse, characterized strains. A new starter (OI) combining 6 raw milk strains of lactococci, recently isolated and characterized, was tested in parallel with the current starter by making 12 Ossau-Iraty raw milk cheeses at 3 farmhouses under the conditions prevailing at each farm. Compliance of the sensory characteristics with those expected by the Ossau-Iraty professionals, physicochemical parameters and coliforms were quantified at key manufacturing steps. The new starter OI gave cheeses having proper compliance but having lower compliance than the S1 cheeses under most manufacturing conditions, while managing coliform levels equally well as starter S1. This lower compliance relied more on the absence of *Streptococcus thermophilus* in starter OI, than on the nature of the lactoccocal strains present in starter OI. The study also shows that variations in 5 technological parameters during the first day of manufacture, within the range of values applied in the 3 farmhouses, are powerful tools for diversifying the scores for the sensory characteristics investigated.

1. Introduction

Since a small number of companies produce just a few LAB starters for the food industry, the same starters are currently used for manufacturing cheeses of different varieties and also of the same variety. This is the case for PDO Ossau-Iraty cheese, a variety of ewe's milk cheese manufactured in South western France (Feutry et al., 2012a), and especially for Ossau-Iraty raw milk cheeses manufactured in farmhouses or small dairies. The microbial diversity that gives raw milk its advantages for cheese-making, which depends on both the native milk microbiota and traditional practices, including inoculation practices, is therefore reduced (Montel et al., 2014) among Ossau-Iraty cheeses. One way to overcome this decrease in microbial diversity without using undefined starters that may lead to acidification defects is to formulate new starters combining diverse, characterized strains.

Defined starters combining *Lactococcus lactis* strains (*LC* strains) have been developed for various varieties of ewe's milk cheeses (Casalta et al., 2005; Centeno et al., 2002; Gomez et al., 1999; Poveda et al., 2014). Their suitability for cheese manufacture has been estimated on pasteurized ewe's milk manufactured in 'controlled conditions' (Gomez et al., 1999) rather than on raw ewe's milk manufactured in 'uncontrolled conditions' (Casalta et al., 2005). In addition, the variability among the conditions currently used for manufacturing raw milk cheeses at small scales has not been taken into account. *Streptococcus thermophilus* (*ST*) was detected in different varieties of ewe's cooked cheeses belonging to the Pecorino family (for example Mannu et al., 2002; Randazzo et al., 2008), and was absent from the starters designed for cheese varieties similarly processed as Ossau Iraty, namely without

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curd heating to high temperature.

As for many other cheese varieties, the *LC* population plays an essential role for Ossau-Iraty cheeses since it grows to high number (9 $\log_{10}$ CFU g^{-1}) during the first day of manufacture (Feutry et al., 2012a). Over the past two decades there has been an increasing interest in screening lactococci from natural dairy products in order to isolate strains with improved or novel properties for their potential application in the dairy industry, but also for manufacturing traditional products. These studies have highlighted the wide diversity that exists among wild strains, which is greater than among commercial or reference strains (Sánchez et al., 2000). Wild *LC* strains (43) were isolated from 20 raw ewes' milks sampled in 20 farms of the Ossau-Iraty cheese area (Feutry et al., 2012b). They have been characterized genetically and technologically. They show a wide diversity of DNA fingerprints and interesting technological potentials.

The aim of this study was to evaluate the suitability of a new mixed-strain starter for farmhouse manufacture of Ossau-Iraty raw milk cheese. The new starter was composed of 6 *LC* strains selected among the above-mentioned 43 strains. Twelve Ossau-Iraty cheeses were manufactured at 3 farmhouses, under the usual manufacturing conditions prevailing there, to assess whether the new starter ensures correct acidification, sustains safety and achieves the desirable sensory characteristics in the mature cheeses, at least as well as the commercial starter currently used.

2. Material and methods

2.1. Starters

2.1.1. Starter OI

Six *LC* strains previously isolated from 6 ewe's milk samples produced in 5 farms of the PDO Ossau-Iraty cheese area (Feutry et al., 2012b) were combined to make mixed-strain starter OI. Strains were chosen on the basis of i) their high acidifying activity ($\triangle$ pH 0–24 h $\geq$ 1.9; additional Table A), ii) their ability to grow under a thermal gradient cycle usually used in the manufacture of the Ossau-Iraty cheese, iii) their ability to grow in combinations of 2 strains, iv) their phage-resistance and e/their non-lysogeny (Feutry et al., 2012b). All strains are Prt+ since they contain the gene coding for cell envelope proteinase (Feutry et al., 2012b).

The starter was composed of 4 strains of genotype *Lc. lactis* subsp. *lactis* (L7, L14O, L16, L24) and 2 strains of genotype *Lc. lactis* subsp. *cremoris* (C9, C15). The strains were identified and typed by combining subspecies-specific PCR and Rep-PCR techniques (Feutry et al., 2012b); their phenotypic taxonomic traits (data not shown) were those associated with their genotypic subspecies (Férnandez et al., 2011).

They were maintained at − 80 °C in cryotubes (AES, Combourg, France) until use. To standardize the final cell concentration of *LC* in the vat milk, the protocol described by Casalta et al. (1995) was used. Each strain was grown separately at 30 °C on reconstituted (10%) and pasteurized (63 °C, 30 min) ewe's milk powder (Udipal, Onet-le-Château, France) for 24 h. *LC* cells were quantified by plating the cultures on L-M17 medium (Callon et al., 2004).

2.1.2. Starter S1

Starter S1 combined 3 strains of *Lc. lactis* subsp. *lactis* (L1, L8, L14S), 1 strain of *Lc. lactis* subsp. *lactis* biovar *diacetylactis* (D15), 1 strain of *Lc. lactis* subsp. *cremoris* (C3) and 1 strain of *St. thermophilus* (S15) (Feutry et al., 2012b; additional Table A). All strains are Prt^{+}. Their acidifying activity as assessed by $\triangle$ pH 0–24 h varied from 0.9 to 2.3 pH units (additional Table A). Strain D15 is lysogenic and strain L14S has some phage sensitivity (Feutry et al., 2012b). Starter S1 was added as lyophilized powder directly to the vat milk according to each cheese maker's usual practices and the supplier's recommendations.

2.2. Cheese manufacture

2.2.1. Experimental design

Twelve cheeses were manufactured with starter OI (n = 6) or starter S1 (n = 6) on 2 different days (1, 2) in 3 farmhouses (A, B, C) located in Ossau-Iraty cheese production area. These farmhouses were chosen for the microbial load of their milk (<5.0 $\log_{10}$ aerobic mesophilic microorganisms per mL and <3.0 $\log_{10}$ coliforms per mL), indicating a high quality for milk payment. Coliforms were at 1.5 ± 0.4 $\log_{10}$ CFU mL^{-1} and mesophilic aerobic microorganisms at 4.4 ± 0.2 $\log_{10}$ CFU mL^{-1}. Individual cheeses were named according to farm, day of manufacture and starter. The cheeses were thus named A1-OI, A1-S1 to C2-OI, C2-S1.

2.2.2. Manufacturing conditions

Vat milk was inoculated at 1‰ (v/v) with equal quantities of the 6 cultures of individual OI strains or with the lyophilized S1 starter, giving 6.0 $\log_{10}$ CFU mL^{-1}, the usual starter concentration used by the cheesemakers. The milk came from 2 milkings, an evening one and next morning's, as usual. The process generally used for Ossau-Iraty (Fig. 1) was applied.

2.3. Physicochemical analyses

2.3.1. pH

pH was determined by inserting directly into the cheese a pH electrode (Hanna model HI 98240 pHmeter, Hanna Instruments, USA) connected to a pHmeter (WTW 340-i, Nova Analytics, France). It was recorded every 10 min from molding (M) to just before salting (JBS), and then at days 8, 30 and 120 of ripening (day 8, day 30 and day 120).

2.3.2. Lactose and galactose

Lactose and galactose concentrations were determined in duplicate according to the Boehringer Mannheim method (Meylan, France). Results were expressed in g/100 g dry matter. Samples

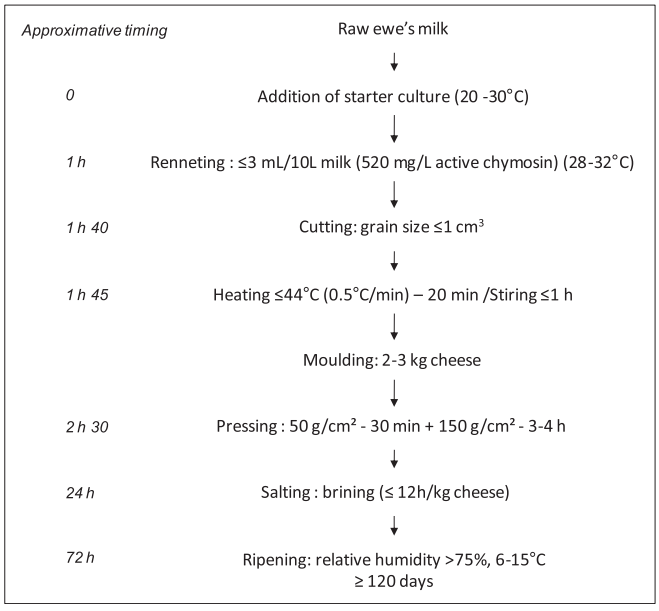

Fig. 1. Scheme of the major steps of the Ossau-Iraty manufacturing process.