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The effect of reduced sodium chloride content on the microbiological and biochemical properties of a soft surface-ripened cheese

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ABSTRACT

Many health authorities have targeted salt reduction in food products as a means to reduce dietary sodium intake due to the harmful effects associated with its excessive consumption. In the present work, we evaluated the effect of reducing sodium chloride (NaCl) content on the microbiological and biochemical characteristics of an experimental surface-ripened cheese. A control cheese (1.8% NaCl) and a cheese with a reduced NaCl content (1.3% NaCl) were sampled weekly over a period of 27 d. Reducing NaCl content induced microbial perturbations such as the lesser development of the yeast *Debaryomyces hansenii* and the greater development of the gram-negative bacterium *Hafnia alvei*. This was accompanied by changes in proteolytic kinetics and in profiles of volatile aroma compounds and biogenic amine production. Finally, the development of the spoilage microorganism *Pseudomonas fragi* was significantly higher in the cheese with a reduced salt content. **Key words:** salt reduction, cheese microflora, aroma compound, biogenic amine

INTRODUCTION

Excessive sodium intake is known to cause high blood pressure and its associated heart diseases (Mohan and Campbell, 2009). In Europe, mean daily sodium intakes range from approximately 3 to 5 g (approximately 8 to 11 g of salt) and are well in excess of dietary needs (approximately 1.5 g of sodium/day for adults; EFSA, 2006). The World Health Organization has proposed a limit of dietary sodium intake by restricting daily salt intake to less than 5 g/d (WHO, 2003). In European and North American countries, processed foods, including cheese products, are estimated to contribute over

70% to dietary sodium intake. Thus, sodium reduction in the human diet is currently one of the main objectives for public health agencies (Elliott and Brown, 2007) and, consequently has become a major challenge facing the food industries. In France, dietary guidelines recommend a 20% reduction of salt in foods to obtain a target of 6.5 to 8 g of salt intake per day (ANSES, 2012). Cheese is perceived as being a food source that is high in sodium, with amounts that vary depending on the type of cheese. In fact, cheeses such as soft, semi-hard, and hard cheeses contain between 0.5 and 2.5% sodium chloride (NaCl), and blue-type cheeses contain between 3 and 5% NaCl.

Sodium chloride fulfills many important functions in cheese: it modifies the physical properties of the cheese curd and rind, controls the growth of the cheese-ripening microflora, limits the development of undesirable species (comprising both pathogens and spoilage microbes), and contributes to taste and overall consumer satisfaction (Guinee and Fox, 2004; Cruz et al., 2011). Several studies have been conducted to characterize the effect of reducing NaCl content or partially substituting NaCl with KCl on various quality aspects of hard cheeses (Shrestha et al., 2011a,b; Grummer et al., 2012; Hystead et al., 2013; Rulikowska et al., 2013; Porcellato et al., 2014), white brined cheeses (Melilli et al., 2004; Ayyash et al., 2011; Ayyash and Shah, 2011a,b,c; Kamleh et al., 2012; Osaili et al., 2014; Thibaudeau et al., 2015), and cream cheeses (Møller et al., 2012). However, there is still a lack of information regarding soft cheeses and, specifically, surface-ripened cheeses.

Soft cheeses represent 40% of the total ripened cheese produced in France. Such products generally contain NaCl at levels that vary from 1.5 to 2.3%. The quality of surface-ripened soft cheeses essentially relies on good development of a rind microbial community, originating from several reservoirs (Irlinger et al., 2015). In soft smear-ripened cheeses, the surface microflora is composed of various species of yeasts, as well as gram-positive and gram-negative bacteria. Salt level has an effect on the growth of bacteria isolated

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from surface-ripened cheeses, including *Brevibacterium*, *Corynebacterium*, *Microbacterium*, and *Staphylococcus* (Mounier et al., 2007). Thus, NaCl content could be a major technological factor controlling underlying ecosystem assembly and behavior. Modifying NaCl content in soft smear-ripened cheeses may affect all biochemical processes sustained by surface microorganism activity, including proteolysis and the production of various secondary metabolites (e.g., aroma compounds and biogenic amines). Furthermore, by changing the indigenous microflora, the natural barrier effect against undesirable species may be altered as well.

The objective of this study was to investigate the effect of reduced NaCl content on microbiological balance with respect to both the cheese-ripening microflora and a spoilage microorganism (*Pseudomonas fragi*), proteolysis, aromatic qualities, and the biogenic amine production profile of an experimental surface-ripened cheese similar to Munster-Livarot-type cheese.

MATERIALS AND METHODS

Strains and Growth Conditions

The 9 microorganisms that composed the model microbial community were *Corynebacterium casei* UCMA 3821 (obtained from the Laboratoire des Microorganismes d'Intérêt Laitier et Alimentaire, Caen, France), *Brevibacterium aurantiacum* ATCC 9174, *Geotrichum candidum* ATCC 204307 (both from the American Type Culture Collection, Rockville, MD), *Arthrobacter arilaitensis* CIP 108037 (from the collection of the Institut Pasteur, Paris, France), and *Staphylococcus equorum* Mu2, *Hafnia alvei* GB001, *Lactococcus lactis* ssp. *lactis* S3+ and its protease-negative variant S3–, *Kluyveromyces lactis* 3550, and *Debaryomyces hansenii* 304 (from the culture collection of the Génie et Microbiologie des Procédés Alimentaires, INRA, Thiverval-Grignon, France). The spoilage microorganism, *Pseudomonas fragi* 1E26, was kindly provided by C. Delbes-Paus (from the culture collection of the Unité de Recherches Fromagères, INRA, Aurillac, France). All strains were originally isolated from cheese. The lactic acid bacteria *Lactococcus lactis* ssp. *lactis* S3+ and S3– were grown for 24 h at 30°C under static conditions in M17 lactose (0.5%) broth (Biokar Diagnostics, Beauvais, France), inoculated at 3% in reconstituted skim milk (100 g/L, Difco Laboratories, Detroit, MI), and then incubated for 16 h at 30°C. All other bacteria were grown under aerobic conditions (using a rotary shaker at 150 rpm) at 25°C for 48 h in 50-mL conical flasks containing 10 mL of brain heart infusion broth (Biokar Diagnostics). The yeasts were grown under the same conditions, ex-

cept that potato dextrose broth (Biokar Diagnostics) was used as the growth medium.

Cheese Production

Sterile model surface-ripened cheeses were produced in a manner similar to that described in previous studies (Mounier et al., 2008; Dugat-Bony et al., 2015). Such a model cheese has been shown to be representative of Munster-Livarot-type cheese in terms of its sensory properties (Bonaïti et al., 2005). Briefly, pilot-scale cheese production (coagulation, cutting, draining, and molding of the curd) was carried out under aseptic conditions in a sterilized 3-m³ chamber, as previously described (Mounier et al., 2008). Only *Lactococcus lactis* ssp. *lactis* S3+/S3– were inoculated at this stage. One kilogram of the resulting cheese curd was crumbled under sterile conditions and mixed 3 times with 234 mL of 90 or 64 g/L NaCl for 10 s at maximum speed using a Waring blender to obtain a homogeneous cheese paste with a salt content of approximately 18 (control level) and 13 (reduced level) g/kg, respectively. Freshly prepared microbial solutions (2×10^9 cfu/mL in physiological saline solution for bacteria, and 2×10^7 cfu/mL for *D. hansenii* and *K. lactis*, and 2×10^4 cfu/mL for *G. candidum*) were then added to the cheese. The 5 ripening bacteria were inoculated at a concentration of 10^6 cfu/g of cheese; *D. hansenii* and *K. lactis* were inoculated at 10^4 cfu/g of cheese; and *G. candidum* was inoculated at 10^2 cfu/g of cheese. In some experiments, *P. fragi* was inoculated at 10^4 cfu/g of cheese. Twenty-five grams of the inoculated cheese paste was then transferred to sterile crystallizing basins with a diameter of 5.6 cm. The model cheeses were ripened for 1 d at 17°C and 93% relative humidity and then for 26 d at 12°C and 97% relative humidity. At least 3 cheeses for each salting condition were taken at regular time intervals during the ripening process (0, 6, 13, 20, and 27 d). The entire cheese was homogenized at 4°C under sterile conditions, and 1 g was sampled and transferred to a sterile container for microbiological analysis. Cheeses were then stored at –80°C until quantitative PCR assay, biogenic amine, and aroma compound analysis.

Physicochemical Analysis

Samples were analyzed for pH, water activity (a_w), and NaCl content. Cheese pH was measured after homogenization of the entire cheese (both surface and core) with a BlueLine 27 surface electrode (Schott, VWR, Fontenay-sous-Bois, France). Water activity was measured on 5 g of cheese using an electronic water activity meter (Novasina, Labmaster, Lachen, Switzerland).

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