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Contribution of *Enterobacteriaceae* to sensory characteristics in soft cheeses made from raw milk

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Abstract

Microbiological and sensory methods were used to analyze 22 soft cheeses, of which 19 were made from raw milk, one was made from both raw and pasteurized milk and two were made from pasteurized milk. Moderate correlations (r-value 0.5–0.6 and p-value <0.01–0.05) were found between the levels of *Enterobacteriaceae* 37 °C and the intensity of the sensory characteristics "bitter", "metallic", "pungent", "manure" and "ammonia". The present study indicates that it is possible to predict high levels of *Enterobacteriaceae* in soft cheeses made from raw milk using only the human senses (odor and taste).

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Keywords: soft cheeses; pasteurization; sensory profile; *Enterobacteriaceae*; raw milk.

1. Introduction

Soft cheeses made from raw milk contribute a diversity of flavors that some consumers perceive positively [1] and also associate with artisanal production [2]. However, several food-borne outbreaks have been traced back to such cheeses [3, 4, 5]. Microbiological limits are used to determine if the food is satisfactory from a public health perspective. The European Union (EU) has set limits for the number of coagulase-positive staphylococci per gram of cheese for cheeses made from raw milk [6]. There are also limits for the number of *Listeria monocytogenes* – up to 99 colony-forming units (CFU) per gram of food is generally accepted [6].

Cheeses made from raw milk have a higher flavor intensity than cheeses made from pasteurized milk [7, 8, 9]. Furthermore, numerous studies have shown that the production of volatile compounds varies considerably between bacterial isolates and is strain dependent [10, 11, 12, 13]. Cheese made from raw milk is therefore particularly complex due to the varying composition of microbes [14, 15]. Most of the studies that compare flavor compounds and bacteria in cheese use chemical analytical instruments such as gas chromatography and mass spectrometry [12]. In contrast, our study is based on quantitative descriptive sensory methods. Sensory methods study the sensory attributes of products, e.g. cheese, giving a profile consisting of basic taste, flavor and/or texture for the analyzed product. This profiling is carried out by selected and trained assessors, which form a panel with the purpose of

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objectively evaluating the products [16].

The formation of flavor compounds due to microbial metabolism during ripening may be grouped into primary and secondary metabolic pathways. The primary metabolic pathways include lipolysis, proteolysis and metabolism of residual lactose, lactate and citrate [17]. Free fatty acids can be precursors to flavor compounds such as ketones, acids, alcohols and esters [10]. These flavors are especially important in soft cheeses [18]. Peptides and amino acids do not generally impact the flavor characteristics in cheese, other than the basic tastes such as bitter [18]. Further conversion is required for flavor formation [19]. Metabolism of residual lactose and of lactate and citrate is a rapid process during the early stages of ripening [17]. Further conversion can contribute to various flavor compounds such as acetaldehyde, diacetyl, acetoin and acetate [19]. These flavors are especially important in yoghurt [18]. The secondary metabolic pathways include metabolism of fatty acids and of amino acids [17]. Amino acids are degraded through transamination reactions and various flavor compounds are then produced from one or two additional reactions [20]. The flavor compounds resulting from this process have significant impact on the resulting flavor in cheese [13]. For example, some acids can contribute to the sensory characteristics “rancid”, “putrid” and “sour”, which contribute to the perception of ripened cheese [20].

The aim of the present study was to test the hypothesis that it is possible to predict the number of *Enterobacteriaceae* using human senses.

2. Materials and methods

This study included 19 raw milk cheeses, one cheese made from both raw milk and pasteurized milk and two cheeses made from pasteurized milk. The raw milk cheeses (n = 19) and the cheese made from both pasteurized and raw milk (n = 1) were purchased from a cheese store in Stockholm, Sweden. The cheeses made from pasteurized milk (n = 2) were purchased from a local grocery store in Grythyttan, Sweden. The cheeses were stored in refrigerators (+4 °C) at the School of Hospitality, Culinary Arts and Meal Sciences, Örebro University, until analysis. All cheeses were analyzed before the best-before date according to the cheeses’ labels.

The cheeses (n = 22) were examined for aerobic microorganisms 30 °C, *Enterobacteriaceae* 37 °C, *Enterobacteriaceae* 44 °C, enterococci, staphylococci, *Bacillus cereus* and *Listeria monocytogenes* using the plate count method. The media used were tryptone glucose yeast extract agar, violet red bile glucose agar, Slanetz and Bartley agar, Baird-Parker agar, BACARA agar and Ottaviani Agosti agar (bioMérieux, France). Furthermore, four cheeses each contributed five typical *Enterobacteriaceae* 37 °C and 44 °C isolates, that is, a total of 10 isolates from each cheese. The total of 40 isolates were identified using API 20 E strips (bioMérieux, France). The results were recorded using the dedicated apiweb version 5.1 software (https://apiweb.biomerieux.com by bioMérieux, France), which yielded a profile and an identification rate in %.

The sensory evaluation by Quantitative Descriptive Analysis (QDA) [16] was conducted by a sensory panel of students (n = 14) at the School of Hospitality, Culinary Arts and Meal Sciences, Örebro University, who participated voluntarily. The assessors had experience in sensory evaluation with lectures in physiology of the senses, perception and sensory methods. The evaluation was carried out in a sensory laboratory conforming to ISO standards [21]. One training session prior to the evaluation was carried out to calibrate the assessors. They discussed and agreed on intensity levels of three different cheeses (two made from raw milk, one made from pasteurized milk). The sensory characteristics used (n = 10) were based on previous studies [9, 22, 23] and included the basic tastes “sweet”, “sour”, “salt” and “bitter” and the flavors “earthy”, “manure”, “mold”, “ammonia”, “metallic” and “pungent”. The intensity was assessed using line scales with labeled end points ranging from low intensity (1) to high intensity (9). The cheeses were evaluated in random order in replicates of one. Data collection was carried out using EyeQuestion version 3.9.7 software (Logic8 BV, The Netherlands). Univariate statistical methods were used to analyze the data. The analysis was performed using PanelCheck version 1.4.0 software (http://www.panelcheck.com by Nofima, Norway) for two-way ANOVA with samples and assessors as factors (two-way ANOVA, 1-rep) and for Principal Component Analysis with sensory characteristics (mean values) for all products.

Data from plate counting and QDA were compiled using Excel 2010 version 14.0 (Microsoft Corporation, USA). The statistical analysis was performed using Statistica version 12.0 software (http://statsoft.com by StatSoft, USA) for correlation (r-value and p-value) and visualized by scatter plots, with the levels of bacteria and the intensity of sensory characteristics as factors.