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Changes of lactose, lactic acid, and acetic acid contents in Serra cheese during ripening

Abstract Changes in the quantities of lactose, lactic acid and acetic acid in Serra cheese were monitored using a triplicate two-way factorial design over a ripening period of 35 days (sampling at 0, 7, 21 and 35 days) throughout the cheesemaking season (sampling in November, February and June). The amount of lactose in total solids of cheese (TS) decreased slowly from 6.17% to 0.21% (w/w_{TS}) as ripening time elapsed. As a result of sugar metabolism, the lactic acid content increased from 0.07% at day 0 to 2.10% (w/w_{TS}) by 35 days, whereas the acetic acid content increased from 0.00% to 0.24% (w/w_{TS}) during the first week. The lactose content was statistically correlated with the lactic acid content but not with the acetic acid content.

Key words Acidification · Maturation · Raw milk Ovine cheese

Introduction

Serra da Estrela cheese (Serra cheese), the most famous and most appreciated Portuguese semi-soft cheese manufactured by traditional methods, is manufactured manually in the Eastern Centre of Portugal at the farm level typically from October to June and using raw ovine milk coagulated with a crude aqueous extract of dried thistle flowers (*Cynara cardunculus* L.) without any added starter. Cheeses manufactured from raw milk may constitute a potential health hazard due to the indigenous microflora which is always qualitatively and/or quantitatively unknown to some degree. It has often been claimed that 60 days of cheese ripening should be allowed to elapse to ensure that viable pathogens are sufficiently reduced in number so as to fall below the threshold that is acceptable for human safety. Despite the fact that Serra cheese is required to mature for at least 45 days and is usually not sold before 60 days, such microorganisms may produce toxins which remain in the product. Strict controls over the pH and starter activity have been used in an attempt to suppress the growth of, and/or the toxin production by, microorganisms that cause food poisoning [1], either by creating unfavourable environments or through ecological competition for available nutrients.

Considering that acidification plays an important role in cheesemaking, the aim of the present research effort was to monitor lactose depletion and the concomitant generation of lactic and acetic acids. Attempts to correlate the amounts of such organic acids with the quantity of lactose in Serra cheese were also made.

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Materials and methods

Cheese manufacture and sampling. Three batches of twelve 0.5-kg cheeses were prepared in a small, certified dairy farm in the *Appélla-tion Serra Controllée* region in three sequential periods within the cheesemaking season (i. e. November, February and May) from raw ovine milk according to traditional practices [2]. Three cheeses of each batch were randomly taken on the day of manufacture and after 7, 21 and 35 days of ripening.

Chemical analyses. The moisture content was determined according to the atmospheric oven method at 100 °C for 24 h [3]. Lactose content was determined according to the method of Acton [4] and was expressed as the percentage in total solids of cheese (%, w/w_{TS}). Aqueous extracts of lactic and acetic acids were obtained by homogenizing 10 g of each cheese sample with 30 ml of a 0.5 M solution of perchloric acid (Merck, Darmstadt, Germany) for 3 min in a Stomacher Lab blender 400 (Seward Medical, London, UK), pouring the solution into a closed jar, and filtering it through a 0.22-µm Syrfil filter (Nucleopore, Cambridge Mass., USA) after it had been left to stand for 2 h at refrigeration temperatures. Concentrations of lactic and acetic acids were determined by HPLC according to the method of Oliemann [5] and were expressed as the percentage in total solids of cheese (%, w/w_{TS}).

Statistical analyses. Statistical treatment of the results obtained from the two-way factorial design (4×3) replicated three times was done via analysis of variance and Scheffé's *F* comparison test. The linearity of the relationship between the amounts of lactic and acetic acids and the

Table 1 Changes in contents (%, w/w_{TS}), and associated standard errors, of lactose, lactic acid, and acetic acid in Serra cheese during ripening

	Ripening time (days)			
	0	7	21	35
Lactose	6.17 ± 0.47	4.60 ± 0.58	0.38 ± 0.11	0.21 ± 0.08
Lactic acid	0.07 ± 0.05	1.15 ± 0.12	2.10 ± 0.29	1.99 ± 0.24
Acetic acid	0.00 ± 0.00	0.24 ± 0.03	0.24 ± 0.04	0.22 ± 0.05

amount of lactose was ascertained by calculation of Pearson's correlation coefficients and associated 95% confidence intervals, as well as by visual inspection of scatterplots of the data. All statistical analyses were performed with the Statview 4.0 statistical package [6].

Results and discussion

The lactose content in cheese (total solid basis, TS) exhibited a statistically significant decrease ($P < 0.05$) as ripening time elapsed. Scheffé's F test indicated that the lactose content in the cheese decreased significantly from 0 to 21 days ($P < 0.05$), but no significant differences were found between 21-day-old and 35 day-old-cheeses (see Table 1). However, this process of lactose depletion was relatively slow (it occurred over a time scale of weeks) when compared with a similar process in other cheeses such as Cheddar (which occurs over a time scale of a few hours). This is so because Serra cheese is manufactured without any addition of starter (in contrast to the manufacture of Cheddar cheese), and it is known that addition of starters results in a rapidly decreasing cheese pH. Analysis of variance of data pertaining to the measurements of lactic acid and acetic acid indicated that the amounts (total solids basis, TS) of each acid were statistically different ($P < 0.05$) from each other, and that the quantity of lactic acid was significantly higher than that of acetic acid throughout ripening and throughout the cheesemaking season (see Table 1). Scheffé's F test indicated that the lactic acid content in cheese (TS basis) increased significantly from 0 to 21 days ($P < 0.05$), but no significant differences were found between 21-day-old and 35-day-old cheeses. Additionally, the lactic acid content was found to be statistically correlated with lactose content (TS basis), considering that the 95% confidence interval of Pearson's correlation coefficient, i.e.]–0.857, –0.961[, did not overlap zero and that the scatterplot suggested an acceptable linear dependency between those variables (plot not shown). Stabilization of the lactic acid content after 21 days agrees with the observation that lactose is virtually depleted at this time (as mentioned above). The pattern of variation of acetic acid content during ripening was different from that of lactic acid (see Table 1): the acetic acid content in cheese (TS basis) increased significantly during the first week of ripening ($P < 0.05$), but stabilized thereafter. On the other hand, the acetic acid content should not be considered to be correlated with the lactose content because the scatterplot indicated weak linear dependency between those variables (plot not shown); although the 95% confidence interval of Pearson's correlation coefficient does not include zero (i.e.]–0.814, –0.428[), the size of the sample analysed is large enough such that the correlation coefficient is virtually always significant [6] irrespective of its value.

Most lactose in milk (≈98%) is removed in the whey. Under normal circumstances, the residual lactose is metabolized during early stages of ripening mainly via the action of lactic acid bacteria (LAB). Hydrolysis of lactose is obtained via different biochemical routes of sugar transport and utilization [7, 8], depending on the LAB strain, because of different metabolic regulatory schemes and sequential utilization of the sugars available in the medium [8]. Lactate is preferentially produced from lactose by starter bacteria, whereas acetate may be obtained from either lactose or citrate mainly by *Leuconostoc* spp., or from amino acids by lactobacilli in particular [7]. In our previous study [9], we reported that LAB and coliforms were the dominant microorganisms during Serra cheese ripening and that the numbers of LAB stabilized after 21 days whereas the numbers of coliforms declined after 7 days. In light of this, it appears that the time course of changes of lactic acid content is explained by the metabolism of lactose during the exponential growth phase of LAB present in Serra cheese, whereas the time pattern of the acetic acid content is explained either by the metabolism of citrate by heterofermentative LAB present in Serra cheese (such as *Leuconostoc lactis* and *Leuc. mesenteroides*) or by the metabolism of lactose during the exponential growth phase of non-LAB present in cheese. Finally, considering that acidification in Serra cheese proceeds slowly during ripening, and also that addition of a starter caused an accelerated decrease of *Enterobacteriaceae* viable numbers during the ripening of Manchego cheese [10], a similar cheese manufactured from raw ovine milk, and a significant reduction of the numbers of *Escherichia coli* in ovine milk [11], it appears that acceleration of acidification during early stages of ripening is in order when attempting to improve safety of Serra cheese for general human consumption.

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