Changes of Mineral Concentrations in Serra Cheese during Ripening and Throughout the Cheesemaking Season

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Abstract: Seasonal changes of the ash content and mineral concentrations in Serra cheese were studied over a typical 35-day ripening period. Statistically significant differences (at the 5% level) exist between the ash content and the concentrations of Na, K, Ca, P, Mg and Zn in cheeses during ripening. The highest concentrations of Na was obtained in cheese ripened for 7 days, whereas the concentrations of K, Ca, P, Ng and Zn decreased significantly during ripening. For 35-day-old cheeses, concentrations of Na, K and Cu were lowest and concentration of P was highest for cheeses manufactured in May. The concentration of Ca was lowest for cheeses manufactured in February. On average, the most concentrated minerals (in g kg^{-1} of total solids, TS) in 35-day-old Serra cheese were Na (18·56), Ca (9·70) and P (7·92) and, at a lower level, K (1·70) and Mg (0·96). Only trace levels (in mg kg_{TS}^{-1}) of Zn (94·33), Cu (2·26) and Mn (1·25) were detected. A high mineral nutrition quality was thus ascribed to 35-day-old Serra cheese based on the average nutritional densities: 4·8 for Ca, 4·0 for P, 1·1 for Mg, 3·4 for Na, 2·4 for Zn, 0·4 for Cu, 0·2 for Mn and 0·2 for K.

Key words: calcium, magnesium, zinc, copper, manganese, sodium, potassium, phosphorus, dairy, sheep, lactation

INTRODUCTION

Since milk production by sheep is limited to only a few countries, mainly located in the Mediterranean basin, data concerning composition of sheep's milk and composition of dairy products manufactured therefrom are much scarcer than those of cow's counterparts. Available reports (Anon 1981) indicate that the average concentration in minerals of sheep's milk is considerably higher than that of cow's milk; hence, dairy products obtained from sheep's milk are expected to possess a higher nutritional value than those obtained from cow's milk. The composition of cheese in terms of minerals is rather variable and depends on such factors as: (i) initial composition of the milk (García-Olmeda *et al* 1981), which in turn depends on the breed, stage of lactation, physiological condition of the animal, composition of the feed and environmental factors (Anon 1981); (ii) cheesemaking procedures (Feeley *et al* 1972), which are different for different cheese types; and (iii) ripening conditions (Coppini *et al* 1979; Le Graet and Brulé 1988), which include not only the temperature and relative humidity prevailing in the maturation room but also protocols of surface salting and washing.

The most important and famous variety of cheese in Portugal manufactured with sheep's milk following traditional procedures is Serra cheese; this type of cheese is manufactured on the farm level only from raw milk without starter using a crude rennet from plant origin (ie the dried flowers of the wild thistle, *Cynara cardunculus* L). The plant rennet possesses a strong and unspecific proteolytic action which leads to extensive breakdown of caseins in the cheese matrix within *ca* 1 month of ripening and thus to development of a soft,

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buttery texture (Macedo *et al* 1993). Despite the fact that this unique cheese is considered a food specialty by most gourmets, concerns pertaining to safety and nutritional value have been raised lately, especially by consumers in North European and North American countries. Whereas the former issue has been dealt with previously (Macedo *et al* 1995, 1996) with a focus on microbiological items, the latter issue (especially in terms of minerals) has not yet received formal attention by researchers and has, therefore, provided a driving force for the research work reported in this communication. Although extensive information on mineral composition of (pasteurised) cow's milk cheeses is available worldwide, information on mineral composition of (raw) sheep's milk cheeses is scarce, eg reports encompassing a few Spanish cheeses (Marcos *et al* 1983; Martin-Hernandez *et al* 1992).

The present study attempts not only to contribute to the characterisation of the mineral profile of Serra cheese by the end of the normal ripening period but also to proceed one step further via statistically assessing the influence of the time variables (ie ripening time and time within the cheesemaking season) on the aforementioned profile. In view of the importance of minerals for human health, the nutritional density of Serra cheese in terms of several minerals after ripening for 35 days is also presented in this report, with the goal of helping potential consumers decide on inclusion of this cheese in their diet.

MATERIALS AND METHODS

Cheese manufacture and sampling

Three batches of twelve 0·5-kg cheeses were prepared by a selected dairy farmer in the *Appéllation Serra Controllée* region in three sequential periods within the cheesemaking season (November, February and May). These three periods were selected so as to represent as close as possible the various stages of the lactation season (which usually starts in October and ends in May), the various feeding regimes (determined mainly by the weather conditions) and the various ripening conditions (constrained chiefly by the local weather). Cheesemaking followed traditional practices: after the temperature of raw sheep's milk (previously filtered through a clean piece of cotton cloth) had reached 28°C, crude kitchen salt was added to the milk at the ratio of 12 g litre^{-1}, and the mixture was stirred until complete solubilisation; thistle flowers (previously dried on the shade in the open air and carefully ground) in an amount enough to give the ratio 0·4 g litre^{-1} of milk were then mixed with tap water until a brown suspension resulted and filtered through a fine, clean piece of cotton cloth, and the clear filtrate was added to the milk and gently stirred for *ca* 5 min; the milk was allowed to rest for *ca* 1 h at 28°C until complete coagulation had occurred; the curd was then manually cut by stirring the coagulum with the bare hand for *ca* 10 min, after which the curd pieces were poured into a fine cloth bag and lightly pressed for *ca* 10 min so as to help in the expression of whey; drainage was completed via pressing of the fresh cheese while in the plastic perforated mould via a 10-kg metal block for *ca* 6 h in the original position and another 6 h after having been turned upside down; final salting was done by rubbing the whole outer surface of the cheese with kitchen salt at a ratio of 0·04 g cm^{-2} of cheese surface; the cheeses were finally placed in maturation chambers with temperature set to 9°C and relative humidity set to 95%; after 1 week the cheeses were transferred to a second ripening chamber without control of temperature and relative humidity, where they were turned upside down daily and washed periodically with tap plain water.

Three cheeses of each batch were taken at random during the ripening period on the day of manufacture proper and after 7, 21 and 35 days and transported under refrigerated conditions (*ca* 4°C) to our premises. After having removed the rind, analytical samples were homogenised and frozen at *ca* −30°C in Whirl-pak™ vacuum packages (Cole-Parmer, Chicago, IL, USA) until analysis was in order.

Chemical analysis

The moisture and salt in each cheese sample were determined as described by Case *et al* (1985) using the atmospheric oven method at 100°C for 24 h (EHRET, Emmendingen, Germany) and the modified Volhard method, respectively. The ash content in cheese was determined using a gravimetric method after thermal treatment in a muffle furnace (Ney, Yucaipa, CA, USA) (Anon 1984) at a temperature of 500°C in order to prevent significant losses of low-boiling elements, eg zinc (Wong *et al* 1977).

To a fraction of the ash thus obtained, 2 ml of 1 M aqueous solution of nitric acid (Merck, Darmstad, Germany) was added, and the solution thus obtained was dried on a thermostatted hotplate. The ash was then redissolved in 10 ml of 1 M aqueous solution of nitric acid in a volumetric flask. This concentrated feedstock solution was subsequently stored in stoppered polypropylene tubes under refrigeration at *ca* 4°C until analysis could be performed.

The concentrations of Ca and Mg (in diluted aliquots obtained from the feedstock solution) and the concentrations of Zn, Cu and Mn (in the original feedstock solution) were determined using a GBC 902 double-beam atomic absorption spectrophotometer (GBC Scientific Equipment, Melbourne, Australia) with an air/acetylene flame. Standard solutions for atomic absorption spectroscopy at 1000 ppm (Spectrosol R™,

BDH Chemicals, Poole, UK) were used to generate calibration curves. Lanthanum (III) (Merck) was added to the final diluted solutions of Ca and Mg at a concentration of 500 ppm in order to avoid interference by P (Fresno *et al* 1995).

The concentrations of Na and K (in diluted aliquots obtained from the feedstock solution) were determined using a flame emission photometer (Jencons Scientific, Bedfordshire, UK) with an air/butane flame. Standard solutions for flame emission photometry at 1000 ppm (Spectrosol R™, BDH Chemicals) were used to generate calibration curves.

The concentration of P (in diluted aliquots obtained from the feedstock solution) was determined by a photometric method via assay of the molybdenum blue complex derivative (Anon 1990) using a fraction of the ash obtained from the cheese sample as described above. Potassium dihydrogen phosphate (Merck), previously dried to constant weight, was used to generate the calibration curve.

Statistical analysis

The Statview™ 4.0 statistical package (Abacus Concepts, Berkeley, CA, USA) was used for statistical treatment of the experimental results generated, pertaining to ash content and individual mineral concentrations (total solid (TS) basis) of cheeses manufactured at different times within the cheesemaking season and ripened for different times, via analysis of variance for the whole dataset and Fisher's protected least significance difference test for pairwise comparison (Haycock *et al* 1992). Both these methodologies could be safely applied because the experimental errors (as evaluated from sets of three replicates) were independent from one another and normally distributed (Box *et al* 1978). The aforementioned analyses were employed to test, on the 5% level of significance, the null hypothesis that no differences existed between concentrations of samples obtained from cheeses manufactured at different times within the cheesemaking season and ripened for different times.

RESULTS AND DISCUSSION

Mineral concentrations

Inspection of Table 1 indicates that the most concentrated minerals in Serra cheese are Na, Ca and P (all of the same order of magnitude); concentrations of K and Mg were *ca* ten-fold lower. Only trace levels of Zn, Cu and Mn could be detected (see Table 2); the concentrations of Zn were *ca* 100-fold lower than those of the

TABLE 1
Changes in concentration (g kg_{TS}^{-1}), and associated standard error, of major minerals and ash in Serra cheese during ripening and at different periods within the cheesemaking season: November (1st row), February (2nd row) and May (3rd row)

Minerals	*Ripening time (days)*			
	0	*7*	*21*	*35*
Sodium (Na)	10·81 ± 0·34	21·04 ± 0·92	20·11 ± 0·31	19·85 ± 0·51
	8·85 ± 0·90	22·82 ± 1·45	20·52 ± 0·20	19·64 ± 0·36
	9·02 ± 0·78	21·06 ± 1·03	17·63 ± 0·53	16·19 ± 0·20
Potassium (K)	2·13 ± 0·10	1·99 ± 0·15	1·77 ± 0·08	1·73 ± 0·12
	2·37 ± 0·19	1·99 ± 0·10	1·96 ± 0·18	1·88 ± 0·09
	1·75 ± 0·19	1·55 ± 0·22	1·51 ± 0·08	1·49 ± 0·08
Calcium (Ca)	12·97 ± 1·10	12·63 ± 1·25	11·15 ± 1·48	10·50 ± 0·89
	10·54 ± 1·16	9·45 ± 0·98	9·43 ± 0·48	8·54 ± 0·35
	12·13 ± 1·46	11·30 ± 0·27	10·52 ± 1·00	10·07 ± 0·59
Phosphorus (P)	8·75 ± 0·40	8·38 ± 0·37	7·55 ± 0·36	7·59 ± 0·27
	9·00 ± 0·15	8·73 ± 0·09	7·73 ± 0·40	7·80 ± 0·19
	9·40 ± 0·09	9·21 ± 0·15	9·18 ± 0·09	8·38 ± 0·28
Magnesium (Mg)	1·23 ± 0·10	1·06 ± 0·12	1·03 ± 0·02	1·00 ± 0·15
	1·26 ± 0·04	1·21 ± 0·20	1·21 ± 0·03	0·97 ± 0·04
	1·08 ± 0·04	0·97 ± 0·05	0·85 ± 0·07	0·92 ± 0·14
Total (ash)	61·10 ± 2·27	78·97 ± 4·22	73·80 ± 2·02	75·03 ± 1·80
	60·73 ± 3·52	88·40 ± 6·46	82·13 ± 2·63	79·00 ± 1·39
	60·40 ± 1·15	77·87 ± 1·37	73·77 ± 2·84	82·97 ± 1·54

TABLE 2

Changes in concentration (mg kg_{TS}^{-1}), and associated standard error, of trace minerals in Serra cheese during ripening and at different periods within the cheesemaking season: November (1st row), February (2nd row) and May (3rd row)

Minerals	*Ripening time (days)*			
	0	*7*	*21*	*35*
Zinc (Zn)	99·33 ± 3·05	84·67 ± 8·02	77·33 ± 3·06	79·33 ± 13·05
	120·00 ± 13·45	113·33 ± 1·16	110·67 ± 5·69	98·00 ± 7·21
	123·33 ± 5·77	121·00 ± 10·15	119·00 ± 1·00	105·67 ± 10·07
Copper (Cu)	3·90 ± 0·85	2·90 ± 0·46	3·10 ± 0·46	2·50 ± 0·40
	3·47 ± 0·55	3·33 ± 0·55	3·00 ± 0·89	2·73 ± 0·15
	2·50 ± 0·70	2·20 ± 0·35	2·10 ± 0·79	1·53 ± 0·15
Manganese (Mn)	1·27 ± 0·06	1·17 ± 0·15	1·23 ± 0·06	1·23 ± 0·06
	1·30 ± 0·10	1·40 ± 0·10	1·40 ± 0·17	1·23 ± 0·16
	1·37 ± 0·15	1·37 ± 0·06	1·40 ± 0·20	1·30 ± 0·10

dominating elements, whereas the concentrations of Cu and Mn were *ca* 10 000-fold lower. Similar concentration levels have been reported by Marcos *et al* (1983) for Spanish cheeses which are also manufactured from raw ovine milk via enzymatic coagulation without the addition of starter (if acid coagulation were employed, acid production would lead to a shift of minerals from colloidal to soluble state) and with addition of similar amounts of salt, although with somewhat different extents of wheying. Fresno *et al* (1995) concluded that manufacturing technology (which is characterised by the combination of specific patterns of coagulation, salting and wheying) has more influence on the concentrations of minerals than the source of milk.

The results provided by the ANOVA using the whole data set indicate that ripening time statistically affects the concentrations (in TS basis) of Ca ($P < 0{\cdot}001$), P ($P < 0{\cdot}0001$), Mg ($P < 0{\cdot}0001$), Zn ($P < 0{\cdot}0005$), Cu ($P < 0{\cdot}01$), Na ($P < 0{\cdot}0001$) and K ($P < 0{\cdot}001$); conversely, ripening time does not statistically affect the concentration of Mn ($P > 0{\cdot}5$). The strongest effect of ripening is observed with respect to Na, which is chiefly contributed by sodium chloride (recall that in the manufacture of Serra cheese crude kitchen salt is added to milk prior to coagulation and to the surface of the cheese after pressing). Between 0 and 7 days of ripening, Na diffuses away from the surface towards the bulk of the cheese using water as continuum for molecular transport; hence, its concentration is expected to increase significantly, and this expectation is consubstantiated by the experimental observation of *ca* 12·1 g kg_{TS}^{-1} ($P < 0{\cdot}0001$, as obtained from Fisher's protected least significance difference test); conversely, K will be lost during wheying, and, since no pool of K is made available at the surface (as happened with Na via NaCl), its concentration decreases significantly in cheese by *ca* 0·24 g kg_{TS}^{-1}, $P < 0{\cdot}0001$). Between 7 and 21 days of ripening, not only the moisture content of cheese decreases but also its surface is periodically washed with tap water with consequent leaching of small amounts of Na (by *ca* 2·2 g kg_{TS}^{-1}, $P < 0{\cdot}0001$). Although Na and K are usually present in the soluble fraction of cheese, such minerals as Ca, P, Mg, Zn and Cu are preferentially linked to the casein micelle (insoluble) fraction; however, as the pH in cheese decreases during ripening, migration of those minerals towards the soluble fraction is observed, and so losses in the residual whey can take place (Moreno Rojas *et al* 1994). Macedo *et al* (1996) reported that, between 0 and 21 days of ripening, significant decrease in the pH of Serra cheese is observed, and this change may account for the significant decrease in the concentrations of Ca (by 1·5 g kg_{TS}^{-1}, $P < 0{\cdot}005$), P (by 0·9 g kg_{TS}^{-1}, $P < 0{\cdot}0001$), Mg (by 0·2 g kg_{TS}^{-1}, $P < 0{\cdot}0005$) and Zn (by 11·9 mg kg_{TS}^{-1}, $P < 0{\cdot}005$). Between 21 and 35 days, the rind becomes thicker and so the loss of whey is very small; during this time-frame, no statistical differences were obtained for the content of the mentioned minerals.

The concentrations of Zn, Ca, Mg, K and Na in fresh cheeses were statistically different during the cheesemaking season ($P < 0{\cdot}05$, as concluded from the results of the ANOVA); cheeses at day proper of manufacture showed the lowest concentrations of Zn in November, of Ca in February and of K and Mg in May. Assuming that essentially the same cheesemaking protocol was followed irrespective of the time of manufacture, this observation can probably be accounted for by variations in milk compositions arising from the qualitatively and quantitatively different metabolic rates (due to different physiological state of the animals and different environmental temperatures), as well as different characteristics of the feeding available (Bell and Whittier 1965). In fresh cheeses the concentrations of Na were significantly higher in November than in February and

May ($P < 0{\cdot}05$, as concluded from Fisher's protected least significance difference test), which were statistically similar; these variations could be partially explained by the salting method used in Serra cheesemaking (salt is added empirically to milk at the very beginning of cheese manufacture and to surface of the cheese after pressing). The concentrations of P, Cu and Mn in cheeses at 0 days did not exhibit statistically significant variations at the 5% level throughout the cheesemaking season. The concentrations of Ca, P, Cu, K and Na in 35-day-old Serra cheeses were significantly different throughout the cheesemaking season ($P < 0{\cdot}05$). The concentrations of N, K and Cu by the end of ripening were statistically lower in May than in November and February, which were similar at the 5% level of significance; these could probably be explained by the lower moisture contents of cheeses manufactured in May than those manufactured in November and February as reported by Macedo *et al* (1996), and so by higher losses of these minerals with whey. The concentration of Ca in 35-day-old cheeses was statistically lower in February, whereas the concentration of P in 35-day-old cheeses was statistically higher in May than in the other 2 months; these variations probably can be explained by the fact that the lowest pH values in cheeses ripened in February favour migration of these minerals to the soluble fraction, whereas the highest pH values in cheeses ripened in May favour the reverse. The concentrations of Mg, Zn and Mn in 35-day-old cheeses did not show evidence of statistically significant variation throughout the cheesemaking season (at the 5% level of significance).

Ash concentration

The ANOVA indicated that the variation of ash content in cheese on a TS basis changed significantly with time of ripening. Regardless of the cheesemaking period, the ash content (see Table 1) increased significantly during the first 7 days of ripening ($P < 0{\cdot}0001$, using Fisher's protected least significance difference test), decreased significantly between 7 and 21 days of ripening ($P < 0{\cdot}001$) and tended to stabilise thereafter with no significant variation ($P > 0{\cdot}05$). The variations of ash content during ripening are mainly due to the variations of the major minerals (eg Na, Ca and P) in Serra cheese during ripening; during the first week of ripening, the ash content increased mainly because of increases in the concentration of Na as a result of salt addition to the surface of cheese; between 7 and 21 days, the ash content decreased because the concentrations of Na, Ca and P decreased due to losses of these minerals in whey (which continues to be expressed although at slower and slower rates); after 21 days of ripening, no significant variations in ash content could be detected because the aforementioned two phenomena (ie salt in and whey out) did not take place to significant extents.

The ash content of cheeses, manufactured in November, February and May, at 0 days of ripening were similar ($P > 0{\cdot}05$, as obtained from use of Fisher's protected least significance difference test). Although these observations for cheese are apparently not consistent with the data reported by several researchers for milk (Shalichev and Tanev 1967; Williams *et al* 1976; Misic and Petrovic 1976), ie lower values of ash content during earlier stages of lactation and increases up to *ca* 14% at the final stages, it should be borne in mind that: (i) salt (which provides Na, the major element of ash) is added empirically to milk at the very beginning of cheese manufacture, and consequently helps in leveling off the initial ash content of milk; and (ii) the extent of whey drainage and curd pressing may be not uniform for different batches of cheese milk processed at different times within the lactation period (despite the standardised procedures followed throughout this experimental research work), and so the initial composition of cheese may be different. The ash content of 35-day-old cheeses manufactured in May was significantly higher than that of cheeses manufactured in November and February, which were statistically similar to one another (at the 5% level of significance). This observation may be accounted for by the fact that 35-day-old cheeses manufactured in May showed, on average, highest concentrations of Ca and P; these higher concentrations were likely due to slower losses of these minerals via whey because their soluble form was prevented as a result of slower acidification of cheese manufactured in May than in November or February (Macedo *et al* 1996).

Nutritional considerations

Figure 1 depicts the average nutritional densities of the various minerals studied in 35-day-old cheeses. The average nutritional density of cheese is defined as the ratio of mineral mass intake to energy intake brought about by consumption of 100 g of cheese, divided by the ratio of mineral recommended daily mass intake (ie 3750 mg for K, 2200 mg for Na, 800 mg for Ca, 800 mg for P, 350 mg for Mg, 15 mg for Zn, 3·8 mg for Mn and 2·5 mg for Cu for a male adult) to recommended daily energy intake (ie 2700 kcal for a male adult) (Anon 1974). Inspection of Fig 1 indicates that Serra cheese is an excellent source of Ca, P and Zn, a well-balanced source of Mg and a deficient source of Cu and Mn. Since this type of cheese has a high percentage of Na but a low percentage of K (like several Spanish cheeses manufactured from ovine milk), it is particularly recommended for people with skeleton and neurological problems but not for people suffering from anemia or hypertension. The period within the cheesemaking

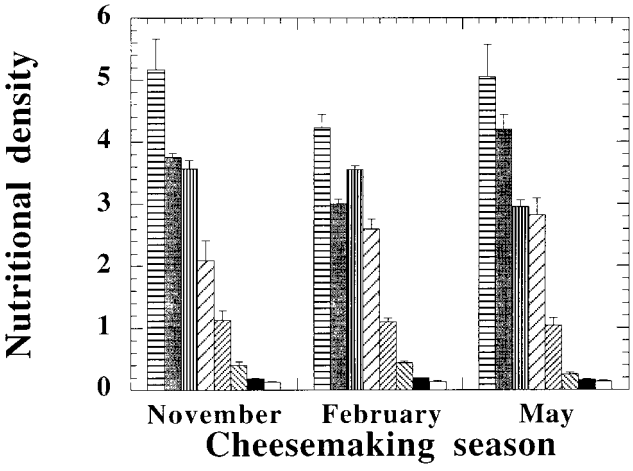

Fig 1. Changes in the nutritional densities of Ca (▤), P (▩), Na (▥), Zn (▨), Mg (▨), Cu (▧), K (■) and Mn (□), in 35-day-old Serra cheese in different periods within the cheesemaking season.

season had statistically significant effects at the 5% level upon the nutritional densities of all minerals except Mg and Mn. Cheeses manufactured in May displayed the lowest nutritional densities for Cu, K and Na but the highest nutrional density for P and Zn. Cheeses manufactured in February displayed the lowest nutritional density for Ca. Reasoning from these observations, it appears that cheeses manufactured and ripened in May are better mineral sources than those manufactured in the other two periods.

CONCLUSIONS

The results in this study indicate that (i) there are significant increases of ash and Na in cheese but significant decreases of Ca, P, Mg, Zn and K during ripening, and that (ii) the period within the cheesemaking season has a statistically significant effect upon the concentrations of Ca, P, K, Cu and Na. Fully ripened Serra cheese is an excellent source of Ca, P, Mg and Zn, and so such cheese may be considered as a high-quality food from a mineral nutrition standpoint.

ACKNOWLEDGEMENTS

The authors are grateful to the members of the technical board of ANCOSE (the Portuguese Breeders Association for Serra da Estrela Sheep) for their cooperation encompassing the local manufacture and transport of the cheeses, and to the technical staff of CEQA (the Food Quality Extension Service of the College of Biotechnology) for their permission and help in utilising analytical equipment. Financial support for the author (A C Macedo) was provided by a PhD fellowship (CIENCIA BD-1741/91-IF, Portugal). Financial support for the research work was partly obtained via project grant MAQUETTE: Improvement of Traditional Cheeses and their Technology (AI, Portugal) and project grant IMPACTO (PRAXIS XXI, Portugal).

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