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Biochemical characteristics of two types of unripe Spanish cow's milk cheese (Cebreiro and Pasiego varieties)

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A detailed study of two varieties of unripe Spanish cheeses (Cebreiro and Pasiego) has been carried out for the first time. The overall chemical composition, the most relevant physicochemical parameters and the classical nitrogen fractions were determined in 24 batches of each variety. The chemical compositions of both varieties are fairly similar to those described in the literature for other unripe cheese varieties. Their low NaCl and ash contents are, above all, outstanding. The low pH of Cebreiro cheese (4·29 ± 0·20) is worth noting. The pH value of Pasiego cheese (5·59 ± 0·60) is similar to that found in other unripe cheeses obtained by enzymatic coagulation. Both varieties show very high Aw values which is to be expected in cheeses with a high moisture content, low NaCl content and very little protein degradation. The values determined for the different nitrogen fractions in both types of cheese indicate a very slight proteolysis.

Linear discriminant analysis was applied to analytical data in order to differentiate both varieties. According to this statistical technique, D-lactic acid, NaCl, Ca, P, and ammonia nitrogen contents and pH and Aw values were the major discriminating variables involved in the differentiation. The correctly classified cases were 100%.

INTRODUCTION

The Spanish Catalogue of Cheeses (Anon, 1990) records 16 varieties of fresh cheeses made in this country, of which only Burgos cheese (Marcos *et al.*, 1983*b*; Chavarri *et al.*, 1985; Núñez *et al.*, 1986; García *et al.*, 1987; Medina *et al.*, 1992), Villalón cheese (Marcos *et al.*, 1983*b*; Chavarri *et al.*, 1985) and Cádiz and Málaga cheeses (Alcalá *et al.*, 1982; Esteban *et al.*, 1982; Millán *et al.*, 1982*a,b*; Marcos *et al.*, 1983*b*) have been studied and are now industrially elaborated.

Biochemical and microbiological characteristics of the other cheeses have not been studied — a fact which makes their quality very difficult to improve and standardize and causes the production of these cheeses to stagnate and in some cases decrease. These are cheeses produced exclusively by artisanal procedures from raw milk, which means that quality is very variable. If these varieties are to be promoted and their production increased, it would be necessary to establish a manufacturing technology using pasteurized milk which would allow products to be obtained with total sanitary guarantee and would maintain the characteristics and attributes of quality cheeses made using traditional procedures. This calls for a study from both the biochemical and the microbiological point of view.

Both Cebreiro cheese and Pasiego cheese are included among the varieties not yet studied. Cebreiro cheese is made in the north-east of Spain (Galicia) using raw cow's milk. It has a shape similar to that of a mushroom. It is soft, spreadable, with voids and is whitish in colour, friable and crumbly. It is presented in units of between 800 and 1500 g.

Pasiego cheese is also made from raw cow's milk in the north of Spain (Cantabria). It is round and flat in shape. It is soft, white in colour, has a semi-sweet flavour and small unequally distributed holes. It is presented in units of between 100 and 600 g.

The aim of this work is to contribute to the chemical characterization of both varieties by determining the main chemical compounds and the most relevant physicochemical parameters in a representative number of samples of each type, to explore transformations of the protein fraction using the determination of classical nitrogen fractions, and an attempt is made to clarify the existing biochemical differences between the varieties making use of discriminant analysis.

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MATERIALS AND METHODS

Samples

Twenty-four units of Cebreiro cheese and 24 units of Pasiego cheese, made by experienced cheesemakers from the production regions following the traditional cheesemaking methods, were used in this work.

When making Cebreiro cheese, whole raw cow's milk held at a temperature of 30–35°C is coagulated by adding about 5 ml of commercial calf rennet of 1/10 000 strength per 100 l of milk; at times small quantities of whey from previous batches are added together with the rennet. After curdling, the curd is cut and left for 24 h so that the whey is separated. Afterwards the curd is placed in cheesecloths for the whey drainage to be carried out. Finally a stone is placed on the cheesecloth to eliminate the residual whey. The period of time that the curd remains in the cheesecloth varies between 12 and 24 h. Afterwards the curd is taken out of the cheesecloth, a variable quantity of salt is added according to individual taste and the mass thoroughly kneaded. The moulding is carried out by placing the curd in cheesecloths and then in bottomless circular moulds, leaving part of the curd out on the top. Afterwards the cheesecloth is tied and a weight is placed on top in order that the whey be eliminated and the cheese adopt its characteristic form. The time the cheese remains in the mould varies between 0·5 and 1 h.

When making Pasiego cheese, whole raw cow's milk is used which is coagulated at a temperature of 30°C adding 35–40 ml of commercial calf rennet of 1/10 000 strength per 100 l of milk. The curdling finishes after 1 h and then the curd is cut and placed in bowls where it remains for about 16 h; the whey is removed from time to time. Afterwards it is placed on a board where stems of *Nardus stricta* L. have previously been placed, which mark the surface of the cheese and help to complete the drainage of the whey. The cheese is left on the board for about three days and is turned from time to time. While on the board, it is salted on both sides using small quantities of solid salt. Sampling was carried out by following the Standard FIL-IDF 50:1969.

Analytical methods

The analysis of the cheese samples was carried out by the following procedures: moisture, FIL-IDF 4A:1982; protein, FIL-IDF 25:1964; fat, FIL-IDF 5B:1986; lactose, FIL-IDF 43:1967; ash, FIL-IDF 27:1964; NaCl, FIL-IDF 17A:1972; phosphorus, FIL-IDF 33C:1987. Calcium contents were measured by the Raadsveld and Klomp (1971) procedure. D- and L-lactic acids were analysed by the enzymatic method (Noll, 1974) by using the Boehringer Kit (Boehringer Mannheim GmbH, Mannheim, Germany). The AOAC 14022: 1975 method was followed for pH determinations and the AOAC 16228:1975 method for the titratable acidity determinations. Aw values were measured in a DECAGON CX-1 Water Activity System apparatus (Decagon Devices Inc., Pullman, USA) and calculated from the chemical-composition data by using the Marcos *et al.* (1981) and López *et al.* (1990) equations. Total nitrogen (TN) was determined by the Kjeldahl method in a Tecator 1007 + 1002 apparatus (Tecator AB, Höganäs, Sweden). The Vakaleris and Price (1959) procedure was followed for the extraction of soluble nitrogen (SN) and the Johnson method (Lichstein & Oginsky, 1965) for its determination. This latter method was also used to determine the non-protein nitrogen (NPN) after precipitating the proteins with trichloroacetic acid at 12%. The amino nitrogen ($N\text{-}NH_2$) and the ammonia nitrogen ($N\text{-}NH_3$) were analysed as described by Ordóñez (1974). The total nitrogen minus the non-protein nitrogen gave the protein nitrogen (PN). The total nitrogen minus the soluble nitrogen gave the casein nitrogen. The soluble nitrogen minus the non-protein nitrogen gave the proteose-peptone fraction. The non-protein nitrogen minus the amino nitrogen and minus the ammonia nitrogen gave the oligopeptides nitrogen.

Statistical methods

With the aim of differentiating the two cheese varieties from their biochemical parameters, linear discriminant analysis is used. The objective of the discriminant analysis is to weigh, and combine linearly, the discriminating variables (in our case the analytical parameters) in such a way that the groups (in this study the two types of unripe cheese) are forced to be as statistically distinct as possible. In order to do so, the weight coefficients of the discriminant linear function are obtained by maximizing the ratio of the among-groups to within-groups variances. Once discriminant function is obtained, it is possible to calculate its value for each sample (discriminant score) and, from this value, to differentiate the two varieties of cheeses. No evidence of the importance of each variable in the differentiation is assumed in advance.

In order to calculate the discriminant function, the discriminant analysis module of the computer programme SPSS/PC$^+$ version 4.0 (Norusis, 1990) was used. The Wilks method (Bisquerra Alzina, 1989) was used to select the variables which formed part of the discriminant function.

RESULTS AND DISCUSSION

Biochemical characteristics

Table 1 shows the chemical compositions of both varieties. Moisture, fat, protein and lactic acid contents are fairly similar in both types and are found within the range of the values observed for other unripe cheeses (Marcos *et al.*, 1983*b*; Arispe & Westhoff, 1984). The NaCl, ash, calcium and phosphorus contents are, in both varieties, lower than those found in other unripe cheeses (Marcos *et al.*, 1983*b*). The very slight salting,

Table 1. Chemical composition of Cebreiro (n = 24) and Pasiego (n = 24) cheeses[a]

	Cebreiro cheese			Pasiego cheese		
	Average	Range	S.D.	Average	Range	S.D.
Moisture	54·66	43·31–66·00	5·95	59·30	49·67–71·47	6·27
Fat	24·23	15·42–33·47	4·41	22·46	14·41–31·06	5·74
Protein	17·6	12·9–23·4	2·43	13·8	10·2–19·3	2·44
Ash	1·31	0·95–1·62	0·16	1·85	1·43–2·56	0·33
Lactose	1·56	0·00–2·48	0·68	2·03	0·86–3·94	0·87
D-lactic acid	0·137	0·009–0·315	0·097	0·078	0·000–0·220	0·060
L-lactic acid	0·521	0·131–1·023	0·204	0·728	0·000–1·162	0·352
NaCl	0·69	0·37–1·12	0·21	0·40	0·12–0·91	0·21
Ca	0·137	0·073–0·350	0·087	0·405	0·302–0·525	0·050
P	0·247	0·174–0·342	0·040	0·262	0·169–0·397	0·066
Ca/P	0·545	0·291–1·332	0·300	1·628	1·056–2·404	0·404

[a] Components expressed as g/100 g of cheese.

sometimes non-existent, influences the low NaCl and ash contents.

Calcium content in Cebreiro cheese is especially low (0·137 ± 0·087%). This phenomenon can be explained by the acidification of the curd during elaboration. Several authors (Kindstedt & Kosikowski, 1988) have described that, in cheese, calcium is more sensitive than phosphorus to acidity and its losses are greater when pH decreases. Kindstedt (1985), working on Mozzarella cheese observed that, when pH of whey dropped from 6·55 to 5·65, the calcium losses increased by approximately 2 mmol/kg of whey for each 1 mmol/kg of whey which increased the losses of phosphorus.

The low losses of phosphorus, when whey acidity increases, are probably due to the high proportion of covalently bound phosphate in the casein micelle. Almost 40% of phosphate in casein micelles is esterified to αs_1, αs_2, β and K caseins and this phosphate is not solubilized when whey pH is decreased (Walstra & Jenness, 1984). On the other hand, the calcium in casein micelles is found completely in ionic combinations with charged residues of casein, citrate and esterified and non-esterified phosphate (Walstra & Jenness, 1984), and it is much more susceptible to solubilization when pH drops. The low molecular weight of calcium with regard to phosphate ion could also be a cause for its greater mobility (Kindstedt & Kosikowski, 1988).

The curd acidification is shown in the Ca/P ratio values which are lower than 1 (0·545 ± 0·300 in Cebreiro cheese). The values of this ratio in Pasiego cheese (1·628 ± 0·404) are similar to those calculated for other unripe cheeses (Marcos *et al.*, 1983*b*) and indicate low losses of calcium in the whey as corresponds to cheeses elaborated using a predominantly enzymatic coagulation.

As is to be expected in fresh cheeses, both varieties have lactose. This compound is more abundant in Pasiego cheese (2·03 ± 0·87%) than in Cebreiro cheese (1·56 ± 0·68%). The greatest degree of whey drainage in Cebreiro cheese is, without doubt, the main factor responsible for its lower lactose content.

Table 2 shows the values of the most relevant physicochemical parameters. The low pH values of Cebreiro cheese is outstanding (4·29 ± 0·20) which is due to the fact that this cheese is obtained using a predominantly acid coagulation. Pasiego cheese shows higher pH values (5·58 ± 0·60), similar to those measured in other unripe cheeses (Esteban *et al.*, 1982; Marcos *et al.*, 1983*b*; Arispe & Westhoff, 1984) and normal in fresh cheeses obtained using a predominantly enzymatic coagulation.

Table 2. Physicochemical parameters of Cebreiro (n = 24) and Pasiego (n = 24) cheeses

	Cebreiro cheese			Pasiego cheese		
	Average	Range	S.D.	Average	Range	S.D.
pH	4·29	4·03 to 4·77	0·20	5·58	4·69 to 6·88	0·60
Titratable acidity[a]	1·46	0·81 to 2·39	0·39	1·76	0·40 to 2·42	0·67
Aw^b	0·988	0·983 to 0·992	0·002	0·990	0·980 to 1·000	0·005
Aw^c	0·993	0·988 to 0·996	0·002	0·996	0·992 to 1·000	0·001
Aw^d	0·983	0·979 to 0·987	0·002	0·991	0·987 to 0·995	0·002
Aw^c–Aw^b	0·005	−0·001 to 0·009	0·002	0·005	−0·004 to 0·016	0·005
Aw^d–Aw^b	−0·004	−0·001 to −0·010	0·002	0·0009	−0·006 to 0·009	0·004

[a] Expressed as g of lactic acid/100 g of Total Solids.
[b] Measured in a DECAGON CX-1 Water Activity System apparatus.
[c] Calculated by using the Marcos *et al.* (1981) eqn.
[d] Calculated by using the López *et al.* (1990) eqn.

Table 3. Nitrogen fractions of Cebreiro (n = 24) and Pasiego (n = 24) cheeses[a]

	Cebreiro cheese			Pasiego cheese		
	Average	Range	S.D.	Average	Range	S.D.
TN[b]	2·76	2·03–3·66	0·38	2·15	1·60–3·03	0·38
PN	97·1	94·7–98·5	1·18	96·1	93·2–97·8	1·67
NPN	2·92	1·46–5·27	1·18	3·89	2·17–6·78	1·67
SN	7·49	4·04–14·4	2·88	12·4	4·62–22·5	5·77
Casein N	92·5	85·6–96·0	2·88	87·6	77·6–95·4	5·77
Prot.-pept. N	4·57	1·91–9·75	1·96	8·49	2·33–16·4	4·27
N-NH_2	0·67	0·29–1·75	0·34	0·67	0·00–2·08	0·67
Oligopeptides N	1·84	0·49–3·88	0·93	2·84	1·35–4·70	1·07
N-NH_3	0·40	0·00–0·86	0·20	0·38	0·07–0·97	0·25

[a]Expressed as g/100 g of TN.
[b]Expressed as g/100 g of cheese.

The Aw values which both types of cheese show are very high and on the same lines as those determined in similar varieties. These values are to be expected in cheeses with a high moisture content, low NaCl content and low protein degradation.

Little information exists on the water activity of unripe cheese due to the difficulty in measuring this parameter in the 0·98–1·00 range. Labuza *et al.* (1976), on comparing the values obtained using seven different methods, showed the inaccuracy of the Aw experimental measurements when working in a range of between 0·96 and 1·00. This is why, together with the instrumental measurements carried out, it was also decided to calculate the water activity values of 48 cheeses from biochemical parameters using the equations

$$Aw = 1 - 0{\cdot}033 \times m \text{ (Marcos } et\ al.\text{, 1981) and}$$
$$Aw = 0{\cdot}9719 - 0{\cdot}0044\ [NaCl] + 0{\cdot}0041\ pH$$
(López *et al.*, 1990).

When the first equation is used, average values of 0·005 units greater than those experimentally determined, are obtained. Only in one unit of Cebreiro cheese and four units of Pasiego cheese was the calculated value lower than that experimentally determined one. This shows that NaCl is not the only solute which acts as a depressor of the water activity in these cheeses, a phenomenon already observed by other authors (Marcos *et al.*, 1983*a*) working on cheeses with similar characteristics. The correlation coefficients between Aw values and NaCl contents, expressed as g/100 g of moisture, were r = –0·18 for Pasiego cheese and r = –0·45 for Cebreiro cheese. These figures corroborate the small part played by NaCl in the depression of water activity in these cheeses. This phenomenon could be influenced by the low salt contents of both varieties.

When the Aw values are calculated using the equation proposed by López *et al.* (1990), the figures obtained in Cebreiro cheese were on average 0·004 units lower than those experimentally determined. In Pasiego cheese this equation gave values higher than those experimentally determined in 13 of the 24 units studied, a value equal to that experimentally determined in two cheeses and values higher than those determined in the remaining nine.

With regard to the depressing effect, which the other chemical components cause, on the Aw in both varieties: in Pasiego cheese the nitrogen compounds of low molecular weight seem to have an important depressing effect judging from the existing correlation coefficients calculated between the values of water activity and soluble nitrogen (r = –0·69), nonprotein nitrogen (r = –0·76) and amino nitrogen (r = –0·79) contents, when these compounds are expressed as g/100 g of moisture. No significant correlation was observed in Cebreiro cheese which suggests a special intervention of some component in the depression of water activity.

The results obtained allow us to demonstrate once more the inaccuracy of the systems used in determining water activity and to reaffirm previous observations, made by other authors (Labuza *et al.*, 1976), of the doubtful validity of the third decimal figure in the data given in the literature on water activity in different foods.

Table 3 shows the values of different nitrogen fractions in the two varieties of cheese. The values obtained indicate a low protein degradation as is to be expected in unripe cheeses.

The values of nonprotein, amino and ammonia nitrogen, indicators of proteolysis intensity, are within the range of those observed in other Spanish unripe cheeses (Marcos *et al.*, 1983*b*). With regard to the extension of the proteolysis, indicated by the soluble nitrogen value, it is similar in Pasiego cheese to the rest of the cheeses mentioned. However, Cebreiro cheese shows average values of soluble nitrogen which are considerably lower. This is fundamentally due to its low content of large-sized peptides (proteose-peptone fraction) and could be attributed to the small quantities of rennet used in its elaboration. It is worth recording that the rennet which remains in the curd after whey drainage is responsible for the initial attack on caseins, forming large-sized peptides which are later degraded by the action of microbial and milk-autochthonous enzymes. Gripon *et al.* (1975), after chromatographic study of pH 4·6 soluble nitrogen present in aseptic curds, which were made as described by Le Bars *et al.* (1975), observed that the peptides with a molecular weight lower than 3000 Daltons only represented 28% of this fraction; 50% were made up of peptides with molecular weights of between

Table 4. Some correlation coefficients (*r*) obtained from the correlation matrix for variables analysed in the 48 cheeses

Variables	*r*
Moisture—Protein	–0·58
Moisture—Fat	–0·91
Moisture—Lactose	0·53
Moisture—Aw	0·54
Protein—Lactose	–0·55
Fat—Ash	–0·50
Fat—Aw	–0·51
Fat—SN	0·50
Fat—N-NH_2	0·50
Fat—Proteose-peptone N	0·55
Ash—pH	0·72
Ash—N-NH_2	–0·54
Lactose—pH	0·57
pH—Aw	0·50
Aw—N-NH_2	–0·58
SN—NPN	0·89
SN—N-NH_2	0·83
SN—Proteose-peptone N	0·98
SN—Oligopeptides N	0·84
NPN—N-NH_2	0·86
NPN—Proteose-peptone N	0·79
NPN—Oligopeptides N	0·95
N-NH_2—Proteose-peptone N	0·76
N-NH_2—Oligopeptides N	0·71
Proteose-peptone N—Oligopeptides N	0·74

3000 and 5000 Daltons; and 20% were made up of peptides with a molecular weight higher than 5000 Daltons. O'Keefe *et al.* (1976) equally showed that soluble nitrogen at pH 4·6 in aseptic Cheddar cheese fundamentally contains large-sized peptides.

Another reason for this circumstance could be the inhibition of the unspecific rennet activity at pH values observed in Cebreiro cheese. Noomen (1978) proved that the most favourable conditions for rennet action on αs_1-casein in the cheese occurs at pH values of about 5. Similar observations were made by Mulvihill and Fox (1977) working *in vitro*. With regard to the action on β-casein, Mulvihill and Fox (1978) proved that the rennet effect is also strongly influenced by pH and that this casein is optimally hydrolyzed to give way to peptide β-I at a pH value of 6·4. Noomen (1978) also proved that, in the pH range between 4·85 and 5·75, β-casein degradation by rennet action in the cheese was slight, but significantly increased at higher pH values. In agreement with the observations of these authors, pH values observed in Cebreiro cheese (4·29 ± 0·20) were not the most favourable for rennet action on the caseins.

The most relevant interdependences between analysed components (correlation coefficients higher than 0·5) drawn from the pooled within-group correlation matrix are shown in Table 4. Most of these correlations involved nitrogen fractions.

Discriminant analysis

The following discriminant function was obtained:
D = 8·75 (D-lactic acid) + 2·17 (NaCl) – 8·10 (Ca) + 8·39 (P) – 2·40 (pH) + 216·35 (Aw) – 1·72 (N-NH_3) – 203·53.

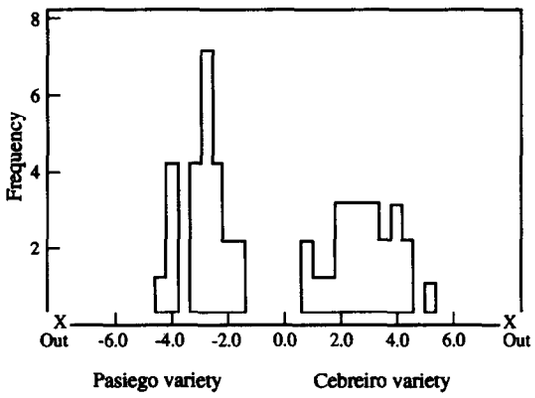

Fig. 1. Histogram of the discriminant scores of Cebreiro and Pasiego cheeses.

The Cebreiro variety cheeses show positive discriminant scores with values between 0·68 and 5·35 and an average value of 2·88. The Pasiego variety cheeses show negative discriminant scores with values between –1·52 and –4·43 and an average value of –2·88.

Figure 1 shows the histogram of discriminant scores versus frequency for each cheese variety. It can be observed that there is no overlapping of the groups, which indicates that the two cheese types are clearly discriminated. This observation is ratified by the classification summary which is shown in Table 5. The percentage of well-classified cases is 100% which indicates the effectiveness of the discriminant function.

Some of the variables involved in the discrimination of the two cheese types are directly related to the elaboration technology: NaCl contents depend on the intensity of salting; Ca contents and pH value depend on the type of coagulation used (acid or enzymatic). D-lactic acid content is probably related to the type of flora present, studies carried out by Thomas & Crow (1983) on Cheddar cheese show that isolated pediococci and homofermentative lactobacilli have two stereospecific lactate dehydrogenases which cause the × transformation of L(+)-lactate to D(–)-lactate.

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Table 5. Classification results

Actual group	No. of samples	Predicted group membership: Cebreiro cheese	Pasiego cheese
Cebreiro cheese	24	24	0
		100·0%	0%
Pasiego cheese	24	0	24
		0%	100·0%

Percent of 'grouped' cases correctly classified: 100·00%

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