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Influence of animal and vegetable rennet on the physicochemical characteristics of *Los Pedroches* cheese during ripening

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Abstract

The effects of rennet type (animal or vegetable) on the physicochemical characteristics (moisture, fat, protein, lactose, lactic acid, NaCl, ash, water activity, pH and nitrogen fractions: SN, NPN, AAN and NH_3-N) of "Los Pedroches" cheese manufactured with pure ewes' milk were investigated. Changes were observed during 100 days of ripening. Analysis of the covariance was carried out, using these chemical composition data, expressed as a function of the dry matter. The rennet type influenced moisture, protein, and water activity values (higher in the cheeses manufactured with animal rennet), fat and SN (higher in cheeses manufactured with vegetable rennet). There was a significant rising trend in the levels of lactic acid, ash, NaCl, and nitrogen fractions during ripening, while a significant decrease was observed in the moisture, lactose, pH and a_w values.

Keywords: Los Pedroches cheese; Vegetable rennet; Physicochemical composition; Ripening course

1. Introduction

Los Pedroches cheese, one of the most representative Spanish artisanal ewes' milk types, is manufactured in the north of the Andalucía region (Córdoba, Spain). Its production is seasonal (from December to May), and it can be described as an uncooked hard-paste and fatty-ripened cheese, obtained from raw Merino ewes' milk, having no addition of lactic cultures and being normally clotted with vegetable rennet. During ripening, a sequence of physical, chemical, microbiological and organoleptic changes occurs which gives rise to a cheese with an aroma and slightly hot and creamy taste; this is very desirable to consumers, especially if the cheese is manufactured with vegetable rennet. Los Pedroches cheese is included in the list of *traditional foods* presented by Spain to the European Union.

Some aqueous extracts of dry flowers from wild thistles that belong to various species of the genus *Cynara* L. have been used to produce several varieties of Spanish, Portuguese, French and Italian ewe cheeses (Barbosa, Valles, Vassal, & Mocquot, 1976). The species *Cynara cardunculus* L., that grows spontaneously in the Mediterranean and Macaronesian regions (Madeira and Canary Islands), is normally used to produce the Portuguese cheeses Serra and Serpa (Macedo, Malcata, & Oliveira, 1993; Vieira de Sá & Barbosa, 1972), and the Spanish cheeses, Los Pedroches, La Serena, Torta del Casar (from ewes' milk), Los Ibores (from goats' milk) and Flor de Guía (mixture of ewes' and cows' milk; Fernández-Salguero, Sanjuán, & Montero, 1991). The other species of wild thistle, the *Cynara humilis* L., is more abundant and it is used for some artisanal cheeses too, especially when *C. cardunculus* is scarce. In some cases they are used together.

Heimgartner, Pietrzak, Geertsen, Brodelius, da Silva, and Pais (1990), purified and characterized the proteinases of *C. cardunculus*. They are three aspartic proteinases called "Cyprosins" (Cordeiro, Jakob, Puhan, Pais, & Brodelius, 1992), or "Cardosins" (Pires et al., 1994), where the number 3 is the one that shows the most similar characteristics to chymosin, showing considerably

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higher clotting activity and specific hydrolysis of proteins, more in ewe's milk than in cow's milk one. This accords with the fact that the typical cheeses manufactured in Spain and Portugal with *C. cardunculus*, either from ewe's or goat's milk, do not show a marked bitter flavour, while cheeses produced from cow's milk tend to have a bitter taste (Barbosa et al., 1976).

In a detailed study about the chemical changes that take place during ripening of Los Pedroches cheese manufactured with animal and vegetable rennet (Sanjuán, 1992), the influence of some factors on the clotting time using vegetable rennet (*Cynara* sp.; Sanjuán & Fernández-Salguero, 1994; Sanjuán, Millán, Fernández-Salguero, Castelo, Cardona, & Estupiñán, 1994) as well as the changes in the fatty acids (Sanjuán, Millán, Gómez, & Fernández-Salguero, 1995), the mineral composition (Sanjuán, Saavedra, Millán, Castelo, & Fernández-Salguero, 1998) and the principal nitrogenous components (Fernández-Salguero & Sanjuán, 1999), were investigated. Several studies have been also made on the biochemistry of the ripening process of Los Pedroches cheeses clotted with animal rennet (Fernández-Salguero, 1975, 1978a, 1978b, 1978c) and on the characterization of commercial Los Pedroches cheese (Fernández-Salguero, Esteban, & Marcos, 1977; Fernández-Salguero & Marcos, 1977; Marcos, Esteban, & Fernández-Salguero, 1977). Carmona (1994) studied the influence of processes such as the addition of starter cultures (Carmona, Sanjuán, Gómez, & Fernández-Salguero, 1999), or use of different ripening conditions, on the proteolysis and sensory quality of these cheeses. Vioque, Gómez, Sánchez, Mata, Tejada, and Fernández-Salguero (2000) compared chemical and microbiological characteristics in Los Pedroches cheese, manufactured with both extracts from flowers (*Cynara cardunculus* and *Cynara humilis*) as milk coagulants.

The evolution of the physical and chemical characteristics of Los Pedroches cheese (moisture, fat, protein and fractions, lactose, lactic acid, ash, salt, water activity and pH) has been studied in this work during 100 days of ripening; the influence that the type of rennet used in these cheeses manufacture (animal or vegetable) was also determined. This work provides valuable preliminary scientific knowledge to be used, in subsequent studies, to characterize the vegetable rennet (*Cynara* sp.) used in the manufacture of some European ewes' cheeses (French, Spanish, Italian and Portuguese).

2. Materials and methods

2.1. Cheesemaking procedure

A producer from Los Pedroches Valley area (Córdoba, Spain) manufactured, at a traditional factory, four batches of 10 cheeses each, from unpasteurized Merino ewes' milk to which no starter cultured was added. These were matured in the winter–spring characteristic environmental conditions of the production zone. Starting from the milk of 1 day's milking, half of it was clotted using an animal rennet (commercial rennin powder, Ha-bo from Chr. Hansen) according to the instructions of the manufacturer, and the other half, using a vegetable rennet (aqueous extract of the thistle *Cynara cardunculus* L.), to give two batches of 10 cheeses each. Another two batches were produced on a different day but under the same conditions.

The aqueous extract from the thistle *C. cardunculus* L. was prepared from 70 g of dried flowers in 1 l of water, leaving it to macerate during 24 h and adding about 8 ml of this extract per litre of milk in order to obtain the clotting in 80–90 min at 30 °C ± 1 °C. After pressing, the salting was achieved by rubbing dry salt into the surface of the cheeses. The ripening took place at a temperature from 5 to 10 °C and the relative humidity during the first 12–15 days of the lactic fermentation ranged from 70 to 80%; later, the temperature ranged from 10 to 20 °C and the relative humidity remained between 75 and 85%. The temperature and relative humidity were measured by means of a thermohydrograph.

At intervals during ripening (2, 4, 7, 14, 22, 30, 43, 60, 80 and 100 days), one cheese from each of the four experimental batches was carried to the laboratory for analysis.

2.2. Physicochemical analysis

The moisture content was determined indirectly by desiccation in a hot air incubator, to constant dry weight (British Standards Institution, B.S. 770, 1963), following the technique described by Kosikowski (1970). We have used AOAC methods (1980) to determine the protein (16.245), the acidity expressed as lactic acid (16.247), ash (16.241) and total chlorides (NaCl; 16.244), modifying the Volhard method with the Kosikowski (1970) silvermetric technique as described by Fernández-Salguero (1975). The fat content was estimated by the Gerber method as described by the Netherlands Standard NEN 3059 (1969). Lactose was determined by the Somogyi method for reduced carbohydrates. The metabolising energy (heat value) was calculated by applying the conversion factors proposed by the UK Ministry of Agriculture, Fisheries and Food, Food Standards Committee (1976).

The water activity (a_w) was measured with a Decagon Devices CX-1 dew-point hygrometer (Pullman, St. Paul, Minnesota, USA). The pH was measured with a Beckman 3800 digital pH-meter on 1:1 (w/v) aqueous extracts of cheese.

The nitrogen fractions, including soluble nitrogen (SN) at pH 4.6, non-protein nitrogen (NPN) in 12% TCA, and ammonia nitrogen (NH_3-N), were prepared

and analysed as described by Fernández-Salguero et al. (1991) and Sanjuán (1992). Amino acid nitrogen (AAN) was determined by the procedure of Reiter, Sorokin, Pickering, and Hall (1969).

All samples were analysed in duplicate.

2.3. Statistical analysis

The following analysis of the covariance model was used for each physicochemical variable considered:

$$(\mathrm{M})y_{ijk} = \alpha_i + \beta\ t_j + e_{ijk};\ \ i = 1, 2; j = 1, \ldots, 10; k = 1, 2$$

where y_{ijk} denotes the physicochemical variable value of the kth experimental cheese, manufactured with ith rennet at the jth control; α_i represents the effect of the rennet (animal or vegetable), t_j is the ripening time in the control j, and e_{ijk} is the random error that we suppose normally distributed with mean zero and standard deviation, σ. In order to analyse if there was an effect of the rennet type, the contrast with the null hypothesis $H_{\alpha 0}$:$\alpha_1 = \alpha_2$ was carried out. In the same way, the null hypothesis $H_{\beta 0}$:$\beta = 0$ was contrasted to determine if there was a tendency during ripening, and an estimation point of this parameter was obtained.

The statistical calculations have been made using the statistical package SAS (1988).

3. Results and discussion

Table 1 shows the values of the raw chemical composition of Los Pedroches cheeses produced with animal and vegetable rennet, obtained over 100 days of ripening time. In the same way, the NaCl, a_w and pH values of these cheeses are given in Table 2. Total nitrogen (TN) and different soluble fractions (SN, NPN, AAN and NH_3-N, all as percentages of TN) are showed in

Table 1
Changes in the chemical composition and metabolisable energy of Los Pedroches cheese produced with animal[a] or vegetable[a] rennet, during ripening

Ripening days	Moisture (g/100 g cheese)		Fat (g/100 g DM)		Protein (g/100 g DM)		Lactose (g/100 g DM)		Lactic acid (g/100 g DM)		Ash (g/100 g DM)		ME (kcal) (g/100 g DM)	
	A	V	A	V	A	V	A	V	A	V	A	V	A	V
2	50.50	51.63	49.81	49.16	38.11	37.73	3.89	4.36	0.92	1.02	7.27	7.72	616	610
4	49.25	48.17	48.60	52.00	41.0	37.6	1.59	2.00	1.36	1.29	7.49	7.13	607	626
7	48.38	48.41	51.38	51.01	39.11	38.2	0.26	1.91	1.59	1.60	7.67	7.30	620	620
14	47.89	46.78	47.93	54.48	41.9	36.32	0.00	0.00	2.08	2.12	8.13	7.07	599	637
22	46.02	44.95	49.98	52.79	40.6	37.90	0.00	0.00	1.71	1.96	7.71	7.34	613	628
30	43.65	42.47	52.32	52.77	38.63	37.63	0.00	0.00	1.89	1.90	7.15	7.70	625	626
43	43.88	40.28	48.69	53.21	40.8	37.5	0.00	0.00	2.49	2.14	8.03	7.20	602	630
60	40.48	40.10	49.96	51.04	39.50	38.53	0.00	0.00	2.63	2.82	7.91	7.61	608	614
80	38.26	36.63	47.99	52.00	40.93	37.7	0.00	0.00	2.82	2.74	8.26	7.60	596	619
100	35.25	34.10	48.19	50.10	40.8	38.3	0.00	0.00	2.84	3.01	8.22	8.61	597	604

DM, dry matter; ME; metabolisable energy; A, animal; V, vegetable.
[a] Mean values for four experiments from two cheeses obtained under the same conditions.

Table 2
Changes in NaCl content, a_w and pH of Los Pedroches cheese produced with animal[a] or vegetable[a] rennet, during ripening

Ripening time (days)	NaCl (g/100g dry matter)		a_w		pH	
	Animal	Vegetable	Animal	Vegetable	Animal	Vegetable
2	2.88	3.19	0.979	0.975	5.6	5.6
4	2.66	2.74	0.976	0.971	5.4	5.4
7	2.64	2.94	0.974	0.972	5.4	5.4
14	3.28	3.34	0.960	0.964	5.3	5.3
22	3.19	3.08	0.962	0.959	5.4	5.4
30	2.98	3.60	0.960	0.952	5.4	5.4
43	3.32	3.08	0.953	0.948	5.3	5.5
60	3.56	3.18	0.942	0.943	5.2	5.2
80	3.63	3.30	0.937	0.930	5.1	5.2
100	3.10	3.73	0.929	0.908	5.1	5.1

a_w, water activity.
[a] Mean values for four experiments from two cheeses obtained under the same conditions.

Table 3
Changes in TN and the different soluble nitrogen fractions (SN, NPN, AAN and NH3-N) in Los Pedroches cheese batches obtained with animal[a] rennet or vegetable[a] rennet during ripening

Ripening time (days)	TN (g/100 g DM)		SN (g/100 g DM)		NPN (g/100 g DM)		AAN (g/100 g DM)		NH_3-N(g/100 g DM)	
	A	V	A	V	A	V	A	V	A	V
2	5.97	5.92	12.9	28.2	5.10	6.32	1.39	1.75	0.30	0.32
4	6.42	5.89	13.4	35.0	6.00	6.82	1.64	2.28	0.48	0.50
7	6.13	5.99	15.0	39.7	6.84	7.10	1.73	2.78	0.60	0.64
14	6.56	5.69	26.3	42.1	12.6	9.86	2.40	3.61	0.50	0.64
22	6.36	5.94	27.2	46.8	11.4	15.6	3.06	4.55	0.82	1.03
30	6.06	5.90	34.9	44.4	14.3	12.3	4.70	5.00	1.55	1.66
43	6.39	5.87	35.5	45.5	17.7	15.9	6.64	5.40	2.33	2.49
60	6.19	6.04	42.1	61.4	18.6	20.35	7.37	6.53	2.87	3.63
80	6.42	5.90	34.7	53.0	25.6	22.1	10.7	10.5	3.10	3.54
100	6.39	6.00	35.7	49.5	23.6	23.5	13.8	12.5	4.38	5.04

TN, Total nitrogen; SN, soluble nitrogen; NPN, non-protein nitrogen; AAN, amino acid nitrogen; NH_3-N, ammonia nitrogen; DM, dry matter; A, animal; V, vegetable.

[a] Mean values for four experiments from two cheeses obtained under the same conditions.

Table 4
Estimations and contrasts for the parameters of the model (M)

Variable	*P* values		β-Estimate
	$H_{\alpha 0}$:$\alpha_1=\alpha_2$	$H_{\beta 0}$:$\beta=0$	
Moisture (1)	0.0348	0.0001	−0.155±0.008
Fat (2)	0.0005	0.2141	−0.012±0.010
Protein (2)	0.0001	0.2745	0.008±0.007
Lactose (2)	0.5152	0.0006	−0.022±0.006
Lactic acid (2)	0.8301	0.0001	0.018±0.002
Ash (2)	0.0819	0.0006	0.008±0.002
NaCl (2)	0.3709	0.0010	0.006±0.002
a_w	0.0001	0.0001	−0.0006±0.00002
pH	0.4291	0.0001	−0.004±0.0006
SN (3)	0.0001	0.0001	0.225±0.040
NPN (3)	0.8150	0.0001	0.192±0.012
AAN (3)	0.5480	0.0001	0.113±0.004
NH3-N (3)	0.0640	0.0001	0.044±0.002

$H_{\alpha 0}$:$\alpha_1=\alpha_2$, Rennets comparison; $H_{\beta 0}$:$\beta=0$, Trend in the ripening time. (1), g/100 g cheese; (2), g/100 g dry matter; (3), g/100 g total nitrogen; SN, soluble nitrogen; NPN, non-protein nitrogen; AAN, amino acid nitrogen; NH_3-N, ammonia nitrogen.

Table 3. The contrasts and estimations referred to in Section 2.3, for each of the variables considered, are showed in Table 4. Figs. 1–5 show, in a graphic way, the effect produced by the type of rennet over the ripening time, but only in those variables in which a significant difference (Table 4) between animal and vegetable rennet, was found (moisture, fat, protein, a_w, and SN). The data and the corresponding fits by means of the covariance model (*M*) (*2.3.*), are showed in these figures. To obtain a better observation about the effects produced by the ripening and the rennet used (animal or vegetable), the results are expressed as g/100 g of dry matter.

It is noticeable that the effect produced by the rennet type (animal or vegetable) used to manufacture Los Pedroches cheese, had a significant influence on the water content, with a *P*-value of 0.0348 (Table 4), showing, in general, higher values in the cheeses where animal rennet was used (Table 1, Fig. 1). It could be deduced that the vegetable rennet made a structure with the milk components that retained less liquid during the de-wheying stage of the cheese.

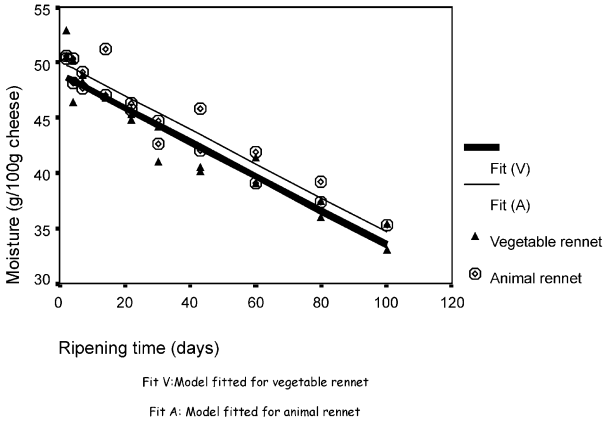

Fig. 1. Effect of type of rennet (animal or vegetable) on the moisture content along ripening time of Los Pedroches cheese.

Fig. 1 shows a decreasing trend in the ripening time for moisture with a *P*-value of 0.0001 (Table 4), in batches produced with animal and vegetable rennet. This is due to the natural de-wheying tendency and to the influence of the drying environmental conditions.

Based on the mean values of M/NFS (moisture in non fat solids) reached at the end of the ripening time of this study (60–100 days) and according to the FIL/IDF (1981), the cheeses either manufactured with vegetable (55.1±2.83 g/100 g) or animal rennet (54.8±2.29 g/100 g), are considered *hard cheeses*.

Fat in the cheese is a major component that influences, not only its physical properties, but mainly the

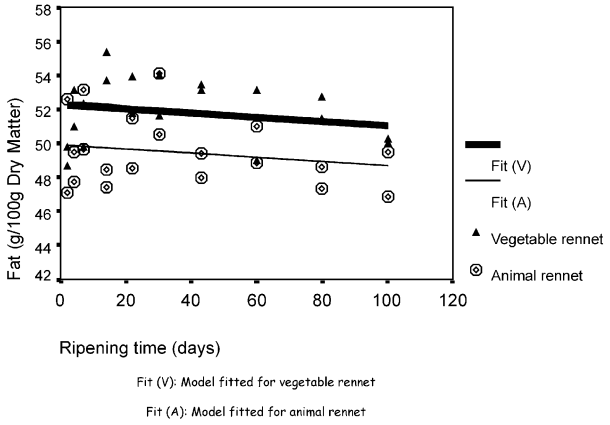

Fig. 2. Effect of type of rennet (animal or vegetable) on the fat content along the ripening time of Los Pedroches cheese.

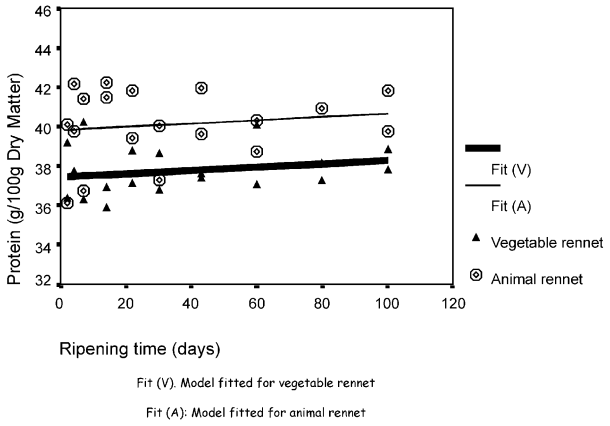

Fig. 3. Effect of type of rennet (animal or vegetable) on the protein content along the ripening time of Los Pedroches cheese.

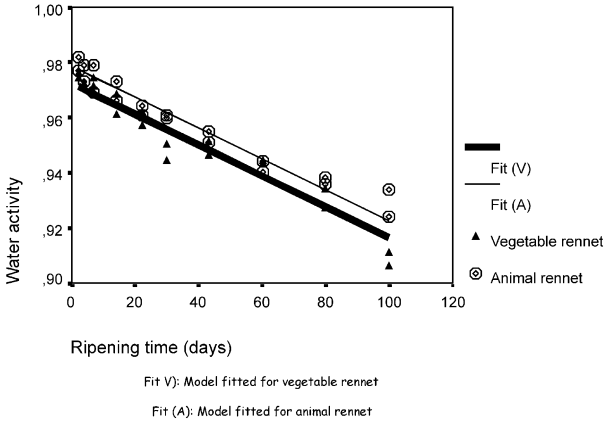

Fig. 4. Effect of type of rennet (animal or vegetable) on the a_w along the ripening time of Los Pedroches cheese.

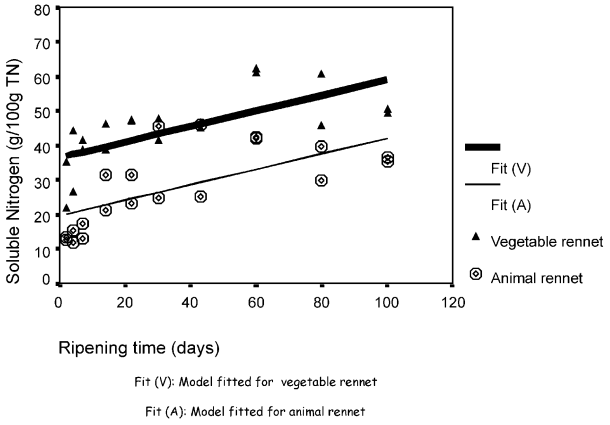

Fig. 5. Effect of type of rennet (animal or vegetable) on the soluble nitrogen (SN) content along the ripening time of Los Pedroches cheese.

development of the flavour. The statistical study (Table 4) showed a significant difference for the fat content between cheeses manufactured with animal and vegetable rennet (with a *P*-value of 0.0005). This component was higher in the cheeses manufactured with vegetable rennet (Table 1, Fig. 2). Since all the cheeses were manufactured from the same milk, there seems to be a stronger capacity for capturing fatty components in the curd with the vegetable rennet. This higher fat rate may explain the finer texture and greater greasiness described for the cheeses produced with vegetable rennet when they were sensorially evaluated (Sanjuán, 1992).

The percentage of fat in dry matter in the cheeses at the end of the studied period (60–100 days), manufactured with both type of rennets, indicates that they can be ranked as *fatty cheeses* (CAE, 1991).

The difference found in the protein contents of the cheeses produced by the two rennets was highly significant (*P*-value of 0.0001, Table 4); higher values were showed in those manufactured with animal rennet (Table 1, Fig. 3). This fact, together with the significant difference found for the a_w of these cheeses, higher when elaborated with animal rennet (Table 2, Fig. 4), suggests greater proteolytic activity from the vegetable enzyme rennet (cyprosin) than from the animal one (chymosin), producing more depressor compounds of low molecular weight (peptones, peptides and aminoacids) which reduces the a_w.

In contrast to the results of this study, some authors have found lower fat contents in cheeses produced, under the same conditions, with vegetable rennet than with animal rennet, by using apple leaves (*Calotropis procera*; Aworth & Muller, 1987) or ash gourd proteinase (Gupta & Eskin, 1977). Aworth and Muller (1987) even found more moisture and protein in those cheeses produced with vegetable rennet.

Quantitatively, lactose is the most important carbohydrate in milk. Its presence is transitory in the cheese, since it disappears quickly from the curd due to the

glycolytic fermentation by the micro-organisms producing mainly lactic acid, which determines the cheese acidity and favours rennet action, clot retraction and dewheying, giving the curd certain physical characteristics that influence the final flavour and texture. It also inhibits the growth of some types of micro-organisms that produce gas and undesirable flavours and aromas. Table 1 shows that lactose appeared in the first controls in both kinds of batches, diminishing progressively until the 7th day of ripening. This decrease is significant (*P*-value of 0.0006, Table 4) along the ripening time. According to Webb and Johnson (1965), the presence of lactose will only be possible in the first 2 weeks of ripening in some soft cheeses.

Lactic acid, in contrast to lactose, increased in a significant way (*P*-value of 0.0001, Table 4) with ripening of the cheeses, by transformation of the initial lactose present in the cheese. However, it is noticed that there is a slight increase in lactic acid until the 100th day of ripening (Table 1), instead of the disappearance of lactose from the 14th day. This phenomenon is due to the metabolism of part of the glucose from the lactose hydrolysis, by the hexose-diphosphate route, which produces lactic acid during the whole ripening course (Dixon, Fox, & Daly, 1980).

Ash in the cheese is composed of salt, usually added to the curd, and the mineral components derived from the sodium, potassium, calcium and magnesium chlorides phosphates and citrates, that compose the milk salts and intervene in its organic and inorganic constitution. During ripening, the ash values (Table 1) increased progressively with a *P*-value of 0.0006 (Table 4) in batches manufactured either with animal or vegetable rennet.

Fernández-Salguero (1975), analysing batches of Los Pedroches cheese manufactured with animal rennet, noticed a moisture decline from 54 to 36 g/100 g of cheese, and values of 19.7–24.1 g/100 g of cheese for protein over 2 months of ripening. The mean values found in the mentioned work at the end of the studied period (67 days), were 31.9 g/100 g of cheese for fat, 1.5 g/100 g of cheese for lactic acid and 6.2 g/100 g of cheese for ash. These values were quite similar to those found in the present study for the same ripening time, excepting for ash, which was lower in our cheeses.

The addition of sodium chloride to the cheese contributes to the flavour in the final product and selectively regulates the microbial activity, too, controlling the development of undesirable organisms (Loren & Free, 1970).

Changes in NaCl content showed a rising tendency in both types of batches (*P*-value 0.001, Table 4), due to the influence of environmental conditions, pH of the cheese, grain size when cut, and initial moisture in the curd (Geurts, Walstra, & Mulder, 1980), on the capture of salt taken during ripening. The main cause of the high variability observed in the salt content during ripening of Los Pedroches cheese (Table 2), in this case is, without any doubt, the fact that it is an added product (dry salting) and therefore its rate is regulated mainly by the manufacturing technology. These salt values are lower than the ones found by Fernández-Salguero (1975) in Los Pedroches cheese during 2 months of ripening. Actually, the tendency is to manufacture less salty food products, which probably explains these observed differences for the same cheese variety.

Water activity and pH are factors that have a great influence on cheese stability and they condition the development of the micro-organisms, the enzymatic activity and the rate of the biochemical reactions during the ripening time.

The effect produced by the rennet type (animal or vegetable) in manufacture of Los Pedroches cheese, had a significant influence on the a_w content, with a *P*-value of 0.0001 (Table 4). Also, it is noticeable that the a_w mean values decreased progressively in both kinds of batches (*P*-value of 0.0001, Table 4).

Though, initially, the values of this variable in both types of batches were similar (Table 2, Fig. 4), they declined until the end of the maturity appreciably, and more so with vegetable rennet. The decline is a consequence of factors such as a decrease in the moisture content, the salt diffusion from the surface towards the inside of the cheese, the progressive formation of compounds of low molecular weight, such as lactic acid at the beginning of the process, and of non-protein N compounds in more advanced maturing stages.

The change in the mean pH values observed, was similar for the cheese batches produced with either animal or vegetable rennet (Table 2). In both, the pH follow in the ripening time in a general and significant downward trend (*P*-value 0.0001, Table 4). The values declined until the 14th maturing day (as a consequence of the lactic acid production, due to the microbial fermentation from the lactose content), then, rose until the 30th and the 43rd day in animal and vegetable batches, respectively (owing to the microbial metabolisation of lactic acid and the production of ammonia as a consequence of the deamination), and from then onwards, it fell until the last determination, probably due to the production of several carbonated compounds, from the energetic metabolism of lactic bacteria. The same pH course has been observed by several authors (Fernández-Salguero, 1975; Mansour & Alais, 1972; Pérez-Elortondo, Aldamiz-Echobarria, Albisu, & Barcina, 1998) although there are references showing other pH tendencies during cheese ripening.

The values of a_w and pH found at the end of the ripening time in the cheeses studied, together with the combination of the metabolic products and salt levels, classifies Los Pedroches cheeses, in general, as preserved products.

In a study carried out by Núñez, Fernández del Pozo, Rodriguez-Marín, Gaya, and Medina (1991) on the effect of animal and vegetable rennets on the characteristics of ewes' cheese of La Serena (Extremadura, Spain) during 60 days ripening, as in our case, a greater amount of moisture was detected in the cheeses produced by animal rennet and a declining course of it in both types of batches. The salt changes (NaCl) had a rising trend on the inside of the cheeses manufactured with animal or vegetable rennet, with a tendency to equilibrium on the outside. Sousa and Malcata (1997), in a study comparing the effects of animal and plant rennets on characteristics of ovine cheese, concluded that the type of rennet had no significant effect on cheese composition (moisture, fat, protein, salt and pH).

The proportion of total SN has traditionally been regarded as a "ripening index" for cheese as it reflects the extent of proteolysis.

It is noticed (Fig. 5), that SN values, differed markedly between the cheeses coagulated with the two types of rennet. Casein hydrolysis was found to be much more extensive and faster in cheese made with vegetable rennet (Table 3). After 2 days of ripening, the SN values for the cheeses made using vegetable rennet were more than double those of samples produced with animal rennet. The amount of SN increased gradually until 60 days, after which it levelled off in both types of cheese. The mean SN values for the last three samples analysed (at 60, 80 and 100 days after manufacture) showed that cheese made with vegetable rennet contained about 30% more soluble nitrogen than that made with animal rennet, with *P*-value 0.0001 (Table 4).

The low initial levels of SN in the cheese made using animal rennet are the result of the curdling mass undergoing low microbial activity at the early stages of ripening; also, the protein solubilization observed can be ascribed to the action of proteolytic enzymes in the rennet, which give rise to the formation of high-molecular weight products. On the other hand, the high levels of SN found in our cheese batches produced using vegetable rennet suggest an increased proteolytic activity of cyprosins rather than the involvement of microbial endo- and exo-proteases, since the difference was observed throughout the ripening process.

Interestingly, NPN values and their changes during ripening were very similar for both types of rennet (Table 3). The similarity of NPN contents between the two types of cheese suggests that the production of nitrogenous compounds of low molecular weight is independent of the type of rennet used; these compounds arise largely from the activity of microbial enzymes present in the cheese.

The influence of the ripening time on NS and NPN of Los Pedroches cheeses shows a significant rising trend (*P*-value 0.0001, Table 4).

A previous study (Fernández-Salguero, 1978a) on Los Pedroches cheeses, which were manufactured with animal rennet and ripened for 67 days at a higher ambient temperature, showed higher SN and NPN levels.

Malcata and Freitas (1995), in a study about the influence of some conditions on the characteristics of Picante da Beira Baixa cheese, concluded that vegetable rennet increased proteolysis compared with animal rennet. The SN and NPN fractions in La Serena cheese, which is very similar to Los Pedroches cheese and is produced using *C. cardunculus* L. proteinases, after 60 days of ripening, were found at levels similar to ours (Fernández del Pozo, Gaya, Medina, Rodriguez-Marín, & Núñez, 1988). A different study of La Serena cheese during ripening (Núñez et al., 1991) showed higher levels of SN and NPN than those obtained in this work although, consistent with the present results, the NPN levels were similar for the two types of rennet, and the NS values showed that cheeses made with vegetable rennet contained more SN after 60 days of ripening than cheese produced with animal rennet. These results are not in agreement with those obtained by Sousa and Malcata (1997), showing NPN values significantly higher in cheeses manufactured with animal rennet than those made with *C. cardunculus* at 70 days of ripening.

Unlike levels of SN, the AAN values, with both types of rennet (Table 3), initially increased more markedly in the cheese made with vegetable rennet but then levelled off in both types of cheese. The levels of AAN continued to rise throughout the study (*P*-value 0.0001, Table 4) in such a way that amino acids were the most quantitatively significant compound group in the NPN fraction after 80 days of ripening.

La Serena has reported contents (expressed as nitrogen soluble in phosphotungstic acid, PTA-N) above those in Table 3 (Fernández del Pozo et al., 1988; Núñez et al., 1991).

NH_3-N values also increased from the beginning of ripening (Table 3) in cheese made with animal and vegetable rennet (*P*-value 0.0001, Table 4). The low proportion of ammonia nitrogen at the beginnig of ripening suggest that deaminase activity was quite low, which can be ascribed to the pH of these cheeses (Table 2) being well below the optimum value for the enzyme (Sanjuán, 1992).

The degrees of proteolysis, in terms of NPN or its main components (peptides, aminoacids and ammonia), were similar in cheeses produced using animal or vegetable rennets.

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