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Characteristics of Burgos and Hispánico cheeses manufactured with calf rennet or with recombinant chymosin

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Recombinant chymosin coagulated milk more rapidly than calf rennet in the manufacture of Burgos cheese, but the type of coagulant had no effect on cheese yield, moisture or pH. Higher levels of residual α_{s1}- and β-casein were found for Burgos cheese made with recombinant chymosin, whereas no differences in soluble nitrogen fractions nor in cheese flavour were detected.

Milk coagulation time was shorter with calf rennet than with recombinant chymosin in the manufacture of Hispánico cheese. Cheese yield, moisture, pH, soluble nitrogen fractions and residual α_{s1}-casein in Hispánico cheese were not influenced by the type of coagulant, but residual β-casein was higher in cheese made with recombinant chymosin. Rheological and sensory characteristics of Hispánico cheese made with recombinant chymosin did not differ from those of calf rennet cheese.

INTRODUCTION

Calf rennet is usually preferred by cheese manufacturers to other milk coagulants because of its high chymosin (EC 3.4.23.4) content, which results in a more specific milk coagulating activity. There is a worldwide shortage of calf rennet due to the increase in cheese production and to a decrease in the number of veal calves slaughtered.

Immobilization of coagulating enzymes (Carlson, 1984), molecular cloning of chymosin (Nishimori *et al.*, 1981; Harris *et al.*, 1982) and extraction of chymosin from older beef cattle (Pszczola, 1989) are the main approaches followed in order to overcome the shortage of calf rennet. Chymosin synthesized by *Escherichia coli* (Green *et al.*, 1985) and *Kluyveromyces lactis* (Bines *et al.*, 1989) has been used successfully in Cheddar cheesemaking.

Burgos cheese is a white variety manufactured in Spain from a mixture of pasteurized cows' and ewes' milk without lactic starters, and consumed within 3–4 days (Nuñez *et al.*, 1989). Its microbiological quality is well documented (Chavarri *et al.*, 1985; García *et al.*, 1987), but no information on changes in its chemical characteristics during manufacture and storage is available.

Hispánico cheese is a semi-hard variety manufactured from a mixture of pasteurized cows' and ewes' milk to which lactic starters are added (Anon., 1987), and ripened for 30–60 days. Its ripening has not been studied.

The objective of the present work was to investigate the main changes in the characteristics of Burgos and Hispánico cheeses made with calf rennet or with recombinant chymosin, during manufacture and storage.

MATERIALS AND METHODS

Cheesemaking

Burgos cheese was manufactured in duplicate trials from 120 litres of milk (80 litres Friesian cows' milk + 40 litres Manchega ewes' milk), pasteurized at 75°C for 15 s, cooled to 30°C and distributed into three 40-litre vats. $CaCl_2$ (4 g per vat) was added. Vat 1 was coagulated with 5·33 ml Maxiren 15 L (Gist-Brocades NV, Delft, The Netherlands), vat 2 with 5·33 ml Hansen's standard rennet with a declared content of 55%

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chymosin and 45% bovine pepsin (CHL-Lacta, Madrid, Spain) and vat 3 with 5·33 ml Miles 15 000 rennet with a declared content of 70% chymosin and 30% bovine pepsin (Marschall España S.A., Madrid, Spain). Maxiren 15 L (RC), Hansen's rennet (A) and Miles rennet (B) were analyzed by manufacturers using IDF Standard 110A (International Dairy Federation, 1987). Curd was cut 40 min later into 2 cm cubes, left unstirred for 5 min and scooped into cylindrical moulds. After whey drainage for 2 h in a room at 4°C without pressing, cheeses were brine-salted (10% NaCl) for 10 min at 4°C and held for four days at 4°C.

Hispánico cheese was manufactured in duplicate trials from 180 litres pasteurized (75°C/15 s) milk (120 litres Friesian cows' milk + 60 litres Manchega ewes' milk). After milk distribution into three 60-litre vats, 6 g $CaCl_2$ and 600 ml lactic starter culture (Hansen's CH NOl, CHL-Lacta, Madrid, Spain) grown in skim milk were added to each vat. Rennet (A, B or RC) was added (8 ml per vat) 30 min later. After 35 min, the curd was cut into 6–8 mm cubes and scalded at 37°C for 15 min while stirring. Whey was drained off and three cheeses per vat were obtained. They were pressed for 20 h at 10°C, salted for 24 h at 10°C in 20% NaCl brine and ripened for 60 days at 12°C.

Coagulation parameters and cheese yield

Coagulation of milk from each cheesemaking trial was followed in duplicate by means of an Hellige thrombelastograph model D (Hellige GmbH, Freiburg im Breisgau, FRG), at 30°C. Parameters determined were r (minutes to reach 1 mm amplitude), $r + k$ (minutes to reach 20 mm amplitude) and A_{40} (millimetres of amplitude after 40 min).

Burgos cheese was weighed in duplicate two and four days after manufacture with 0·1 g accuracy using a Mettler PE 6000 balance. Hispánico cheese was weighed 24 h after manufacture and on days 15, 30, 45 and 60. Yield was expressed as kg cheese dry matter/100 litres milk.

Chemical analyses

Cheese moisture, pH and soluble N fractions (pH 4·6-, 12% trichloroacetic acid- and 1% phosphotungstic acid-soluble N) were determined in duplicate as described previously (Nuñez *et al.*, 1986). Soluble N fractions were expressed as a percentage of total N.

Residual casein fractions were determined in duplicate by polyacrylamide gel electrophoresis as described previously (Gaya *et al.*, 1990).

Residual caseins were expressed as percentages of levels in milk, taking into account the N content of samples for the correction of densitometric reading of peak areas.

Rheological determinations

Breaking force, apparent elastic modulus (E_{ap}) and hardness were measured from compression curves (six determinations/cheese) obtained using an Instron Compression Tester 1122, as described by Gaya *et al.* (1990).

Sensory evaluation

Cheese flavor quality and intensity were determined by 10 trained panellists on a 0–10 point scale , as indicated previously (Gaya *et al.*, 1990).

Statistical treatment of data

Analysis of variance was performed on data obtained using program BMDP 8V (BMDP Statistical Software, Los Angeles, CA, USA) with rennet type and cheese age as the main effects. Tukey's test (Steel & Torrie, 1980) was used for comparison of means.

RESULTS AND DISCUSSION

Milk coagulation and cheese yield

A significant effect of type of rennet on coagulation parameters r ($P < 0·001$), $r + k$ ($P < 0·05$) and A_{40} ($P < 0·05$) was observed in the manufacture of Burgos cheese (Table 1). RC gave the shortest coagulation time ($r + k$ = 31·3 min) and the firmest coagulum (A_{40} = 25·6 mm). Curd pH 45 min after rennet addition was in the range 6·48—6·54; the differences were too small to have significantly influenced milk coagulation.

In Hispánico cheese manufacture (Table 1) a significant effect of type of rennet on r ($P < 0·001$), $r + k$ ($P < 0·01$) and A_{40} ($P < 0·05$) was also found. Milk coagulation with RC occurred at a slower rate than

Table 1. Milk coagulation parameters[a] in the manufacture of Burgos and Hispánico cheeses[b] with recombinant chymosin (RC) or with calf rennet (brands A or B)

Cheese	Parameter	Coagulant		
		RC	A	B
Burgos	r	15·4 ± 0·5	16·8 ± 1·5	15·3 ± 1·6
	$r + k$	31·3 ± 2·5	39·6 ± 2·9	35·6 ± 3·2
	A_{40}	25·6 ± 2·3	20·8 ± 2·3	22·6 ± 2·1
Hispánico	r	16·6 ± 1·2	14·5 ± 1·7	14·6 ± 1·6
	$r + k$	32·0 ± 1·4	31·2 ± 2·4	28·7 ± 1·5
	A_{40}	27·8 ± 1·9	25·8 ± 1·8	28·5 ± 2·4

[a] Parameters: r, minutes to reach 1 mm amplitude; $r + k$, minutes to reach 20 mm amplitude; A_{40}, millimetres of amplitude after 40 min.
[b] Mean values of duplicate determinations on two cheesemaking trials.

Table 2. Yield[a], moisture and pH of Burgos cheese[b] manufactured with recombinant chymosin (RC) or with calf rennet (brands A or B)

	Days after manufacture	Coagulant		
		RC	A	B
Yield	2	9·51 ± 0·47	10·29 ± 0·63	11·42 ± 0·33
	4	9·40 ± 0·62	10·37 ± 0·03	9·91 ± 0·73
H_2O (%)	2	74·7 ± 0·0	73·6 ± 2·5	72·2 ± 1·8
	4	73·6 ± 1·3	71·7 ± 0·5	74·5 ± 1·9
pH	2	6·43 ± 0·11	6·50 ± 0·14	6·48 ± 0·16
	4	6·46 ± 0·12	6·42 ± 0·14	6·46 ± 0·17

[a] Yield is expressed as kg cheese dry matter/100 litres milk.
[b] Mean values of duplicate determinations on two cheese-making trials.

with rennets A or B, as indicated by r and $r + k$ parameters. Coagulum firmness for RC, as measured by A_{40}, was intermediate between values for rennets A and B.

Milk inoculation with lactic starter culture in Hispánico cheese manufacture resulted in curd pH values 90 min after rennet addition of 6·41 for RC, 6·17 for rennet A and 6·28 for rennet B. Proteolytic activity of pepsin present in rennets A and B might enhance growth of starter bacteria, with a higher pH decrease. Lower pH values reduce coagulation time and increase curd firmness (Kowalchyk & Olson, 1977; Okigbo *et al.*, 1985). The highest pH value observed in the present authors' experiments for RC curd may be partly responsible for the longer coagulation time of milk with recombinant chymosin.

No significant effect of milk coagulant on cheese yield expressed as dry matter, moisture content or pH was detected during cold storage of Burgos cheese (Table 2). Moisture content decreased ($P < 0·05$) during storage. No change in cheese pH was recorded, as no lactic starter had been added to milk.

Cheese yield, expressed as dry matter, moisture and pH of Hispánico cheese were also not influenced by milk coagulant (Table 3). Moisture content decreased significantly ($P < 0·01$) during the ripening of this cheese variety.

Table 3. Yield[a], moisture and pH of Hispánico cheese[b] manufactured with recombinant chymosin (RC) or with calf rennet (brands A or B)

	Days after manufacture	Coagulant		
		RC	A	B
Yield	30	8·02 ± 0·56	7·53 ± 0·37	8·01 ± 0·53
	60	7·72 ± 0·53	7·17 ± 0·32	7·35 ± 0·26
H_2O (%)	30	42·7 ± 0·6	43·7 ± 0·1	42·4 ± 1·4
	60	38·3 ± 2·2	40·0 ± 1·3	40·4 ± 1·5
pH	30	5·08 ± 0·09	5·13 ± 0·01	5·05 ± 0·02
	60	5·11 ± 0·03	5·04 ± 0·08	5·11 ± 0·01

[a,b] As in Table 2.

Previous works on the use of recombinant chymosin reported no significant differences in cheese yield due to the use of this milk coagulant for Cheddar (Green *et al.*, 1985; Bines *et al.*, 1989), Colby (Hicks *et al.*, 1988), Edam (Prokopek *et al.*, 1988), Gouda (van der Berg & de Koning, 1990) or Mozzarella (Rampilli *et al.*, 1990) cheeses.

Proteolysis

Moderate casein breakdown was recorded during Burgos cheese manufacture and storage (Table 4). Degradation of α_{s1}-casein did not progress after manufacture, with similar values for curd and two-day cheese. However, β-casein was significantly lower in two-day cheese than in curd for all rennets.

Higher ($P < 0·001$) levels of α_{s1}- and β-casein were found in Burgos cheese made with RC than with rennets A or B. Pepsin present in calf rennet is a more active and less specific proteinase than chymosin, a fact which may explain the lower levels of residual caseins in calf rennet Burgos cheese. No significant differences were reported, however, for Cheddar, Edam or Gouda cheeses manufactured with RC or calf rennet (Green *et al.*, 1985; Koch *et al.*, 1986; Bines *et al.*, 1989; van den Berg & de Koning, 1990). Mild manufacturing and storage conditions of Burgos cheese, without curd

Table 4. Residual casein fractions and soluble nitrogen fractions[a] of Burgos cheese[b] manufactured with recombinant chymosin (RC) or with calf rennet (brands A or B)

	Days after manufacture	Coagulant		
		RC	A	B
α_{s1}-Casein	Curd	58·7 ± 1·5	51·2 ± 3·6	54·2 ± 6·2
	2	60·3 ± 4·0	51·6 ± 2·4	54·6 ± 3·0
	4	59·8 ± 4·8	52·6 ± 3·5	44·0 ± 1·8
β-Casein	Curd	60·6 ± 2·2	48·3 ± 2·0	48·1 ± 2·8
	2	50·8 ± 4·5	41·3 ± 2·8	39·2 ± 3·6
	4	51·0 ± 3·0	41·9 ± 4·9	34·7 ± 1·1
pH 4·6-soluble N	Curd	22·1 ± 2·8	25·1 ± 3·2	27·3 ± 2·4
	2	18·3 ± 1·3	16·5 ± 0·3	16·1 ± 1·3
	4	17·7 ± 0·2	17·8 ± 0·0	18·1 ± 0·2
TCA-soluble N	Curd	5·71 ± 0·65	5·99 ± 1·13	6·61 ± 1·01
	2	5·69 ± 0·68	5·28 ± 0·71	6·05 ± 0·49
	4	5·19 ± 0·52	4·74 ± 0·60	4·60 ± 0·52
PTA-soluble N	Curd	3·34 ± 0·23	3·81 ± 0·18	3·44 ± 0·36
	2	1·71 ± 0·15	2·03 ± 0·04	2·01 ± 0·19
	4	1·76 ± 0·18	1·97 ± 0·16	2·09 ± 0·21

[a] Residual casein fractions are expressed as percentage on milk casein; TCA, 12% trichloroacetic acid; PTA, 1% phosphotungstic acid.
[b] Mean values of duplicate determinations on two cheese-making trials.

heating, acidification or storage at ripening temperatures, might contribute to a higher pepsin residual proteolytic activity in this variety.

Nitrogen soluble at pH 4·6 and in PTA decreased significantly ($P < 0·05$) during the first two days of cold storage of Burgos cheese (Table 4). Prolonged whey drainage for this variety might account for losses of whey proteins, soluble at pH 4·6, and of low molecular weight N, soluble in PTA.

Levels of soluble N fractions in Burgos cheese made with RC or calf rennet did not differ significantly. Similar results have been obtained for Cheddar cheese and other cows' milk varieties (Green *et al.*, 1985; Meisel, 1988; Prokopek *et al.*, 1988; Bines *et al.*, 1989; Rampilli *et al.*, 1990; van den Berg & de Koning, 1990).

Residual α_{s1}- and β-casein decreased during ripening, mainly during the first 30 days (Table 5). The level of α_{s1}-casein was not influenced by type of rennet. On the contrary, significantly higher ($P < 0·05$) levels of β-casein were found in Hispánico cheese made with RC than in cheese made with rennets A or B (Table 5), perhaps due to the pepsin content of the latter.

All soluble N fractions increased during ripening (Table 5), without a significant effect of rennet on their levels throughout maturation.

Rheology

No compression curves of Burgos cheese could be obtained with the Instron tester, because of the extremely weak texture of this fresh variety which has a moisture content over 70% (Table 2).

Table 5. Residual casein fractions[a] and soluble nitrogen fractions[b] of Hispánico cheese manufactured with recombinant chymosin (RC) or with calf rennet (brands A or B)

	Days after manufacture	Coagulant		
		RC	A	B
α_{s1}-Casein	Curd	57·7 ± 4·9	55·5 ± 4·1	54·8 ± 3·7
	30	21·5 ± 2·8	29·0 ± 3·9	19·6 ± 2·0
	60	21·2 ± 3·2	27·8 ± 3·3	24·6 ± 0·4
β-Casein	Curd	37·7 ± 4·3	33·0 ± 3·8	36·2 ± 3·5
	30	28·0 ± 2·2	25·2 ± 2·2	21·5 ± 0·8
	60	29·9 ± 2·2	25·0 ± 1·8	23·7 ± 2·4
pH 4·6-soluble N	Curd	9·85 ± 0·87	9·01 ± 0·79	8·30 ± 0·40
	30	19·3 ± 0·3	19·3 ± 1·4	18·3 ± 1·7
	60	18·4 ± 1·3	20·3 ± 1·5	19·4 ± 1·5
TCA-soluble N	Curd	3·57 ± 0·41	4·25 ± 0·54	4·48 ± 0·48
	30	9·11 ± 0·59	9·69 ± 0·7	10·5 ± 0·4
	60	10·6 ± 0·8	11·1 ± 0·4	10·6 ± 0·2
PTA-soluble N	Curd	1·32 ± 0·19	1·57 ± 0·26	1·65 ± 0·25
	30	3·65 ± 0·32	3·77 ± 0·44	3·43 ± 0·27
	60	4·13 ± 0·37	5·13 ± 0·03	4·46 ± 0·24

[a,b] As in Table 4.

Rheological characteristics of Hispánico cheese (Table 6) were not influenced by type of rennet. Breaking point values were similar to those obtained for pasteurized milk Manchego cheese ripened at 8–12°C (Gaya *et al.*, 1990), whereas E_{ap} and hardness values were slightly lower than those for Manchego cheese. Green *et al.* (1985) found no significant differences in firmness or elasticity between Cheddar cheese manufactured with RC or with calf rennet at 5 or 18 weeks of age.

Sensory characteristics

The quality of Burgos cheese flavour was not influenced by rennet used. Mean scores for Burgos cheese made with RC, A or B were 6·96 ± 0·92, 7·06 ± 1·05 and 7·11 ± 0·97 after two days, and 7·39 ± 0·91, 7·64 ± 0·79 and 6·91 ± 0·96 after four days. Scores for four-day cheese were significantly ($P < 0·05$) higher than for two-day cheese.

Flavour quality of Hispánico cheese was not influenced by type of rennet, although it improved ($P < 0·001$) with cheese age (Table 6). Most work on cows' milk cheese concluded that there were no differences in flavour quality due to the use of RC (Green *et al.*, 1985; Koch *et al.*, 1986; Bines *et al.*, 1989; Rampilli *et al.*, 1990; van den Berg & de Koning, 1990). The only defect attributed to RC—bitter flavour—occurred in Cheddar cheese made with undiluted *Escherichia coli* RC (Hicks *et al.*, 1988), but it was not found if an a propriate dilution was used.

Intensity of Hispánico cheese flavour was independent of rennet, but increased ($P < 0·01$) with cheese age (Table 6). No differences in flavour intensity between

Table 6. Rheological[a] and sensory[b] characteristics of Hispánico cheese manufactured with recombinant chymosin (RC) or with calf rennet (brands A or B)

	Days after manufacture	Coagulant		
		RC	A	B
Breaking point	30	27·8 ± 2·2	25·8 ± 2·1	26·6 ± 1·6
	60	31·2 ± 1·3	29·5 ± 1·9	33·1 ± 1·4
E_{ap}	30	0·14 ± 0·01	0·13 ± 0·02	0·13 ± 0·02
	60	0·21 ± 0·02	0·17 ± 0·03	0·20 ± 0·02
Hardness	30	0·32 ± 0·03	0·27 ± 0·02	0·29 ± 0·03
	60	0·33 ± 0·02	0·32 ± 0·04	0·41 ± 0·04
Flavour quality	30	5·60 ± 0·67	6·19 ± 1·00	5·96 ± 0·84
	60	6·75 ± 0·94	6·94 ± 1·10	6·94 ± 0·96
Flavour intensity	30	6·32 ± 1·02	5·84 ± 0·96	6·05 ± 1·04
	60	6·30 ± 1·02	6·92 ± 0·83	6·80 ± 0·86

[a] Mean values of six determinations per sample on two cheesemaking trials. Breaking point is expressed in N, apparent elastic modulus (E_{ap}) in N/mm² and hardness in joules.
[b] Mean values from 10 panellists on two cheesemaking trials according to a 0–10 point scale.

RC cheese and calf rennet cheese were reported for either Cheddar or Edam cheese (Green *et al.*, 1985; Meisel, 1988).

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