

Relationships between Abondance cheese texture, its composition and that of milk produced by cows grazing different types of pastures

Christophe BUGAUD^a, Solange BUCHIN^{a*},
Yolande NO L^a, Laurent TESSIER^a, Sylvie POCHET^a,
Bruno MARTIN^b, Jean Fran ois CHAMBA^c

^a Technologie et Analyses Laiti res, INRA, BP 89, 39801 Poligny Cedex, France

^b Unit  de Recherches sur les Herbivores, INRA, Theix,
63122 Saint-Gen s Champanelle, France

^c Institut Technique Fran ais des Fromages, BP 30, 74800 La Roche sur Foron, France

(Received 30 November 2000; accepted 3 February 2001)

Abstract — The relationships between the texture and composition of Abondance cheese (Haute-Savoie, France) and the milk used for its production, were investigated under real conditions of cheese production. Ten different types of pasture, exploited by three producers, were involved. Milk characteristics, linked to the type of pasture, affected cheese texture, as did the cheesemaking process. Cheeses from milk produced on mountain pastures (M, n = 5, 1 500–1 850 m) exhibited different rheological properties from those produced with milk from valley pastures (V, n = 5, 850–1 100 m): they were less elastic and less deformable. Rheological characteristics were mainly linked to the proportion of 18 atoms of carbon unsaturated fatty acids, which was higher in M milks, and to the proteolysis of cheese. Plasmin activity, which was higher in M milks, could enhance primary proteolysis in the corresponding cheeses. Differences in sensory texture, which were greater between M cheeses than between V cheeses, were attributed to variations in moisture and salt content. These differences could be linked to the cheesemaking process and also to the characteristics of milks, such as their pH-value and acidifying ability.

texture / rheology / cheese composition / milk composition / pasture

R sum  — Relations entre la texture de fromages d'Abondance, leur composition, et celle des laits produits par des vaches p turant diff rents types de pelouse. Les relations entre la texture du fromage d'Abondance (Haute-Savoie, France), sa composition et celle du lait utilis  pour sa fabrication ont  t   tudi es dans des conditions r elles de production du fromage. Dix p turages diff rents,

* Correspondence and reprints

Tel.: (33) 3 84 73 63 03; Fax: (33) 3 84 37 37 81; e-mail: buch@poligny.inra.fr

exploit s par 3 producteurs, ont  t   tudi s. Les caract ristiques du lait, li es aux types de p turage ont jou  un r le dans l' laboration de la texture, de m me que les param tres de fabrication. Les fromages fabriqu s   partir de laits produits sur des p turages de montagne (M, n = 5, 1 500–1 850 m)  taient moins  lastiques et moins d formables que les fromages issus de laits produits sur des p turages de vall e (V, n = 5, 850–1 100 m). Ces caract ristiques rh ologiques  taient li es principalement   la teneur en acides gras insatur s   18 atomes de carbone des laits, plus  lev e dans les laits M, et   la prot olyse des fromages. La plasmine, plus active dans les laits M, contribuerait   une prot olyse primaire plus importante dans ces fromages. Les fromages M pr sentaient une plus grande diversit  de texture que les fromages V, en partie associ e   des diff rences de teneurs en eau et en sel. Ces diff rences seraient li es   la technologie mise en  uvre et   certaines caract ristiques du lait comme son pH et son aptitude   s'acidifier.

texture / rh ologie / composition des fromages / composition des laits / p turage

1. INTRODUCTION

The influence of milk production factors on the sensory characteristics of ripened cheeses is of particular importance for cheeses with a "protected denomination of origin" (PDO), where the milk used undergoes few or no modifications before cheesemaking. Among factors influencing milk characteristics, feeding, and particularly the nature of pastures, have been cited as the most important for the cheese quality [16, 40].

Recent studies, performed on different cheese varieties, have pointed out the effects of the forage (method of conservation, botanical composition) on the characteristics of cheeses, including their texture [12, 42, 43]. The studies were carried out under experimental conditions, the cows being fed the forage indoors. Concerning the pastures, very little work has been carried out that shows the effect of the grass grazed by the animals on the characteristics of cheese texture, although Martin and Coulon [24] revealed an association between the characteristics of the pastures (botanical composition, phenological stage) and the texture of Reblochon cheese. Moreover, Buchin et al. [8] have demonstrated an influence of the botanical composition of pasture on Abondance cheese texture. According to the latter, the differences observed in the

rheological and sensory properties of texture may arise from differences in primary proteolysis due, to a certain extent, to the amount of plasmin and plasminogen in cheeses. But no study has investigated the whole chain from pasture to cheese texture.

The aim of the present study was to investigate the relationships between cheese texture and the composition of cheeses and corresponding milks, as well as the types of pastures involved. It is included in a wider project which aims to investigate the effect of the nature of the pasture on cheese characteristics (texture and flavour). The study was realised under real conditions of milk and cheese production, so as to cover a wide botanical diversity of pastures, and enable the investigation of the whole cheese production chain, from pasture to milk and then cheese. Abondance cheese, a semi-hard PDO cheese produced in the north of the French Alps, was chosen as a model. First of all, we studied the relationships between texture properties and the composition of matured cheeses, and then we considered the relationships between the composition of matured cheeses and possible sources of variations in cheese composition, such as milk properties and cheesemaking parameters. The relationships with characteristics of pastures were discussed.

2. MATERIALS AND METHODS

2.1. Experimental conditions

The experiment was carried out during the spring and summer of 1998 in three farms (X, Y, Z) in the Abondance PDO cheese area (Haute-Savoie, France), all producing milk and manufacturing cheeses. The choice of the ten pastures involved in the study was made to ensure as wide a botanical diversity as possible. They were located at between 850 m and 1 100 m for valley pastures (V, n = 5) or between 1 550 m and 1 850 m for mountain pastures (M, n = 5). The characteristics of the pastures (localisation, botanical composition,

phenological stage) were described in Bugaud et al. [9]. Herds were allowed to graze the studied pastures for 4 to 10 days. During the last three days of each grazing period, the parameters of the cheesemaking process were recorded and one cheese per day was sampled for analyses.

As a preliminary stage, we compared the three different systems of production (herd + cheesemaking process) by feeding these herds a common hay (H) and then comparing the cheeses which were produced in each plant. The characteristics of the three herds (size, breed, stage of lactation, milk yield) are described in the experimental design (Fig. 1). More details were given in a previous article [10].

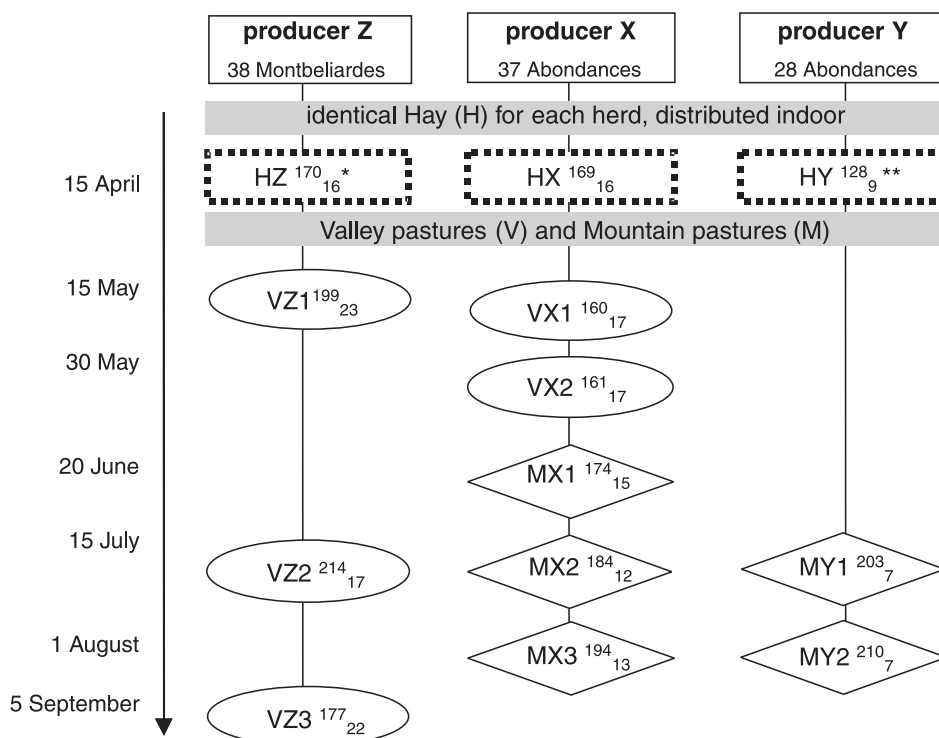


Figure 1. Experimental design.

* HZ^a_b where a was the average stage of lactation and b the milk yield (day).

** The Y studied milk provided exclusively from the morning milking. ($kg \cdot d^{-1}$).

2.2. Cheesemaking

Abondance cheese is a round semi-hard cheese weighing ~9 kg and made using raw milk from Abondance, Tarentaise or Montb liarde cows only. In our study, cheeses were manufactured following the same general scheme, although with certain variations from one farm to another (Tab. I). The milk was warmed to between 30 and 35  C and then inoculated with a mixed thermophilic starter culture. After a 25 min milk maturation period, rennet (containing 700 mg L⁻¹ chymosin) was added at a concentration of 0.15–0.21 mL L⁻¹. Cheesemaker Y also added 0.5 mL L⁻¹ of a natural calf rennet maceration in traditional, boiled and acidified whey. The clotting ability of the natural calf rennet, controlled using a visual method [1], was identical throughout the experiment. Clotting time was assessed visually, and after a firming period evaluated by each cheesemaker, the curd was cut into 3–4 mm curd particles. The curd-whey mixture was stirred for about 7 min, heated to 47–48  C over a period of 45 min, and

then stirred at the same temperature for a further 10–40 min. The mixture was then allowed to rest for 5 min before the curd was removed with a cloth, moulded and pressed for 20 h. The cheeses were then removed from the moulds, left at 13  C for 12 h and then brined at 13  C for 12 h in a saturated brine solution. Cheeses from the three farms were collected and all ripened in the same ripening room (11  C, 95% relative humidity) for six months. They were turned and salted every day for the first 15 days, then three times a week for the following month and then once a week.

For MX1 and HY cheeses (see the signification of acronyms in experimental design Fig. 1), the curd-whey mixture was warmed to 45–46  C instead of 47–48  C.

2.3. Analyses

2.3.1. Cheese texture

Rheological measurements were carried out by the method of uniaxial compression at a constant displacement rate, as de-

Table I. Cheesemaking parameters observed for each cheesemaker.

	Cheesemaker X	Cheesemaker Y	Cheesemaker Z
Composition of starter culture ¹	0.4 mL�L ⁻¹ of <i>St</i> + <i>Ldl</i>	0.4 mL�L ⁻¹ of <i>St</i> + <i>Ldb</i> + 0.5 mL�L ⁻¹ of natural calf rennet	0.4 mL�L ⁻¹ of <i>St</i> + <i>Ldl</i> + 0.3 mL�L ⁻¹ of <i>St</i> + <i>Ldb</i>
Rennet (mL�L ⁻¹)	0.21	0.15	0.17
Clotting time (min)	22 (2) ²	24 (4)	22 (1)
Firming period (min)	13 (3)	8 (4)	8 (1)
Duration of curd whey stirring (min)	12 (4)	4 (1)	2 (1)
Duration of second stirring (min)	30 (8)	23 (9)	13 (3)
Density (kg�m ⁻³) and acidity (�D) of the brine	1170–1190 15–20	1100 50–75	1165–1190 20–40

¹ *St* : *Streptococcus thermophilus*, *Ldl* : *Lactobacillus delbrueckii* subsp. *lactis*, *Ldb* : *Lactobacillus delbrueckii* subsp. *bulgaricus*.

² () : standard deviation.

scribed by No l et al. [31], using a TA-XT2 texture analyser (Rh o, 91160 Champlan, France). Three rheological parameters were evaluated: strain and stress at fracture and the deformability modulus. Fracture strain describes the deformability and fracture stress expresses the mechanical resistance of a cheese. The higher the deformability modulus value, the less elastic the cheese.

The texture properties of ripened cheeses were evaluated by 14 trained panelists. A profile of 5 attributes was used, adapted to this type of cheese. The panelists were trained to assess the degree of each attribute using a structured scale from 1 to 7. Firmness and elasticity were assessed using the method described by Lavanchy et al. [19]. Melting and pasty textures were comparable, respectively, to the solubility and adhesivity defined by Lavanchy et al. [19]. A sandy texture was defined by the presence of grains.

2.3.2. Composition of cheeses

Chemical analyses were carried out on cheeses at one day and at the end of ripening, after the removal of 10 mm of rind. The pH-value and the contents of dry matter (DM), fat (F), total nitrogen (TN), nitrogen soluble in tri-sodium citrate 0.5 mol·L⁻¹ at pH 4.4 (pH 4.4-SN) and nitrogen soluble in phosphotungstic acid (PTA-SN) were measured using the methods described in Ard  and Polychroniadou [3]. Nitrogen fractions were expressed as a percentage in TN (pH 4.4-SN/TN and PTA-SN/TN). The moisture content in non-fat cheese (M/NFC) was calculated as (100-DM)/(100-F) (g·100 g⁻¹ non-fat cheese). The insoluble fraction at pH 4.4 was analysed using polyacrylamide gel electrophoresis with urea [2]. The surface of each peak was expressed as a percentage of the total area. Three ratios were calculated: γ/β -casein, $\alpha_{S1-I}/\alpha_{S1}+\alpha_{S2}$ -casein and $\alpha_{S_{deg}}/\alpha_{S1}+\alpha_{S2}$ -casein, where $\alpha_{S_{deg}}$ -casein was the fraction

of caseins eluting in front of α_{S1-I} -casein. The sodium chloride content was determined by a potentiometric method using a chloride analyser (Model 926, Corning, Halstead) and expressed in g·100 g⁻¹ moisture (NaCl/M). The calcium content, measured in cheese at the end of pressing, was determined by using the complexometric method [33] and was expressed in g·100 g⁻¹ non-fat dry matter (Ca/NFDM).

The temperature and pH of cheeses were measured at moulding after 4 h of pressing and at one day, at the end of pressing.

2.3.3. Composition of milks

The measurements and the values in milk of the protein, fat, urea, calcium and phosphorus contents, the total bacterial cell count, the pH, the plasmin and plasminogen-derived activities, the proportions of 18 atoms of carbon unsaturated fatty acids (C18uns = C18:1 + C18:2 + C18:3) and of β -casein C variant were detailed in Bugaud et al. [10].

2.3.4. Statistical analyses

Differences between cheeses were investigated using analyses of variance (Version 6.12, SAS Institute INC., 1996) in two stages: 1) differences between H, V and M cheeses, 2) differences between cheeses within the H, V or M pastures. The first stage corresponded to a model with one factor and three levels, the second stage to a model with one factor and five levels (for M and V) or three levels (for H).

To describe cheese texture, a principal components analysis (PCA) was performed on the five sensory descriptors and three rheological parameters. Our goal was to characterise the global differences in cheese texture. We therefore performed the aforementioned analyses of variance on the coordinates of cheeses on the first and second axes of the PCA.

The relationships between the variables of cheese texture and cheese composition, on the one hand, and variables of cheese composition and milk composition, on the other hand, were evaluated using the Partial Least Squares regression (PLS) [39], implemented under Splus 3.4[®].

Four cheeses, one from MX1, one from VX1 and two from MY2 were excluded from the statistical analyses because of technical problems which were identified during the cheesemaking process.

3. RESULTS

3.1. Characteristics of matured cheeses

3.1.1. Texture

Figure 2 shows the biplot resulting from the PCA applied to rheological and sensory

variables. The first principal component (PC1) represented 40% of total variability and opposed sensory descriptors, i.e. melting and pasty textures versus sandy texture and to a lesser extent firmness. The second principal component (PC2) represented 31% of total variability and opposed two rheological properties: the deformability modulus and fracture strain. Elasticity and fracture stress contributed equally to both axes.

The differences within cheese replicates (produced by the same cheesemaker, from the same pasture) were globally lower than differences between cheeses produced on different pastures.

Differences were observed between H, M and V cheeses. PC2 separated M cheeses from H and V cheeses ($P < 0.01$). M cheeses had the highest deformability modulus, the lowest strain and stress at fracture. With the exception of VZ3

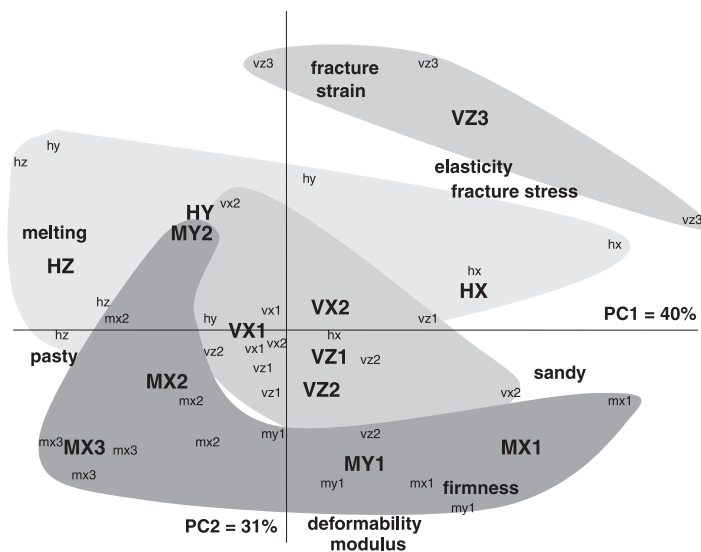


Figure 2. Analysis of the principal components of cheese texture.

In large type: mean of replicates; in small type: replicates.

H: cheese from milk produced with hay. M: cheese from milk produced on mountain pastures. V: cheese from milk produced on valley pastures [9].

X, Y, Z: cheeses manufactured respectively by the X, Y, Z cheesemakers.

For MY2, there was just one replicate (in large type).

cheeses, all V cheeses were located at the centre of the map and could therefore be defined as medium cheeses. H cheeses and VZ3 cheeses exhibited the highest degree of strain and stress at fracture and the lowest degree of deformability modulus.

Differences in texture between M cheeses were greater than those seen between V cheeses, when VZ3 cheeses were excluded. M cheeses differed from each other on PC1 and PC2 ($P < 0.05$). MX1 and MY1 cheeses were the firmest and sandiest. They were opposed to MX3 and MX2 cheeses, which were the least elastic and had the most melting texture. VZ3 cheeses were opposed to the other V cheeses on PC2 ($P < 0.01$): they were the most elastic and had the highest strain and stress at fracture. No differences were observed between the other V cheeses.

PC1 separated HX cheeses from HY and HZ cheeses ($P < 0.05$). HX cheeses were the sandiest and firmest and had the least melting and pasty textures.

3.1.2. Composition

Differences of proteolysis were greater than differences of gross composition between H, M and V cheeses (Tab. II and Tab. III). Only the pH-value was significantly higher in V cheeses than in M cheeses ($P < 0.05$). M cheeses presented the highest values and V cheeses the lowest values for most indicators of proteolysis: the pH 4.4-SN/TN fraction ($P < 0.05$) and the $\alpha_{S1-I}/\alpha_{S1} + \alpha_{S2}$ -caseins and $\alpha_{Sdeg}/\alpha_{S1} + \alpha_{S2}$ -caseins ratios ($P < 0.001$). The PTA-SN/TN fraction was higher in M cheeses than in H cheeses ($P < 0.05$).

Table II. Gross composition of matured cheeses.

forage	cheese	n	M/NFC (g·100 g ⁻¹)	NaCl/M (g·100 g ⁻¹)	pH	F/DM (g·100 g ⁻¹)	Ca/NFDM (g·100 g ⁻¹)
	hz	3	54.7 (0.9)	5.2 ^a (0.2)	5.89 ^a (0.13)	53.7 (0.5)	2.89 ^b (0.10)
hay	hx	3	52.4 (0.9)	5.3 ^a (0.3)	5.64 ^b (0.02)	51.4 (0.7)	2.84 ^b (0.04)
	hy	3	53.3 (1.4)	4.7 ^b (0.3)	5.59 ^b (0.11)	51.8 (1.8)	3.06 ^a (0.08)
variance analysis between H cheeses			ns	*	*	ns	*
	vz1	3	52.1 (0.9)	5.7 (0.6)	5.79 ^b (0.04)	50.8 ^b (0.3)	2.84 ^b (0.09)
valley	vz2	3	53.2 (1.8)	5.7 (0.4)	5.84 ^{ab} (0.06)	53.3 ^a (0.8)	2.93 ^{ab} (0.04)
pastures	vz3	3	53.9 (0.7)	5.3 (0.3)	5.94 ^a (0.09)	51.9 ^b (0.4)	3.04 ^a (0.09)
	vx1	2	52.7 (0.2)	5.5 (0.6)	5.64 ^c (0.01)	51.7 ^b (0.2)	2.84 ^b (0.04)
	vx2	3	51.9 (0.2)	5.7 (0.3)	5.64 ^c (0.03)	51.9 ^b (0.1)	2.78 ^b (0.09)
variance analysis between V cheeses			ns	ns	***	**	*
	mx1	2	51.4 ^b (0.8)	6.2 ^a (0.7)	5.65 ^{ab} (0.08)	51.8 (0.2)	3.04 ^a (0.17)
mountain	mx2	3	53.9 ^a (0.6)	6.6 ^a (0.3)	5.75 ^a (0.04)	51.9 (0.4)	2.81 ^{ab} (0.13)
pastures	mx3	3	53.6 ^a (0.9)	5.4 ^{ab} (0.5)	5.55 ^b (0.06)	51.0 (0.9)	3.04 ^a (0.09)
	my1	3	51.6 ^b (0.7)	4.9 ^{bc} (0.2)	5.51 ^b (0.02)	50.9 (1.1)	2.72 ^b (0.08)
	my2	1	53.0 ^{ab} (-)	3.9 ^c (-)	5.62 ^{ab} (-)	50.2 (-)	2.77 ^{ab} (-)
variance analysis between M cheeses			*	**	**	ns	*
variance analysis between H, M, V			ns	ns	* V > M	ns	ns

M/NFC: moisture in non-fat cheese. NaCl/M: salt in moisture. F/DM: fat in dry matter. Ca/NFDM: calcium in non-fat dry matter measured in 1 day-cheeses. n: number of replicates. ^{a, b, c} Results of Newman-Keuls' test. Variance analysis: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns not significant. (-): standard deviation.

Table III. Proteolysis of matured cheeses.

forage	cheese	n	pH 4.4– SN/TN (g·100 g ⁻¹)	PTA– SN/TN (g·100 g ⁻¹)	γ/β-casein	α _{S1} -I / α _{S1} +α _{S2} - casein	α _{Sdeg} / α _{S1} +α _{S2} - casein
	hz	3	22.6 (1.7)	11.6 (0.4)	2.48 ^a (0.35)	1.02 ^b (0.02)	0.48 (0.07)
hay	hx	3	19.7 (1.7)	10.5 (1.6)	1.49 ^b (0.06)	0.88 ^c (0.08)	0.33 (0.07)
	hy	3	20.6 (0.5)	8.9 (0.9)	1.41 ^b (0.11)	1.43 ^a (0.04)	0.46 (0.05)
variance analysis between H cheeses			ns	ns	**	***	ns
	vz1	3	20.7 (0.8)	13.5 ^a (1.5)	1.94 ^{ab} (0.27)	0.67 ^b (0.03)	0.31 (0.01)
valley	vz2	3	21.2 (0.9)	12.8 ^a (0.3)	2.31 ^a (0.44)	0.77 ^b (0.11)	0.37 (0.07)
pastures	vz3	3	19.7 (1.5)	9.1 ^b (0.9)	1.31 ^b (0.13)	0.83 ^{ab} (0.07)	0.29 (0.04)
	vx1	2	20.7 (0.3)	12.4 ^a (0.1)	1.56 ^b (0.00)	0.88 ^{ab} (0.01)	0.31 (0.00)
	vx2	3	20.4 (1.0)	11.3 ^a (1.1)	1.44 ^b (0.09)	1.03 ^a (0.13)	0.33 (0.05)
variance analysis between V cheeses			ns	**	**	**	ns
	mx1	2	20.9 (2.8)	11.2 (5.8)	1.71 (0.27)	0.97 ^b (0.02)	0.42 ^c (0.08)
mountain	mx2	3	23.1 (0.3)	15.9 (0.8)	2.30 (0.32)	1.19 ^{ab} (0.09)	0.59 ^{ab} (0.06)
pastures	mx3	3	23.2 (0.5)	11.7 (1.5)	2.13 (0.24)	1.14 ^{ab} (0.11)	0.63 ^a (0.01)
	my1	3	20.6 (0.8)	14.1 (1.3)	1.78 (0.11)	1.01 ^b (0.09)	0.46 ^{bc} (0.04)
	my2	1	21.4 (–)	11.4 (–)	1.75 (–)	1.40 ^a (–)	0.58 ^{ab} (–)
variance analysis between M cheeses			ns	ns	ns	*	**
variance analysis between H, M, V			* M > V	* M > H	ns	*** H+M > V	*** M > H > V

pH 4.4-SN/TN: nitrogen soluble in tri-sodium citrate at pH 4.4 on total nitrogen. PTA-SN/TN: nitrogen soluble in phosphotungstic acid on total nitrogen. n: number of replicates. ^{a, b, c} Results of Newman-Keuls' test.

Variance analysis: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns not significant.

(–): standard deviation.

Within the group of V cheeses, VZ3 cheeses had the highest pH value ($P < 0.001$) and calcium content (Ca/NFDM) ($P < 0.05$) and the lowest PTA-SN/TN fraction ($P < 0.01$). VZ2 cheeses had the highest fat content (F/DM) ($P < 0.001$) and γ/β-caseins ratio ($P < 0.01$) and together with VZ1 cheeses, had the lowest α_{S1}-I/α_{S1}+α_{S2}-caseins ratio ($P < 0.01$). VX1 and VX2 cheeses, which did not differ from each other, exhibited the lowest pH value.

Within the group of M cheeses, MX2 and MX3 cheeses had the highest moisture content (M/NFC) and contrasted with MX1 and MY1 cheeses ($P < 0.05$). MX1 and MX2 cheeses had the highest NaCl content (NaCl/M) and contrasted with MY2 cheese ($P < 0.01$). MX3 and MY1 cheeses had the

lowest pH value and contrasted with MX2 cheeses ($P < 0.01$). MY1 cheeses had the lowest calcium content ($P < 0.05$). MX1 and MY1 cheeses had the lowest α_{S1}-I/α_{S1}+α_{S2}-caseins ($P < 0.05$) and α_{Sdeg}/α_{S1}+α_{S2}-caseins ($P < 0.01$) ratios, MX3 the highest α_{Sdeg}/α_{S1}+α_{S2}-caseins ratio. MX2 and MX3 cheeses tended to have a higher pH 4.4-SN/TN fraction, while MX2 and MY1 cheeses tended to have a higher PTA-SN/TN fraction.

HY cheeses had the lowest NaCl content ($P < 0.05$), the highest calcium content ($P < 0.05$) and α_{S1}-I/α_{S1}+α_{S2}-caseins ratio ($P < 0.001$). The Z cheeses had the highest pH value ($P < 0.05$) and γ/β-caseins ratio ($P < 0.01$).

3.2. Relationships between the texture and composition of matured cheeses

The relationships between the texture of matured cheeses and their composition were investigated using PLS regression (Tab. IV). The three rheological variables and five sensory descriptors were predicted by the variables of chemical composition (presented in Tabs. II and III) and by the variable of the proportion of C18uns in milks, which is supposed to be similar in cheeses [25]. The prediction of rheological variables by the model was better (variance

of 64–90%) than the prediction of sensory descriptors (variance of 42–55%, except for sandy texture which had a variance of 81%).

Rheological parameters were first predicted by indicators of proteolysis and by C18uns, and then by the pH-value. Fracture strain and stress were negatively related to C18uns. The higher the indicators of proteolysis, the lower the fracture stress and the higher the fracture strain. The deformability modulus was positively related to the $\alpha_{Sdeg}/\alpha_{S1}+\alpha_{S2}$ -caseins ratio but negatively related to the $\alpha_{S1}-I/\alpha_{S1}+\alpha_{S2}$ -caseins ratio.

Table IV. Partial Least Squares regression-based prediction of cheese texture variables by the composition of matured cheeses.

Variables	explicative variables ¹				variance explained %
	1	2	3	4	
Rheological properties					
Fracture strain	(C18uns) ²	$\alpha_{S1}-I/\alpha_{S1}+\alpha_{S2}$ -casein	pH	PTA-SN/TN	90
Fracture stress	(pH 4.4-SN/TN)	(PTA-SN/TN)	(C18uns)	(γ/β -casein)	64
Deformability modulus	$\alpha_{Sdeg}/\alpha_{S1}+\alpha_{S2}$ -casein	($\alpha_{S1}-I/\alpha_{S1}+\alpha_{S2}$ -casein)	(pH)	pH 4.4-SN/TN	77
Sensory properties					
Sandy	(M/NFC)	(pH 4.4-SN/TN)	NaCl/M	($\alpha_{S1}-I/\alpha_{S1}+\alpha_{S2}$ -casein)	81
Firm	NaCl/M	(M/NFC)	γ/β -casein	pH 4.4-SN/TN	52
Melting	$\alpha_{S1}-I/\alpha_{S1}+\alpha_{S2}$ -casein	(NaCl/M)	pH	$\alpha_{Sdeg}/\alpha_{S1}+\alpha_{S2}$ -casein	54
Pasty	F/DM	γ/β -casein	$\alpha_{Sdeg}/\alpha_{S1}+\alpha_{S2}$ -casein	pH 4.4-SN/TN	42
Elastic	M/NFC	(γ/β -casein)	(F/DM)	(pH 4.4-SN/TN)	55

¹ The explicative variables are ranked in decreasing order of importance of prediction.

² Between () negative contribution.

C18uns: 18 atoms of carbon unsaturated fatty acids; pH 4.4-SN/TN: soluble nitrogen in tri-sodium citrate at pH 4.4 on total nitrogen; PTA-SN/TN: soluble nitrogen in phosphotungstic acid on total nitrogen; M/NFC: moisture in non-fat cheese. NaCl/M: salt in moisture. F/DM: fat in dry matter.

Sensory descriptors were equitably predicted by gross composition and proteolysis. As expected, sandy and firm textures were negatively related to moisture and NaCl contents. These descriptors were also predicted by indicators of proteolysis: the higher the indicators of proteolysis, the lower the sandy texture and the higher the firmness. The melting descriptor was positively related to indicators of proteolysis ($\alpha_{S1-I}/\alpha_{S2}+\alpha_{S2}$ -caseins and $\alpha_{Sdeg}/\alpha_{S2}+\alpha_{S2}$ -caseins ratios) and to pH-value, and negatively related to NaCl content. A pasty texture was positively related to fat content and to indicators of proteolysis. Elasticity was positively related to moisture content and negatively related to indicators of proteolysis (γ/β -caseins ratio and pH 4.4-SN/TN) and fat content. Calcium content was never a major predictor of cheese texture.

3.3. Relationships between the composition of matured cheese and both milk characteristics and technological parameters

Relationships between the composition of matured cheeses and milk and technological variables were investigated using a PLS regression (Tab. V). In order to predict pH 4.4-SN/TN and PTA-SN/TN fractions, γ/β , $\alpha_{S1-I}/\alpha_{S1}+\alpha_{S2}$ and $\alpha_{Sdeg}/\alpha_{S1}+\alpha_{S2}$ -caseins ratios were added to the predictive variables. In all cases, the prediction of composition variables by the model was higher than 66%.

In the first instance, indicators of primary proteolysis were predicted by milk characteristics. As expected, the γ/β -caseins ratio was positively related to plasmin and plasminogen-derived activities. The $\alpha_{Sdeg}/\alpha_{S1}+\alpha_{S2}$ -caseins ratio was also positively related to plasmin activity. As for the $\alpha_{S1-I}/\alpha_{S1}+\alpha_{S2}$ -caseins ratio, the strongest relationship was observed with the urea content of milk. The three caseins ratios were also associated with the conditions of heating (duration and temperature) and

renneting, in particular for the $\alpha_{S1-I}/\alpha_{S1}+\alpha_{S2}$ -caseins and $\alpha_{Sdeg}/\alpha_{S1}+\alpha_{S2}$ -caseins ratios. Both these ratios were positively related to the pH of the milk, while the γ/β -caseins ratio was negatively related to the pH of the cheese at the end of pressing.

The pH 4.4-SN/TN fraction was firstly related to indicators of primary proteolysis and, consequently, to plasmin activity. The PTA-SN/TN fraction was negatively related to acidification parameters like the pH after 4 h of pressing (pH 4 h) and at the end of pressing (pH 1d).

Gross composition variables exhibited stronger relationships with technological parameters than with indicators of proteolysis. Moisture content and pH value were predicted by the conditions of heating and of second-stirring of curd particles. However moisture content was also positively related to the pH of milk. The pH of matured cheese was predicted by acidification parameters. Unexpectedly, NaCl content was first predicted by the total bacterial content and plasmin activity, and then by brine characteristics. As expected, fat content in the cheese was mainly explained by the fat and protein contents of the milk.

4. DISCUSSION

This study was conducted under real conditions of Abondance cheese production, which occasioned certain problems concerning implementations and limits of interpretation. Nevertheless, we were able to establish significant relationships between the texture properties of Abondance cheese and the nature of the pastures involved, through changes in milk and cheese composition. Moreover, the results have shown that the variability between replicates was lower than the variability between cheeses produced from the different

Table V. Partial Least Squares regression-based prediction of the composition of matured cheeses by the milk composition ¹ and the technological parameters ².

Variables	explicative variables ⁵					Variance explained %
	1	2	3	4	5	
Indicators of proteolysis						
γ/β -casein	PLM	(pH 1d) ⁶	PLG	(d-heat)	t-clo	84
α_{S1} -I/ α_{S1} + α_{S2} -casein	(urea)	(Tp-heat)	(Tp-ren)	pH m	pH ren	91
α_{Sdeg} / α_{S1} + α_{S2} -casein	PLM	pH m	(Tp heat)	pH 4h	pH ren	88
PH 4.4-SN/TN ³	α_{Sdeg} / α_{S1} + α_{S2} -casein	α_{S1} -I/ α_{S1} + α_{S2} -casein	(PLM)	γ/β -casein	d-2stir	95
PTA-SN/TN ³	(pH 1d)	(pH 4h)	(P)	γ/β -casein	protein	66
Indicators of gross composition						
M/NFC	(d-heat)	pH m	(t-2stir)	t-clo	(urea)	71
F/DM ⁴	fat	(protein)	–	–	–	88
pH	(d-heat)	(d-2stir)	(pH 4h)	(Tp-heat)	pH 1d	91
NaCl/M	TBC	PLM	(pH 4h)	(A brine)	(β -C variant)	71

¹ Milk characteristics: plasmin (PLM) and plasminogen derived (PLG) activities, calcium (Ca), phosphorus (P), pH (pH m), total bacterial content (TBC), urea content, fat content, protein content (β -C variant).

² Technological parameters:

cheesemaking process: pH at renneting (pH ren), temperature before renneting (Tp ren), of curd heating (Tp heat), clotting time (t-clo), firming period, durations of curd heating (d-heat) and of the second stirring (d-2stir), density (D brine) and acidity (A brine) of brine.

acidification parameters: pH at 4 hours of pressing (pH 4 h) and at one day (pH 1d).

³ pH 4.4-SN/TN and PTA-SN/TN were predicted by milk characteristics, technological parameters and ratios of caseins.

⁴ F/DM were completely predicted by the first two variables.

⁵ The explicative variables are ranked in decreasing order of importance of prediction.

⁶ Between () negative contribution.

pH 4.4-SN/TN: soluble nitrogen in tri-sodium citrate at pH 4.4 on total nitrogen. PTA-SN/TN: soluble nitrogen in phosphotungstic acid on total nitrogen. M/NFC: moisture in non-fat cheese. F/DM: fat in dry matter. NaCl/M: salt in moisture.

pastures. That supports the experimental design we have chosen.

The relationships between cheese texture and composition observed during this study had never previously been demonstrated for this type of cheese. Rheological properties were linked, firstly, to the fatty acid composition and proteolysis, and sec-

ondly, to the pH of the cheese at the end of ripening. The sensory properties of cheese texture were mainly explained by the gross composition (moisture and salt content) and proteolysis of the cheese.

The relationships between C18uns in milk and the rheological properties of Abundance cheese agree with studies on

butter [26, 38], Mascarpone cheese [4] and Morbier cheese [7]. The lower melting point of unsaturated fatty acids may produce a more fluid fatty matter, and consequently softer cheeses. The higher fracture strain and, to a lesser extent, the higher fracture stress of cheeses from hay may lead to greater cohesiveness. These results agree with those found by Coulon et al. [12] and Buchin et al. [7] who compared the firmness of semi-hard cheeses produced using milk from hay and from pasture. Thus, the lower fracture strain of M cheeses could be explained by the higher proportion of C18uns in the milk employed, probably mainly related to the grazing conditions (temperature, altitude, walk of the herd, botanical composition) on mountain pastures [10, 11].

The primary proteolysis in cheese, which may be defined as the changes in β -, γ -, α_s -caseins and peptides, and other minor bands that are detected by polyacrylamide gel electrophoresis [34], is a key point in explaining rheological parameters and, to a lesser extent, sensory measurements of Abondance cheese.

The results obtained with respect to primary proteolysis helped to explain the rheological differences between H, V and M cheeses, being linked firstly to milk characteristics, in particular plasmin. Plasmin hydrolyses β -casein into γ -caseins in cheeses [15] and has been shown to hydrolyse α_{s1} - and α_{s2} -caseins in a model aqueous solution of casein [20, 21]. This proteolytic activity was expressed in our cheeses by the γ/β -caseins and $\alpha_{sdeg}/\alpha_{s1}+\alpha_{s2}$ -caseins ratios. The higher plasmin activity in M milks, probably linked to the specific grazing conditions (altitude, botanical composition) encountered on M pastures [10], may have led to a higher level of primary proteolysis. Thus, the higher degradation of the protein matrix in M cheeses would have induced weakness of the cheese structure, which is known to be related to the following rheological properties: a higher defor-

mability modulus and a lower fracture stress [31]. These rheological patterns contribute to a higher sensory sensation of friability [32]. These results confirm the findings of Mulvihill and McCarthy [30] and Buchin et al. [8], who demonstrated the importance of plasmin content to the primary proteolysis and the rheological properties of cheeses. The higher elasticity and fracture stress of VZ3 cheeses, which had globally the lowest primary proteolysis, could be linked to the lower plasmin and plasminogen-derived activities of the corresponding milks.

The correlation observed between the melting properties of cheese and the extent of α_{s1} -casein breakdown agrees with the literature [18]. Chymosin is one of the enzymes which perform the breakdown of α_{s1} -caseins into α_{s1} -I-caseins [29]. Its activity in cheese is known to be affected by some technological parameters [41]. So, the influence of the temperatures at renneting and at cooking on the α_{s1} -I/ $\alpha_{s1}+\alpha_{s2}$ -caseins ratio in the cheeses of this experiment could be partly explained by the residual activity of chymosin. Another enzyme, cathepsin D, is able to hydrolyse α_{s1} -caseins into α_{s1} -I-caseins [17]. This enzyme, native in milk, may also have been involved in primary proteolysis [8]. However the variation factors of cathepsin D have never been studied. Lastly, no explanation could be given about the role of urea in the hydrolysis of α_s -casein.

The secondary proteolysis, which was defined in our study by the amount of peptides and amino acids soluble in the tri-sodium citrate at pH 4.4 and in phosphotungstic acid [34], was linked to cheese texture, as has already been observed by Steffen et al. [37]. On the one hand, the pH 4.4-SN/TN fraction was principally linked to the proteolytic activity of plasmin. On the other hand, the PTA-SN/TN fraction, which contains the smallest peptides (< 600 DA) and free amino acids, seemed mainly monitored by the

acidification parameters, known to be mostly controlled by the population of starter bacteria. These results confirm the role played by the starter in the secondary proteolysis of matured cheese [6]. Nevertheless, the differences in PTA-SN/TN fraction observed between cheeses manufactured using the same starters (type and quantity) suggest that the endogenous microflora of milk may also have played a role. Beuvier et al. [5] and Demarigny et al. [14] demonstrated the influence of endogenous microflora on the proteolysis and texture of Swiss-type cheese. But the relationships between pasture characteristics and milk microflora remain to be explored.

As expected, some of the gross composition parameters, such as the fat content in cheese, varied in line with milk composition. Others were linked to technological parameters. Indeed, although the cheese-making process was controlled as much as possible, variabilities could occur in the form of interactions between milk characteristics and technological parameters [24].

Differences in the textural characteristics between mountain cheeses can mainly be explained by variations in their moisture and salt content. The higher moisture content of MX2 and MX3 versus MX1, and of MY2 versus MY1 could induce differences in texture [13, 36], producing a less sandy and firmer cheese texture. Technological parameters (duration of heating and stirring) are known to influence the moisture content of cheeses [24, 27]. However, milk characteristics could also affect the moisture content, as was observed in mountain cheeses: the pH of milk is known to affect milk clotting time and indirectly lead to the reduced draining of cheese [23, 28]. The higher pH of milk in MX2, MX3 and MY2 cheeses was concomitant with a higher somatic cell count [10].

Acidification delays were observed after 4 h of pressing in MX3 cheeses. Experimental measurements of acidifying ability showed an acidification delay in MX3

milks in comparison with a blend of MX1 and MX2 milks. This may have been linked to the presence in milk of natural inhibitors of microbial activity, such as lactoperoxidase or thiocyanate [22], the latter being linked to the cyanogenic glucosids present in feed [35]. As a consequence, MX3 cheeses were less dry and softer. An acidification delay was also observed in VZ3 cheeses at the end of pressing but its cause (starters or milk properties) is unclear. It may have contributed to the particular texture of these cheeses.

ACKNOWLEDGEMENTS

This work was partially funded by SUACI-GIS and the Institut National de Recherche Agronomique. We are grateful to J.B. Coulon (INRA, Theix), A. Hauwuy (SUACI-GIS), E. Beuvier (INRA, Poligny) for their critical reading of the paper, C. Achilleos (INRA, Poligny) for advice on rheological measurements, the staff at ITFF (La Roche sur Foron) for advice on processing and assistance with chemical analyses, T. M tais (Les Maisons du Go t, La Roche sur Foron) for the sensory analyses, P. Schlich (INRA, Dijon) for allowing us to use the macro command of biplot developed with SAS Software, and H. Lamprell (INRA, Poligny) for reviewing the English.

REFERENCES

- [1] Anonyme, M thode officielle d'analyse pour la d termination de la teneur en chymosine et en pepsine bovines. C. – D termination du temps de coagulation d'un lait additionn  d'une solution enzymatique, J. Off. R pub. Fr. (1981) 2905–2909.
- [2] Andrews A., Proteinases in normal bovine milk and their action on caseins, J. Dairy Res. 50 (1983) 45–55.
- [3] Ard  Y., Polychroniadou A., Laboratory manual for chemical analysis of cheese, Office for official publications of the European communities, Luxembourg, 1999.
- [4] Battelli C., Pellegrino L., Ostini G., Alcuni aspetti della qualita della panna e del mascarpone industriale da essa ottenuto, Latte 20 (1995) 1098–1112.

- [5] Beuvier E., Berthaud K., Cegarra S., Dasen A., Pochet S., Buchin S., Duboz G., Ripening and quality of Swiss-type cheese made from raw, pasteurized or microfiltered milk, *Int. Dairy J.* 7 (1997) 311–323.
- [6] Bouton Y., Guyot P., Dasen A., Influence des interactions entre le lait et le levain lactique sur l'affinage et la qualit  du fromage Comt , *Int. Dairy J.* 6 (1996) 997–1013.
- [7] Buchin S., Delague V., Duboz G., Berdagu  J.L., Beuvier E., Pochet S., Grappin R., Influence du type de fourrage et du traitement thermique du lait sur les caract ristiques biochimiques et sensorielles d'un fromage de type Morbier, in: *Du terroir au go t des fromages*, 5th plenary meeting AIR 2039- COST'95, Besan on France, 27-28 Septembre 1997, pp. 138–152.
- [8] Buchin S., Martin B., Dupont D., Bornard A., Achilleos C., Influence of composition of Alpine highland pasture on the chemical, rheological and sensory properties of cheese, *J. Dairy Res.* 66 (1999) 579–588.
- [9] Bugaud C., Bornard A., Hauwuy A., Martin B., Salmon J.C., Tessier L., Buchin S., Relation entre la composition botanique de v g tations de montagne et leur composition en compos s volatils, *Fourrages* 162 (2000) 141–155.
- [10] Bugaud C., Buchin S., Coulon J.B., Hauwuy A., Dupont D., Influence of the nature of Alpine pastures on plasmin activity, fatty acid and volatile compound composition of milk, *Lait* 81 (3) (2001) In press.
- [11] Collomb M., B tikofer U., Spahni M., Jeangros B., Bosset J.O., Composition en acides gras et en glycidides de la mati re grasse du lait de vache en zones de montagne et de plaine, *Sci. Aliments* 19 (1999) 97–110.
- [12] Coulon J.B., Verdier I., Pradel P., Effet du mode de pr sentation du m me fourrage (foin ou herbe p tur e) sur les caract ristiques de fromages de Saint-Nectaire affin s, *Lait* 76 (1996) 479–486.
- [13] Delacroix-Buchet A., Trossat P., Prot olyse et texture des fromages   p te cuite press e. I. Influence de l'activit  de l'eau, *Lait* 71 (1991) 299–311.
- [14] Demarigny Y., Beuvier E., Buchin S., Pochet S., Grappin R., Influence of raw milk microflora on the characteristics of Swiss-type cheeses: II. Biochemical and sensory characteristics, *Lait* 77 (1997) 151–167.
- [15] Farkye N.Y., Fox P.F., Contribution of plasmin to Cheddar cheese ripening: effect of added plasmin, *J. Dairy Res.* 59 (1992) 209–216.
- [16] Grappin R., Coulon J.B., Terroir, lait et fromage:  l ments de r flexion, in: *3rd Renc. Rech. Rum.*, Institut de l' levage, Paris, 1996, pp. 21–28.
- [17] Kaminogawa S., Yamauchi K., Miyazawa S., Koga Y., Degradation of casein components by acid protease of bovine milk, *J. Dairy Sci.*, 63 (1980) 701–704.
- [18] Lawrence R.C., Creamer L.K., Gilles J., Texture development during cheese ripening, *J. Dairy Sci.* 70 (1987) 1748–1760.
- [19] Lavanchy P., B rodier F., Zannoni M., No l Y., Adamo C., Squella J., Herrero L., L' valuation sensorielle de la texture des fromages   p te dure ou semi-dure. Etude interlaboratoires, *Lebensm. Wis.s u Technol.* 26 (1993) 59–68.
- [20] Le Bars D., Gripon J.C., Specificity of plasmin towards bovine α_{S2} -casein, *J. Dairy Res.* 56 (1989) 817–821.
- [21] Le Bars D., Gripon J.C., Hydrolysis of α_{S1} -casein by bovine plasmin, *Lait* 73 (1993) 337–344.
- [22] Marshall V.M.E., Reiter B., Comparison of the antibacterial activity of the hypothiocyanite anion towards *Streptococcus lactis* and *Escherichia coli*, *J. Gen. Microbiol.* 120 (1980) 513–516.
- [23] Martin B., Coulon J.B., Facteurs de production du lait et caract ristiques des fromages. I. Influence des facteurs de production sur l'aptitude   la coagulation des laits de troupeaux, *Lait* 75 (1995) 61–80.
- [24] Martin B., Coulon J.B., Facteurs de production du lait et caract ristiques des fromages. II. Influence des caract ristiques des laits de troupeaux et des pratiques fromag res sur les caract ristiques du reblochon de Savoie fermier, *Lait* 75 (1995) 133–149.
- [25] Masson C., Rousseaux P., Decaen C., Variations g ographiques et saisonni res de la qualit  du fromage de Comt , *Lait* 61 (1981) 31–48.
- [26] Middaugh R.P., Baer R.J., Casper D.P., Schingoethe D.J., Seas S.W., Characteristics of milk and butter from cows fed sunflower seeds, *J. Dairy Sci.* 71 (1988) 3179–3187.
- [27] Mietton B., Desmazeaud M., de Roissart H., Weber F., Transformation du lait en fromage, in: de Roissart H., Luquet F.M. (Eds.), *Bact ries lactiques*, Loriga, Uriage, 1994, Vol. 2, pp. 55–154.
- [28] Millet J., Incidence de la teneur en ur e du lait et de la standardisation du pH du lait sur la qualit  d'une p te press e cuite: « Comt  », *Process* 1039 (1990) 56–62.
- [29] Mulvihill D.M., Fox P.F., Proteolytic specificity of chymosin on bovine α_{S1} -casein, *J. Dairy Res.* 46 (1979) 641–651.
- [30] Mulvihill D.M., McCarthy A., Relationships between plasmin levels in rennet caseins and proteolytic and rheological changes on storage of cheese analogues made from these caseins, *J. Dairy Res.* 60 (1993) 431–438.
- [31] No l Y., Zannoni M., Hunter E.A., Texture of Parmigiano Reggiano cheese: Statistical relationships between rheological and sensory variates, *Lait* 76 (1996) 243–254.
- [32] No l Y., Ard  Y., Pochet S., Hunter A., Lavanchy P., Luginb hl W., Le Bars D., Polychroniadou A., Pellegrino L., Characterization of protected denomination of origin cheeses: relationships

- between sensory texture and instrumental data, *Lait* 78 (1998) 569–588.
- [33] Pearce K.N., The complexometric determination of calcium in dairy products, *N.Z.J. Dairy Sci. Technol.* 12 (1977) 113–115.
- [34] Rank T.C., Grappin R., Olson N.F., Secondary proteolysis of cheese during ripening: a review, *J. Dairy Sci.* 68 (1985) 801–805.
- [35] Reiter B., Review of the progress of dairy science: antimicrobial systems in milk, *J. Dairy Res.* 45 (1978) 131–147.
- [36] R hm H., Lederer H., Ginzinger W., Relationship between rheological properties and composition of Emmental cheese. 2. Principal component regression, *Milchwissenschaft* 47 (1992) 620–623.
- [37] Steffen C., Eberhard P., Bosset J.O., Ruegg M., Swiss-type varieties, in: Fox P.F. (Ed.), *Cheese: chemistry, physics and microbiology*, Chapman and Hall, London, 1993, Vol. 2, pp. 83–110.
- [38] Stegeman G.A., Baer R.J., Schingoethe D.J., Casper D.P., Composition and flavor of milk and butter from cows fed unsaturated dietary fat and receiving bovine somatotropin, *J. Dairy Sci.* 75 (1992) 962–970.
- [39] Tenenhaus M., *La r gression PLS. Th orie et pratique*, Ed. Technip, Paris, 1998.
- [40] Urbach G., Effect of feed on flavor in dairy foods, *J. Dairy Sci.* 73 (1990) 3639–3650.
- [41] Van den Berg G., Exterkate F.A., Technological parameters involved in cheese ripening, *Int. Dairy J.* 3 (1993) 485–507.
- [42] Verdier I., Coulon J.B., Pradel P., Berdagu  J.L., Effect of forage type and cow breed on the characteristics of matured Saint-Nectaire cheeses, *Lait* 75 (1995) 523–533.
- [43] Verdier-Metz I., Coulon J.B., Pradel P., Viallon C., Albouy H., Berdagu  J.L., Effect of the botanical composition of hay and casein genetic variants on the chemical and sensory characteristics of ripened Saint-Nectaire type cheeses, *Lait* 80 (2000) 361–370.

To access this journal online:
www.edpsciences.org

