New results in the volatile odorous compounds of French cheeses

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Lait (1990) 70, 89-106

Original article

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(Received 7 December 1988; accepted 10 April 1989)

Summary — The volatile odorous constituents of five French blue cheeses (three Roquefort, a Bleu des Causses and a Bleu d'Auvergne) were isolated by high vacuum distillation, separated by gas chromatographic analysis and subjected to mass spectral analysis. One hundred and twenty nine compounds were identified and quantified. Methyl ketones were the majors compounds for all cheeses and represented 50 to 75% of the total odorous profile. Secondary alcohols, esters were abundant, especially in the Roquefort samples. The Bleu des Causses was rich in sulfur compounds. Lactones, primary alcohols and aldehydes were more abundant in the Bleu d'Auvergne.

Roquefort — Bleu des Causses — Bleu d'Auvergne — volatile compound — flavour

Résumé — Nouveaux développements dans l'étude des composés volatils odorants des fromages français à pâte persillée. *Les composés volatils odorants de 5 fromages à pâte persillée (3 roqueforts fabriqués avec des souches de* Penicillium roqueforti *différentes, 1 Bleu des Causses et 1 Bleu d'Auvergne) ont été extraits par distillation sous vide poussé, séparés par chromatographie en phase gazeuse et analysés par spectrométrie de masse. Cent vingt neuf composés ont été identifiés et quantifiés. Les méthyl-cétones étaient majoritaires dans tous les fromages et représentaient quantitativement 50 à 75% du profil odorant total. Les alcools secondaires et les esters étaient abondants, surtout dans les échantillons de roquefort. Le Bleu des Causses était le plus riche en composés soufrés et le bleu d'Auvergne le plus riche en lactones, alcools primaires et aldéhydes.*

roquefort — bleu des Causses — bleu d'Auvergne — composésvolatil — arôme

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INTRODUCTION

The homologous series of odd-carbon chain methyl ketones have been identified and characterized as the main compounds responsible for the unique flavour of blue cheeses (Anderson, Day, 1966). Most of the studies carried out in the last 20 years have been restricted to the quantitative determination of carbonyl compounds in various blue varieties : American Blue (Schwartz, Parks, 1963), Roquefort (Schwartz *et al,* 1963), Normanna cheese (Norwegian Blue : Svensen, Ottestad, 1969), German Edelpilz (Ney, Wirotama, 1972) and more recently, Stilton (Madkor *et al,* 1987).

The typical blue- or Roquefort-type odour has been found to persist after removal of all monocarbonyls (Schwartz, Parks, 1963). But neutral non-carbonyl volatile compounds of blue cheese have not been frequently studied. No quantitative data have been published. Only several esters and lactones have been reported in blue cheese (Day, Anderson, 1965; Ney, Wirotoma, 1972; Jolly, Kosikowski, 1975).

This paper reports the quantitative analysis of the neutral volatile compounds of different French blue cheeses. Three samples of Roquefort (produced from ewe's milk), a Bleu des Causses and a Bleu d'Auvergne (both produced from cow's milk) were analyzed. It was of interest to determine to what extent the organoleptic differences observed between these cheeses could be explained by the neutral volatiles occurring in the profiles.

MATERIAL AND METHODS

Cheese samples

Three Roquefort samples manufactured under commercial conditions with 3 different *Penicillium roqueforti* strains : PF, PO and PG (Table I), were obtained after 210 d of ripening from the Etablissements du Groupe Société des Caves de Roquefort (Roquefort, France). A Bleu des Causses sample manufactured with the same PG strain was obtained after 100 d of ripening from Valmont (Rodez, France). Except for the milk origin, the manufacturing procedures were similar for both Roquefort and Bleu des Causs-

Table I. Properties of the 3 strains of *Penicillium roqueforti* used in the manufacture of the Roquefort samples (by courtesy of M Assenat, Roquefort, France)
Propriétés des souches de Penicillium roqueforti *utilisées pour la fabrication des échantillons de Roquefort (avec la gracieuse permission de M Assenat, Roquefort, France)*

	Strains		
	PF	*PO*	*PG*
proteolytic activity	low	high	medium
lipolytic activity	low	rather high	medium
mycelium aspect	dark	pale	dark
commercial presentation	good	poor	good
organoleptic properties	neutral flavor	good flavor very typical	good flavor often pungent

es, A Bleu d'Auvergne sample was purchased at a local market after a ripening period of approximately 45 d.

All samples were stored at – 20 °C until studied.

Rapid measure of the total free fatty acids

100 g of cheese, triturated for 15 min at 30 °C, were centrifugated (12 000 *g*) for 10 min at 15 °C. An aliquot (2 g) of the upper fat layer was withdrawn and diluted in 20 ml of a mixture ethanol : diethyl ether (1 : 1, v/v). After addition of 0.5 ml of 1% ethanolic phenolphtalein, the solution was titrated with 0.1 N ethanolic sodium hydroxyde. 20 ml of ethanol : diethyl ether (1 : 1) were neutralized under the same conditions to estimate the background titration.

Separation and measurement of sulfur compounds

The static headspace method, described by Qvist, von Sydow (1976) was used to analyze the volatile sulfur compounds fo the blue cheeses. 50 g of the deep-frozen cheese were homogenized at 0 °C with 100 ml of distilled water in a flask which was then rotated in an inclined position in a water bath at 32 °C to obtain equilibrium.

After 30 min, 200 ml of the vapors surrounding the sample were trapped in an injection loop cooled with liquid nitrogen. Heating of the loop allowed the direct injection of the trapped compounds in a Girdel 3 000 chromatograph (Delsi) equipped with a Tracor sulfur specific flame photometric detector.

The column used was a 6.4 m x 2 mm *id* glass tube packed with 5 % Igepal CO 630 on Chromosorb G. AW DMCS (80/100 Mesh). Oven temperature was programmed from 10 °C to 110 °C at 4 °C/min after an initial isothermal period of 15 min. The nitrogen carrier gas flow was 20 ml/min.

Compounds were identified according to their retention time (calculated relative to methyl disulfide), and by comparison with the retention times of authentic compounds when available. Concentrations of some sulfur compounds could be calculated using external standards (injected under the same conditions).

Extraction of neutral volatile compounds

A vacuum degassing (2.10^{-3} Torr; 5 h 30) and a cold-finger molecular distillation (3.10^{-5} Torr; 4 h 30) were successively carried out. The apparatus and procedures were similar to those described by Forss & Holloway (1967). Three extractions were performed for each type of cheese using each 150 g of ground deep-frozen cheese.

Aqueous distillates, collected in the liquid nitrogen traps and removed from the cold-finger by rinsing with distilled water, were pooled. After adjusting to pH 9 (with sodium hydoxide 2N), the distillates were saturated with sodium chloride and thrice extracted in a separatory funnel with trichlorofluoro-methane (Forane 11, Atochem) : 1 x 45 ml and 2 x 20 ml. The aqueous was odorless.

After drying over anhydrous sodium sulfate, an internal standard was added to the solvent extracts (amyl 2-methyl butanoate : 10^{-2} µl/150 g of cheese *ie*, 0.053 mg/kg), which were then carefully concentrated under reflux to a small volume (250 µl).

Gas chromatography analyses

All separations were performed on a Girdel DI 700 chromatograph (Delsi) equipped with a 30 m x 0.32 mm *id* fused silica capillary column (J&W Scientific Inc.) bonded with DB5 (95% methyl-, 5% phenyl-polysiloxane). The film thickness was 1 µm. The hydrogen carrier gas flow was 50 cm/s. The oven temperature was programmed from 30–220 °C at a 2 °C/min rate. FID detector and split-splitless injector temperatures were maintained at 240 °C.

Retention indices were determined using these conditions. Peak areas were recorded with an Enica 10 integrator (Delsi).

Mass spectrometry

Electron impact mass spectra were recorded using a Nermag R10 - 10 spectrometer (Nermag) coupled with a Girdel 31 chromatograph (Delsi). The fused silica capillary column DB5 (60 m x 0.32 *id*, film thickness 1 μm) was directly connected to the ion source. The operating GC conditions were the same as above. The ion source energy was 70 eV. The temperatures of the ion source and the interface were respectively 150 and 280 °C. The scanning rate was 0.8 s from 25 to 300 amu.

All identifications were made by comparison of the mass spectra with those of reference compounds, when available or with bibliographic data.

RESULTS AND DISCUSSION

Titratable free acidity

The free fatty acids (FFA), which are important compounds in the flavour of blue cheese (Day, 1967), were much more abundant in Bleu des Causses than in all other cheeses (table II). They were twice as abundant in Roquefort PG than in the 2 other Roquefort samples. It has been reported that FFA accumulated during ripening to relatively greater proportions in blue cheese than in most other types of cheese (Woo *et al*, 1984). In order to estimate the extent of lipolysis during ripening, it would have been interesting to know the initial values of total FFA in the milk.

Sulfur compounds

Despite the relative high standard deviations, large variations in concentrations of sulfur compounds between cheeses were obvious (table III). The concentrations of hydrogen sulfide and methanetiol were not calculated because calibration curves of these highly volatile compounds were not established.

Sulfur compounds were much more abundant in Bleu des Causses (especially methyl sulfide and methyl disulfide). Nevertheless, hydrogen sulfide, which is generally reported in all types of matured cheeses, was not found in Bleu des Causses. Similar amounts of hydrogen sulfide and dimethyl sulfide were found in the 3 Roquefort cheeses. Methanethiol and dimethyl disulfide were more abundant in Roquefort PO and PG than in PF, which contained the most important amount of carbon disulfide. However, carbon disulfide was found here at very low levels (1 to 5 ppb) and probably did not contribute to the flavor of blue cheese.

Table II. Free fatty acids contained in the fat fraction of the cheeses expressed in meq/100 g of fat.
Teneur en acides gras libres des fractions lipidiques de chaque fromage (exprimée en meq/100 g de matière grasse).
[a] : PF, PO, PG ; Roquefort: CAU : Bleu des Causses; AUV : Bleu d'Auvergne.

	Cheeses [a]				
	PF	*PO*	*PG*	*CAU*	*AUV*
FFA	4.70	4.65	10.25	17.45	4.70

Table III. Sulfur compounds found in the headspace of the different cheeses. [a] : PO, PF, PG : Roquefort; CAU : Bleu des Causses; AUV : Bleu d'Auvergne; [b] : peak height (average of triplicate analysis); [c] : concentrations (µg/kg of cheese); calculated whenever reference compounds were available; [d] : standard deviation of the 3 replicates.
Composés soufrés présents dans l'espace de tête des différents fromages. [a] : PO, PF, PG : Roquefort; CAU : Bleu des Causses; AUV : Auvergne Blue Cheese; [b] : hauteur de pic (moyenne de trois répétitions); [c] : concentrations (µg/kg de fromage) calculées chaque fois que les composés de référence étaient disponibles; [d] : coefficient de variation des trois répétitions.

Sulfur	PF [a]			PO [a]			PG [a]			CAU [a]			AUV [a]		
Compounds	*H*[b]	*C*[c]	*%*[d]	*H*	*C*	*%*	*H*	*C*	*%*	*H*	*C*	*%*	*H*	*C*	*%*
H_2S	439		38	536		70	243		17	—			—		
CH_3SH	45		25	2 216		34	1 601		32	45 380		18	—		
CH_3SCH_3	1 267	7	20	970	7	42	2 218	11	30	58 345	70	14	256	8	12
CS_2	3 088	5	20	634	3	39	560	2	11	—			95	1	6
unknown	164		47	120		27	512		31	—			69		41
$CH_3S_2CH_3$	155	tr	20	7 702	29	27	8 004	29	9	75 985	90	25	1 819	24	25

The detection threshold of dimethyl sulfide was 0.3 ppb in water and 9 ppb in milk and milk fat (Shankaranarayana *et al*, 1974). Even if the thereshold was not measured in cheese, we supposed that dimethyl sulfide was present in Bleu des Causses at a greater than threshold level (70 ppb), and contributed to the overall aroma of this cheese. In the same way dimethyl disulfide (detection threshold : 3 ppb in water, Shankaranarayana *et al*, 1974) could likely be detected in all samples (except perhaps in Roquefort PG).

Sulfur compounds have not been often reported in the studies of blue cheese aroma. Only methanethiol and hydrogen sulfide have been tentatively identified (Anderson, 1966).

Neutral volatile compounds

General considerations

One hundred and fifty-two compounds were observed in the Forane 11 extracts of the 5 cheese samples. Eighty-one of them were identified and 48 tentatively identified (table IV). The "apparent" concentrations are given in table IV : the aera ratio of each compound *versus* the internal standard was calculated without taking into account extraction recoveries or response coefficients. Estimations of the real concentrations were obtained for the most abundant compounds by adding standards to the Bleu d'Auvergne prior to distillation. The ranges of recoveries were 70–90% for methyl ketones, 50–70% for both primary and secondary alcohols, 50% for alkanals, 60% for lactones and 80–90% for esters, which were in agreement with the previous results of Forss, Holloway (1967) and Stark *et al* (1973).

The average standard deviation was 16.6 %. The highest values were obtained for the less abundant compounds (below 50 µg/kg). This could have been foreseen, owing to the heterogeneity of the samples (already noted by Svensen, Ottestad, 1969) and to the extraction method used. However, variations between samples

Table IV. Volatile compounds of blue cheeses; [a] : concentration (µg/kg of cheese) — average of triplicate analysis; [b] : standard deviation of the 3 replicates; [c] : retention indices determined on DB5 column; * : retention indices identical with those of authentic compounds
*Composés volatils présents dans les bleus; [a] : concentration (µg/kg de fromage — moyenne de trois répétitions; [b] : coefficient de variation des trois répétitions; [c] : indices de rétention déterminés sur DB5; * : indices de rétention identiques à ceux des composés de référence.*

Compounds	*Blue cheeses*										
	PF		*PO*		*PG*		*CAU*		*AUV*		
	C[a]	*%[b]*	*C*	*%*	*C*	*%*	*C*	*%*	*C*	*%*	*IR[c]*
KETONES											
2-butanone	—		—		tr		—		—		600
2-pentanone	1 680	2	565	6	9 430	8	3 440	17	340	10	*686
3-hydroxy 2-butanone	—		—		80	12	—		—		*702
3-methyl 2-pentanone	10	18	10	9	5	24	—		—		*748
2-hexanone	40	7	30	33	145	5	60	12	10	4	*790
5-hepten 2-one	—		—		30	6	5	66	10	14	875
2-heptanone	3 955	6	4 320	32	13 090	18	2 855	8	1 810	2	*897
3-methyl 2-heptanone	5	10	15	4	40	31	5	31	—		940
5 hepten 6-methyl/ 2-one	—		100	18	—		—		40	4	990
3-octanone	65	4	—		15	2	10	25	—		*990
2-octanone	55	8	100	6	210	5	40	8	60	2	*993
1-Me 1-cyclohexen 3-one	—		—		20	10	—		—		1 066
acetophenone	10	18	15	25	—		15	32	—		*1 076
8-nonen 2-one	380	14	340	25	440	16	340	11	610	13	1 092
2-nonanone	3 915	10	7 235	10	10 040	11	1 600	6	4 570	18	*1 100
2-decanone	370	5	460	8	60	3	—		60	3	*1 190
unsatured ketone	—		—		10	22	—		—		1 200
10-undecen 2-one	15	30	35	4	40	14	20	17	—		1 288
6-undecen 2-one	—		—		—		40	10	20	19	1 290
2-undecanone	515	5	990	2	1 090	9	455	14	1 490	10	*1 296
tridecenone	—		—		—		55	30	220	4	1 495

Compounds	Blue cheeses										
	PF		PO		PG		CAU		AUV		
	C[a]	%[b]	C	%	C	%	C	%	C	%	IR[c]
KETONES (cont)											
2-tridecanone	70	26	65	7	115	18	230	24	410	7	*1 500
1-hydroxy 1-methylethyl acetophenone	10	43	10	8	—		—		—		1 520
pentadecanone	—		60	23	30	9	175	26	130	15	*1 704
unsaturated ketone C_{16}	—		—		50	7	—		—		1 798
Total concentration	11 095		14 350		34 940		9 345		9 780	0	
ALCOHOLS											
2-methyl 1-propanol	70	60	85	17	tr		90	34	280	18	*626
1-butanol	10	59	5	21	—		—		—		*660
2-pentanol	600	16	—		1 410	19	1 410	10	290	3	*700
3-methyl 1-butanol	1 030	4	1 460	5	255	7	110	23	3 180	10	*732
2-methyl 1-butanol	—		—		—		—		980	23	*738
1-pentanol	—		—		—		745	27	—		*767
2-hexanol	—		—		5	34	—		—		*816
1-hexanol	50	27	10	7	—		—		10	40	*868
2-heptanol	1 325	4	515	22	2 905	17	785	16	830	1	*900
alcohol C_7	—		—		5	6	—		—		943
1-heptanol	—		—		—		—		10	2	*968
1,5-octadien 3-ol	—		—		—		5	15	—		
1-octen 3-ol	20	12	35	6	20	6	10	62	20	4	*984
2-octanol	—		—		20	13	—		—		*1 010
alcohol	10	40	—		—		—		—		
2-nonanol	710	15	980	14	2 920	22	450	12	970	2	*1 105
phenyl ethanol	140	21	145	7	—		130	5	1 400	5	*1 116
1-decen 3-ol	10	14	25	6	15	7	—		—		1 180
2-decanol	5	14	5	14	—		10	37	10	2	*1 208
2-undecanol	45	17	40	12	115	22	50	18	130		*1 300
Total concentration	4 025		3 305		7 670		3 795		8 110		

Compounds	Blue cheeses										
	PF		PO		PG		CAU		AUV		
	C[a]	%[b]	C	%	C	%	C	%	C	%	IR[c]
PHENOLS											
2-methyl 5-ethyl phenol	5	36	10	24	110	23	—		—		1 115
4-ethyl phenol	30	6	—		20	11	tr		10	18	*1 167
3-propyl phenol	5	5	—		—		—		—		1 273
Total concentration	40		10		130		tr		10		
ESTERS											
methyl butanoate	—		15	4	20	14	25	25	20	5	*720
methyl isovalerate	—		—		—		5	29	—		
ethyl butanoate	50	8	15	22	60	8	55	14	30	14	*803
isopropyl butanoate	5	70	5	21	10	17	10	22	—		*824
sec-amyl acetate	—		—		—		5	32	20	6	
ethyl isolaverate	—		—		—		5	29	—		
n-amyl acetate	—		—		—		10	2	—		
methyl hexanoate	20	27	90	13	80	3	70	22	70	1	*928
methylthio isolaverate	—		—		—		5	2	—		
isobutyl butanoate	5	20	30	34	20	28	10	21	30	3	*959
butyl butanoate	30	38	30	27	—		—		—		*1 002
ethyl hexanoate	75	13	70	10	75	12	70	9	60	9	*1 005
1-Me butyl isobutanoate	—		—		50	5	15	19	—		1 053
isopropyl hexanoate	5	59	5	0	60	19	20	9	—		*1 056
isoamyl isobutanoate	—		—		170	10	85	26	—		1 063
heptyl acetate	—		—		—		—		440	1	*1 075
methyl octanoate	40	14	225	5	195	21	70	22	60	19	*1 124
internal standard											
isobutyl hexanoate	10	1	30	18	40	13	5	30	20	7	*1 150
ethyl octanoate	110	6	—		110	13	80	7	50	9	*1 196
1-Me hexyl butanoate	10	19	20	5	130	15	15	16	10	30	*1 214

Compounds	Blue cheeses										
	PF		PO		PG		CAU		AUV		
	C[a]	%[b]	C	%	C	%	C	%	C	%	IR[c]
ESTERS (cont)											
isoamyl hexanoate	5	25	10	29	70	29	20	20	—		*1 218
methyl nonanoate	5	12	10	3	5	4	5	11	—		*1 226
isopropyl octanoate	5	14	—		50	19	15	9	—		*1 235
isoamyle hexanoate	20	23	10	26	170	21	105	29	320	5	*1 251
amyl hexanoate	10	21	110	8	20	21	10	28	10	4	*1 254
2-phenylethyl acetate	15	17	20	8	—		25	31	20	14	*1 263
methyl 4-decenoate	10	5	35	11	30	19	35	23	30	16	1 315
methyl decanoate	145	28	855	10	635	15	290	29	250	4	*1 324
isopropyl nonanoate	—		—		5	24	10	7	—		*1 330
isobutyl octanoate	10	13	25	6	30	26	15	20	30	3	*1 357
ethyl decanoate	320	5	175	16	195	7	270	23	20	3	*1 397
1-Me hexyl hexanoate	—		—		60	26	10	20	—		1 405
1-Me octyl butanoate	—		80	22	180	18	15	5	—		1 409
ester C_5 octanoate	10	3	—		70	20	20	37	—		1 412
methyl undecanoate	5	27	—		—		—		—		1 424
isopropyl decanoate	15	5	15	14	170	14	100	20	—		*1 434
2-phenylethyl butanoate	—		—		—		—		110	2	*1 440
isoamyl octanoate	35	12	180	24	165	25	115	27	220	6	1 449
amyl octanoate	—		—		—		10	23	10	4	1 454
propyl decanoate	—		—		15	31	—		—		*1 495
methyl dodecanoate	30	19	160	23	120	14	190	36	200	4	*1 527
isobutyl decanoate	20	9	75	3	55	11	40	32	60	8	*1 548
1-Me octyl hexanoate	15	24	—		10	5	—		—		1 588
ethyl dodecanoate	140	18	70	25	135	19	255	22	—		*1 595
1-Me decyl butanoate	—		—		20	8	—		—		1 600
act-amyl decanoate	10	27	5	25	120	15	70	32	—		1 608
isopropyl dodecanoate	5	27	—		20	8	45	30	—		*1 622
isoamyl decanoate	55	7	370	4	250	17	280	37	550	12	*1 647
amyl decanoate	—		—		—		40	38	—		

Compounds	Blue cheeses										
	PF		PO		PG		CAU		AUV		
	C[a]	%[b]	C	%	C	%	C	%	C	%	IR[c]
ESTERS (cont)											
2-phenylethyl hexanoate	10	11	55	13	30	12	100	28	—		* 1650
methyl tetradecanoate	25	12	80	10	40	9	110	38	130	9	*1 728
isobutyl dodecanoate	10	21	—		10	4	20	32	30	18	*1 750
ethyl tetradecanoate	95	35	70	43	—		150	27	150	17	*1 798
ester C_5 dodecanoate	—		—		45	2	—		—		1 805
isopropyl tetradecanoate	—		—		15	10	40	14	—		1 828
isoamyl dodecanoate	—		—		55	5	170	30	—		1 847
methyl hexadecanoate	5	3	40	30	20	32	15	62	—		*1 900
Total concentration	1 390		2 985		3 835		3 155		2 950		
LACTONES											
γ-hexalactone	—		—		5	26	—		—		1 083
γ-octalactone	—		—		30	36	5	25	—		1 264
γ-decalactone	10	9	—		20	6	20	27	40	12	*1 474
γ-dodecenolactone	10	37	45	7	55	15	30	25	150	12	1 664
γ-dodecalactone	30	22	210	11	215	9	370	28	1 030	1	*1 690
δ-dodecalactone	—		—		—		—		700	15	*1 740
δ-tetradecalactone	—		—		—		—		310	1	*1 940
Total concentration	50		255		325		425		2 230		
ALDEHYDES											
3-methyl butanal	—		—		tr		—		160	31	*633
hexanal	—		—		—		—		10	33	*830
benzaldehyde	5	13	10	13	15	14	—		—		*968
phenyl acetaldehyde	—		—		—		—		60	7	*1 070
decanal	—		—		—		—		10	15	*1 225
2,4-decadienal	—		—		—		—		10	20	1 320
Total concentration	5		10		15		0		250		

Compounds	Blue cheeses										
	PF		PO		PG		CAU		AUV		
	C[a]	%[b]	C	%	C	%	C	%	C	%	IR[c]
BENZENIC COMPOUNDS											
methyl benzene	10	14	10	28	—		—		30	30	768
dimethyl benzene	5	11	5	14	—		—		10	5	856
1,1-diMe decyl benzene	10	47	—		—		—		—		1 708
Total concentration	25		15		0		0		40		
ANISOLES											
4-methoxytoluene	80	35	15	2	15	5	5	63	20	5	*1 035
1,3-dimethoxy benzene	10	13	80	9	—		10	26	—		*1 164
3,4-dimethoxytoluene	5	62	—		—		—		—		*1 243
Total concentration	95		95		15		15		20		
PYRAZINES											
2,6-dimethyl pyrazine	20	24	30	19	25	32	10	2	—		*910
2,3,5,-trimethyl pyrazine	—		—		—		—		30	32	*1 015
Total concentration	20		30		25		10		30		
MISCELLANEOUS											
pyridine	—		—		—		—		80	2	
benzothiazole	5	9	—		—		—		—		1 241
indole	5	3	5	15	5	15	—		—		*1 308
Total concentration	10		5		5		0		80		

Compounds	*Blue cheeses*										
	PF		*PO*		*PG*		*CAU*		*AUV*		
	C[a]	*%*[b]	*C*	*%*	*C*	*%*	*C*	*%*	*C*	*%*	*IR*[c]
UNIDENTIFIED COMPOUNDS											
Oxygenated sesquiterpene	70	9	—		—		—		—		1 410
Sesquiterpene	15	7	20	15	—		—		—		1 446
Sesquiterpene	—		—		20	2	—		—		1 452
Sesquiterpene	15	25	30	22	—		—		—		1 455
Sesquiterpene	25	26	10	12	—		—		—		1 512
Oxygenated sesquiterpene	25	24	15	40	—		—		—		1 517
Sesquiterpene	5	7	—		—		—		—		1 532
Sesquiterpene	5	0	—		—		—		—		1 553
Total concentration	5 195		5 980		20		3 925		0		
Total	21 950		27 040		46 980		20 670		23 500		

were always more important than variations between replicates.

The sum of all quantified compounds (last line of table IV) gave an evaluation of the total odorous profile. By applying a correcting coefficient to the main chemical classes (1.43 for methyl-ketones which corresponded to an average recovery of 70%, 1.66 for alcohols and lactones which corresponded to a 60% recovery), a more accurate evaluation of the total odorous profile, *ie* 29.4, 35.6 and 67.3 mg/kg, respectively for the Roquefort samples PF, PO and PG, 27.5 mg/kg for the Bleu des Causses and 34.7 mg/kg for the Bleu d'Auvergne, was obtained. These figures should be considered cautiously since all correcting coefficients could not be calculated, but they allowed a global comparison of the samples. Thus, odorous volatile compounds were more abundant in the three Roquefort cheeses than in the Bleu des Causses sample, the PG cheese being twice as rich as the two other samples. The Bleu d'Auvergne sample was quantitatively intermediate between the Roquefort samples PO and PG.

The predominant components of blue cheese aroma, methyl-ketones and related secondary alcohols, originate from fatty acids (Kinsella, Hwang, 1976). So we tried to relate the total odorous profile (last line of table IV) with the concentration of FFA (table II). An obvious correlation was observed for the Roquefort and the Bleu d'Auvergne samples: both FFA and volatile compounds were twice as abundant in Roquefort PO, PF and Bleud 'Auvergne, and these 3 samples contained similar concentrations of both FFA and volatile compounds. However, despite its higher content in FFA, the Bleu des Causses did not show the richest odorous profile (and especially the highest content in methyl ketones and secondary alcohols). This could be consistent with the observations of Kinsella and Hwang (1976): the rate of release of FFA could be a limiting factor in methyl-ketone production since FFA are toxic to the mycellium of *Penicillium roqueforti* (particularly long-chain fatty acids) and inhibit methyl-ketone formation. However, not only lipolysis rate can affect the odorous profiles. Many other properties of the moud, not measured here, influence the production of aroma compounds in blue cheese (proteolytic activity, growth rate).

Ketones

Methyl-ketones represented about half of the odorous profile for all samples, and accounted for 75% of the odorous profile for Roquefort PG. 2-heptanone and 2-nonanone were the most abundant ketones except for the Bleu des Causses, in which 2-pentanone was predominant and represented 16.6% of the total odorous profile. This ketone was also very abundant in Roquefort PG 20% of the odorous profile). 2-undecanone was important in the Bleu d'Auvergne profile: its concentration was similar to that of 2-heptanone.

Roquefort PF was considered to have a neutral flavour and contained the lowest concentrations of both methyl-ketones and FFA. On the other hand, PG was often judged as good, but a little too pungent or "burning", which could be explained by the abundance of both methyl-ketones and FFA. These results confirmed the general idea that the sensory quality of blue cheese is correlated with these two classes of chemicals. However, the methyl-ketones and FFA content of the Roquefort PO en FF were similar, and only PO was judged to possess the ideal flavour of Roquefort, which indicates the importance of other odorous compounds in the typical flavour of Roquefort.

The quantitative data obtained in this word were compared to the literature data

(table V). All of them had been obtained by derivatization of monocarbonyl compounds to dinitrophenylhydrazones, first described by Schwartz, Parks (1963). Our figures were generally 2 to 10-fold lower than all other results. The concentrations found in Roquefort PG (the highest concentrations in this work) were similar to those obtained by Anderson, Day (1966) for an imported Roquefort sample. However, this cheese was, for them, the poorest sample. The variability of samples of various origins may explain these differences.

A few unsaturated ketones were detected here. Only 8-nonen 2-one had been reported in Normanna cheese (Svensen, Ottestad, 1969). The concentrations were again more important in their work : up to 24 mg/kg instead of 1 mg/kg (maximum value found here in Bleu d'Auvergne cheese). Karahadian *et al* (1985), who found this coumpound in Brie cheese, proposed a mechanism for its formation involving the "classical" oxidative decarboxylation of 9-decenoic acid (emanating from the lipoxygenase-mediated cleavage of polyunsaturated fatty acids).

Low amounts of acetophenone occurred in Roquefort and Bleu des Causses. This compound, which has been found in some washed-rind type cheeses (Adda, Dumont, 1974; Parliment *et al,* 1982) and in Comté cheeses (Dumont *et al,* 1981), where it could be a metabolite of the smear microflora, was reported for the first time in blue cheese.

Alcohols

Alcohols represented 15—20% of the total odorous profile of Roquefort and Bleu des Causses and more than a third for the Bleu

Table V. Methyl-ketones content of blue cheeses of different origin (minimum and maximum value found in each work). A : Normanna cheese (Svensen & Ottestad, 1969) — 22 samples; B : Roquefort cheese (Schwartz *et al,* 1963) — 3 samples; C : Roquefort cheese (Anderson & Day, 1966) — 2 samples; D : American Blue cheese (Anderson & Day, 1966) — 5 samples; E : real concentrations obtained in our work, calculated with a correcting factor of 1.43 (corresponding to a 70% recovery of the methyl ketones).
Quantités de méthyl-cétones trouvées dans différents bleus : données bibliographiques (valeurs minimales et maximales citées dans chaque travail). A : fromage Normanna (Svensen, Ottestad, 1969) — 22 échantillons; B : Roquefort (Schwartz et al, *1963) — 3 échantillons; C : Roquefort (Anderson, Day, 1966) — 2 échantillons; D : Bleu américain (Anderson, Day, 1966) — 5 échantillons; E : concentrations réelles obtenues dans notre travail, calculées avec un coefficient de correction de 1,43 (ce qui correspond à un pourcentage de récupération des méthyl cétones de 70%).*

Chain length	*mg/kg blue cheese*				
	A	*B*	*C*	*D*	*E*
C_5	1.3 - 142.0	3.6 - 20.9	6.5 - 20.9	3.6 - 19.2	0.5 - 13.5
C_7	4.8 - 100.0	17.6 - 71.0	17.9 - 71.8	17.6 - 69.9	2.5 - 18.0
C_9	3.9 - 145.0	19.8 - 88.3	19.8 - 88.3	13.9 - 78.9	2.3 - 14.3
C_{11}	tr - 20.3	2.4 - 29.9	4.9 - 29.9	2.4 - 6.7	0.7 - 2.1

d'Auvergne sample. Secondary alcohols were generally more abundant than primary alcohols, except for Bleu d'Auvergne, for which primary alcohols were twice as abundant.

Odd-numbered secondary alcohols were found in all cheeses. Conversion rates of methyl-ketones to the corresponding alcohols, vary according to the strain and chain length of that strain (Kinsella, Hwang, 1976). However, these rates were similar for Bleu des Causses and Roquefort PG which had been manufactured with the same strain (25, 30 and 10%, respectively, for 2-heptanone, 2-nonanone and 2-undecanone).

Two papers reported a quantitative analysis of secondary alcohols in blue cheese : the concentrations found in our work for 2-pentanol, 2-heptanol and 2-nonanal were similar to those found by Anderson, Day (1966) in American Blue and Roquefort samples. Concentrations obtained by Svensen, Ottestad (1969), for 22 samples of Normanna cheese were, however, 1 to 3-fold higher.

Even-numbered secondary alcohols were also present at very low levels. They probably did not contribute to the blue cheese aroma.

Several primary alcohols found here had been already reported in blue cheeses by Day and Anderson (1965) and Ney and Wirotama (1972), without any quantitative indication. They may impart a fruity, nutty not to the flavour of cheese (Kinsella, Hwang, 1976). 3-methyl 1-butanol, which occurred in both Roquefort PF and PG, was particularly abundant in the Bleu d'Auvergne cheese, where it represented almost 40% of the alcohol class. 1-pentanol was found only in Bleu des Causses. Phenyl-ethanol was found in all cheeses except Roquefort PG, and was 10-fold more abundant in Bleu d'Auvergne (its real concentration in this cheese was 2 mg/kg). With its reminiscent odour of yeast, even at low levels, it contributes to the background flavour and to the typical aroma of blue cheese (Anderson, Day, 1966).

Low amounts of unsaturated alcohols were detected in blue cheeses. Originating from the oxidation of polyunsaturated fatty acids by *P roqueforti*, these compounds have been often reported in Brie or Camembert cheeses (Karahadian *et al*, 1985), where they contributed largely to the aroma. 1-octen 3-ol is also desirable for blue cheese aroma at about 5 to 50 ppm (Moinas *et al*, 1974; Ney *et al*, 1975), but this concentration was not reached in the samples analyzed.

Phenols

The 3 phenolic compounds: 2-methyl 5-ethyl phenol, 4-ethyl phenol and 3-propyl phenol, which occurred in Roquefort samples, have not, as far as we know, been reported before in any kind of cheese. They probably originated from the microbiological breakdown of an amino-acid. Such a mechanism was demonstrated for phenol, one of the major flavour compounds of the surface-ripened cheeses (Parliment *et al*, 1982), which is formed during tyrosine degradation. Incidentally, despite their low thresholds, they were found at such low levels that their contribution to the aroma must be very marginal.

Esters

The most important chemical class for the number of compounds was the ester class. Fourteen of the 57 esters found here were detected by Day and Anderson (1965); the others are reported for the first time in blue cheese. They represented 6–15% of the total odorous profile (15% in Bleu des Causses, which contained 49 esters).

Some of them were found at rather high levels: methyl-decanoate represented 3.2% of the total odorous profile for Roquefort PO, and 1.4 % for both Roquefort PG and Bleu des Causses. Isoamyl-decanoate represented, respectively, 1.4% and 2.3 % of the total odorous profile for Roquefort PO and Bleu d'Auvergne, and ethyl-decanoate, 1.5% of the Roquefort PF profile. In addition, Bleu d'Auvergne contained heptyl-acetate and 2-phenyl-ethyl butanoate, which were not found in the other samples.

Whether ester formation results from a chemical equilibrium or an enzymic mechanism has not yet been elucidated, but esterification is known to be a secondary reaction between primary volatile compounds. The ester content of cheeses has been shown to increase during ripening (Dumont, Adda, 1978). However, despite a ripening period which was twice as long for Roquefort PG than for Bleu des Causses, their ester contents were quantitatively similar.

Esters possess fruity notes and may contribute to the aroma by minimizing the sharpness and bitterness imparted by fatty acids and animes, respectively. This result was obtained by Anderson, Day (1966) with an ethyl-butanoate, methyl-hexanoate and octanoate addition, which reduced the harshness of a synthetic mixture containing methyl-ketones, secondary alcohols and fatty acids. Ethyl-esters of butanoic, hexanoic and octanoic acid were incorporated in a blue cheese flavor patented by Ney *et al* (1975). The flavour specificity of the 3 types of blue cheese studied here could partially originate from the both qualitative and quantitative differences in their ester content. This could, in particular, explain the character of Roquefort PO with regard to the neutral flavour of Roquefort PF; all chemical classes were quantitatively similar, except esters, of which Roquefort PO contained twice as much as Roquefort PF.

Lactones

Lactones were found only at low concentrations in Roquefort and Bleu des Causses, but they represented about 10% of the total odorous profile of Bleu d'Auvergne. The higher content of Bleu d'Auvergne could be related to the use of pasteurized milk, since the heating of milk products has been shown to increase their lactone level.

Both γ- and δ-lactones were found in our samples, γ-dodecalactone being predominant. Blue cheeses have already been shown to contain both δ- and γ-lactones (Jolly, Kosikowski, 1975). But δ-dodecalactone, and above all δ-tetradecalactone, were, in that study, the major lactones formed. Jolly, Kosikowsky (1975) noticed a decrease of δ-lactones during ripening. This phenomenon, also observed by Wong *et al* (1975) in Cheddar cheese, could explain, in our work, the absence of δ-lactones in Roquefort and Bleu des Causses, which had a longer ripening period than Bleu d'Auvergne.

Lactones in cheese are supposed to be formed by hydrolysis of the hydroxy-fatty acids (a normal constituent of milk fat) followed by lactonization (Jolly, Kosikowski, 1975). Other mechanisms have been proposed (Adda *et al*, 1982), but none of them have been proved. The differences in lactone content are therefore directly related to the lactone potential of milk. The amount of lactone precursors, greater for cow's milk than for ewe's milk (Dimick *et al*, 1969), has been shown to be influenced by many different factors such as feed, season, breed, stage of lactation (Dimick, Harner, 1968) or diet of animals (Urbach, 1982).

The lactone concentrations found here were lower than those reported by Jolly, Kosikowski (1975): up to 18 mg/kg of cheese. γ-dodecalactone, which has a detection threshold of about 1 ppm in butter (according to Siek *et al,* 1969), may be important for the background flavour of Bleu d'Auvergne, and perhaps for that of Bleu des Causses and Roquefort PG, if the possible synergic effect of lactones is taken into account. However, the contribution of both δ-dodecalactone and δ-tetradecalactone, which occurred in Bleu d'Auvergne at levels lower than their detection threshold (which were 5.4 ppm in a lactone mixture), is debatable.

Other compounds

A few aldehydes were found in Bleu d'Auvergne. Some of them had been already reported in blue Cheese (3-methyl butanal, phenyl-acetaldehyde: Day, Anderson, 1965; Ney, Wirotama, 1972). Only benzaldehyde was detected in Roquefort. No aldehyde was found in Bleu des Causses. The importance of aldehydes in the flavour of blue cheese has not yet been elucidated. Probably formed by degradation of amino-acids (Kinsella, Hwang, 1976), they may impart an "unclean", harsh and dull flavour (as observed in Cheddar for phenyl acetaldehyde and 3-methyl butanal, by Dunn, Lindsay, 1985).

Several anisole compounds were found: 1,3-dimethoxybenzene, 4-methoxy- and 3,4-dimethoxytoluene. They contribute to the aroma of Roquefort PO and PF with their, respectively, hazelnut-like, floral or sweet notes. Already found in Camembert (Dumont *et al,* 1976), they may originate from the breakdown of lignin occurring in feed.

Undentified compounds

A few presumed sesquiterpenic compounds were found in Roquefort PO and PF. They may have been transferred from the forage to the milk without degradation (Dumont, Adda, 1978). Because they occurred at very low levels and did not exhibit any odor at the sniffing-port of the chromatograph, their contribution to the aroma was negligible.

CONCLUSIONS

Besides carbonyl compounds, always reported as being responsible for the typical blue cheese flavor, many other compounds were found in the odorous profile of all cheeses. Sulfur compounds, esters, anisoles, more numerous in Roquefort and Bleu des Causses, lactones and aldehydes, more abundant in Bleu d'Auvergne, possibly contributed to the aroma and may partly explain the specificity of each cheese. However, other compounds (such as fatty acids and their soaps, amino acids, amines) play a major role in both flavour and taste of blue cheese. The quantification of these compounds could provide further information on blue cheese flavor.

ACKNOWLEDGMENTS

We are grateful to M. Assenat (Laboratoire du Groupe Société des Caves de Roquefort) for supplying the cheese samples.

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