Grana Padano cheese aroma

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Summary. The volatile concentrate obtained from Grana Padano cheese by vacuum distillation was fractionated by continuous liquid–liquid extraction into neutral and acid fractions. Both were analysed by high resolution gas chromatography (HRGC), HRGC–mass spectrometry, and HRGC–olfactometry. A total of 67 components were identified in the neutral extract (22 esters, 13 alcohols, 12 ketones, 6 aldehydes, 5 nitrogen-containing compounds, 3 lactones and 6 miscellaneous compounds) and 16 in the acid extract. Esters were the predominant constituents of the neutral fraction, whose major components were ethyl butanoate and ethyl hexanoate. HRGC–olfactometry of the neutral compounds demonstrated that 23 were odour-active: ethyl butanoate, 2-heptanol, 3-methylthiopropanal, 1-octen-3-ol, ethyl hexanoate and nonanal being the most potent odorants. *n*-Butanoic and *n*-hexanoic acids were the main volatile free fatty acids identified in the acid extract as having an important odour with a high olfactometric index. The backbone of Grana Padano cheese aroma seemed to consist of these acids and 14 potent neutral odorants imparting fruity, green, nutty and coconut notes. The concentration of volatile components responsible for the fruity and green notes was inversely proportional to the length of ripening, whereas the concentration of volatile agents with spicy, nutty and earthy notes tended to increase during maturation. In a comparison of the olfactometric profile, the Grana Padano cheese aroma was found to be more complex than an imitation Grana Padano cheese produced with similar technology but outside the area of the genuine cheese. Some of the main metabolic pathways for the biosynthesis of cheese aroma are reviewed briefly to indicate the possible origin of the compounds identified.

Although 600 volatile agents have been identified in cheese (Maarse & Visscher, 1989), with the exception of blue-veined and surface-moulded cheeses (where the dominant odours are impact compounds) the identity of the odorous molecules perceived during tasting remains to be determined for most varieties. Cheese aroma is still considered the result of a balance between various volatile components which individually do not reflect the overall odour (Adda, 1984).

Among the Italian cheeses, Parmigiano-Reggiano has been the most studied (Dumont *et al.* 1974*a*; Meinhart & Schreier, 1986; Barbieri *et al.* 1994), followed by 'pasta filata' cheeses such as Provolone, bovine or water-buffalo Mozzarella cheese (Moio *et al.* 1993*b*, *c*), as well as 'sweet' and 'natural' blue Gorgonzola (Martelli & Tourn, 1990).

Grana Padano (GP) cheese is an Italian hard cheese with a delicate and

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characteristic aroma, made from raw bovine milk, partly defatted by creaming, with the addition of natural starter, cooked at 53 °C and matured for at least 12 months. Among the GP volatiles, carbonyl compounds have been found to increase during ripening as determined by static headspace analysis (Bottazzi & Battistotti, 1974).

Our analytical approach was to extract cheese volatiles by vacuum distillation, identify the volatile components by high resolution gas chromatography (HRGC) coupled with mass spectrometry, discriminate by sniffing analysis the odorous from the inodorous volatiles and finally to evaluate by Charm analysis the odour active compounds. This paper reports the identification of GP cheese volatile components and the evaluation of their odour intensity using these techniques.

materials and methods

Cheese samples

Three GP cheeses (~ 35 kg) were prepared in a cheese factory (Consorzio Grana Padano, Milano, Italy) within the legal area for production of this cheese. From each cheese after 12, 18 and 24 months three slices were cut, vacuum wrapped and kept in a closed container at −20 °C until the experiments were carried out. One GP cheese produced outside the Appellation d'Origine Contrôlée area at ~ 13 months of ripening was also sampled as imitation Grana Padano cheese.

Extraction and concentration of volatile constituents of the cheese

In recent years the techniques most used in the study of cheese aroma have been those based on direct vacuum distillation of the cheese (Dumont & Adda, 1972). With this procedure it is possible to produce an aqueous distillate containing most of the volatiles, which is subsequently extracted with organic solvents and concentrated. A 150 g portion of the GP sample frozen at −20 °C was powdered while cold and introduced into a 2 l round bottomed flask. Volatile components were distilled under vacuum at a pressure of 0·1 Pa for 6 h as described by Dumont & Adda (1972). The aqueous distillate containing the volatile components was recovered in the liquid nitrogen traps placed between the sample and the vacuum generating system. As internal standard, 11·6 μg methyl decanoate was added (although not for GC–mass spectrometry) and the distillate was brought to pH 9·5 with 2 M-NaOH. The aromatic distillate was then extracted three times for 15 min each with 10 ml distilled dichloromethane. The extract, containing the neutral volatiles, was dried with Na_2SO_4, filtered through glass wool and concentrated by distillation at 50 °C to 100 μl, with a consecutive series of Kuderna–Danish columns of decreasing size. The alkaline solution was then acidified to pH 2 by addition of dilute HCl and extracted again with dichloromethane. The free fatty acids (FFA) were then recovered in the organic extract which was concentrated as described above for the neutral volatiles.

High resolution gas chromatography

HRGC analysis was carried out with an HP 5890 gas chromatograph (Hewlett Packard, Avondale, PA 19311, USA) with a flame ionization detector (FID) and DB-5 (neutral extract) or DB-Wax (acidic extract) fused silica capillary column (30 m × 0·32 mm i.d., film thickness 1 μm; J & W Scientific Inc., Folsom, CA 95630, USA). The oven temperature was programmed from 40 to 210 °C at 3 deg C/min. The temperature of the FID was maintained constant at 240 °C and the temperature of the injector at 230 °C. The hydrogen carrier gas velocity was fixed at 0·37 m/s.

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The area under the peaks was calculated using a Coconut acquisition and processing data system (INRA, Dijon, France). The linear retention indices (LRI) were calculated for each peak using as reference the series of hydrocarbons C_7–C_{24} according to the method of Van den Dool & Kratz (1963). The LRI calculated for each peak was compared with that of pure substance analysed on the same column under the same operating conditions.

Gas chromatography–olfactometry

The aromatic extract was diluted (1 : 3, 1 : 9, 1 : 27, 1 : 81, 1 : 243, 1 : 729, 1 : 2187) and 1 μl was injected into a Hewlett Packard 5890 gas chromatograph with a DB-5 (neutral extract) or a DB-Wax (acidic extract) fused silica capillary column as above. The chromatographic effluent was sniffed at an exit port held at 250 °C. This effluent was mixed with a current of humid air (20 l/min) in order to avoid drying of the mucous membranes of the nose. Sniffing was continued until no more odour was perceived. Every time an odour was detected, its presence was registered on a personal computer. The analysis was repeated on progressively more dilute samples until no further odour was perceived by the operator. At the end of each analysis, each peak or region of the chromatogram corresponding to an odour was marked by a retention time transformed into an LRI. The results obtained from the series of diluted extracts from a cheese sample constituted the Charm chromatogram (Acree *et al.* 1984).

Gas chromatography–mass spectrometry

The electron impact mass spectra were obtained with a Hewlett Packard 5970 mass spectrometer. A DB-5 fused silica capillary column as above was directly connected to the ion source of the spectrometer. The GC conditions were the same as those described for HRGC. The mass spectra were determined at 70 eV, while the ion source and interface were held at 150 and 280 °C respectively.

RESULTS AND DISCUSSION

Identification and measurement of the neutral volatile components

The neutral volatile fraction of the GP cheese after concentration gave an intense fruity odour. The gas chromatogram (Fig. 1*a*) contained 90 separate peaks, 67 of which were identified on the basis of the electron impact mass spectra, LRI and reference compounds. All the volatile components identified, grouped by chemical classes, are reported in Table 1. The quantity of each component was calculated with respect to the internal standard assuming that the extraction efficiency and the GC response were identical for all the compounds separated. The absolute levels of the different classes of volatile compounds of GP cheese compared with those recorded for milk by the same procedure (Moio *et al.* 1993*a*) are shown in Table 2. The esters, ketones, alcohols and lactones were higher in cheese, indicating that these compounds were formed mainly during ripening, while the aldehyde and nitrogen compounds were lower than in milk.

Esters were the main neutral constituents in the aqueous distillate of GP and constituted ~ 41 % of the total neutral volatiles. Ethyl esters of butanoic, hexanoic, octanoic and decanoic acids represented ~ 95 % of the total esters. Ethyl hexanoate, with a distinct odour of unripe apple, was present in the greatest quantity, ~ 60 % of total esters. This odorant in 12 month GP cheese was 10-fold the level found in

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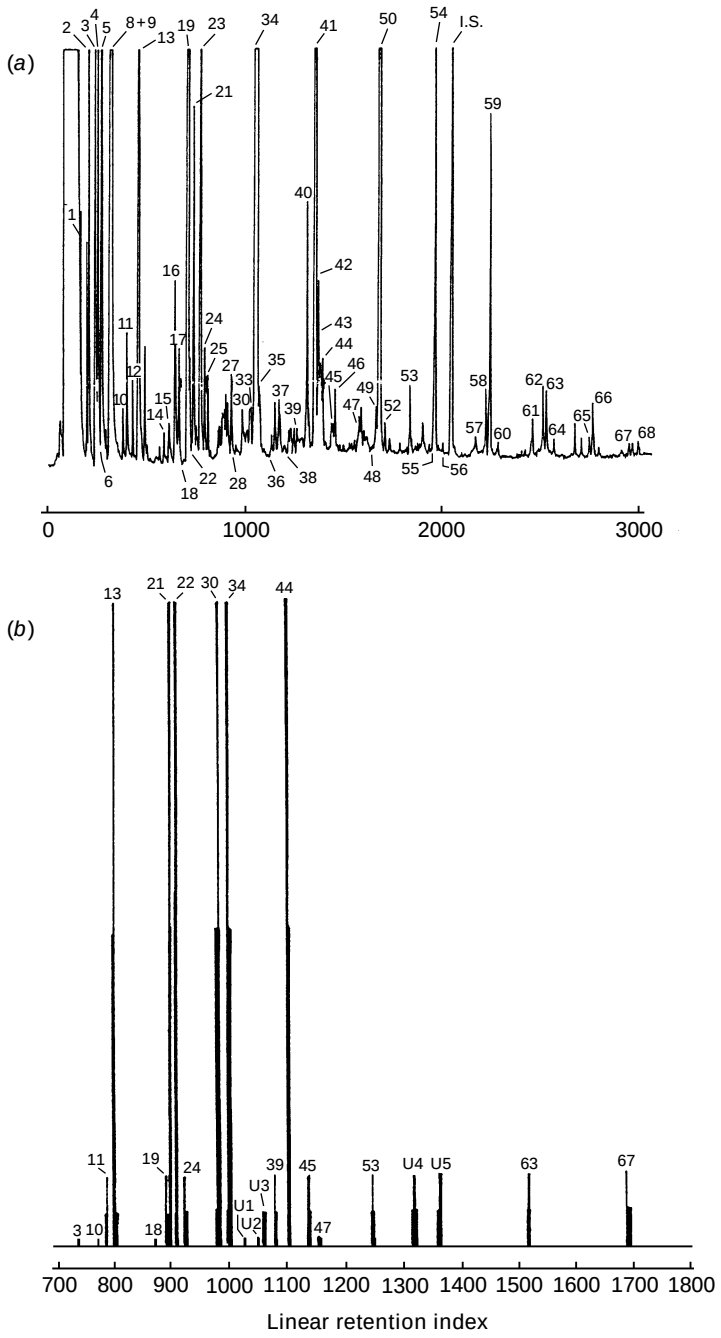


Fig. 1. Comparison of (*a*) gas chromatography–flame ionization detection and (*b*) gas chromatography–olfactory (Charm) response of combined neutral organic extracts of Grana Padano cheese. Assignment and descriptions of peaks are given in Tables 1 and 4.

fresh bovine milk (0·09 mg/kg) and 18-fold that in bovine Mozzarella cheese (0·05 mg/kg), analysed by the same procedure (Moio *et al.* 1993*a*, *b*). Ethyl butanoate was the second most important ester of GP cheese, at about three times the level found in milk (0·12 mg/kg; Moio *et al.* 1993*a*) and five times that found in bovine

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Table 1. *Concentrations of volatile components in the neutral extract of Grana Padano cheese and their olfactory properties*

Peak no.†	Compound	Linear retention index‡ Ref.	Linear retention index‡ Obs.	Identification	Odour descriptor§	Concentration, μg/kg Ripening time 12 months $\bar{X}$	12 months CV	18 months $\bar{X}$	18 months CV	24 months $\bar{X}$	24 months CV
	Esters										
6	Methyl butanoate	719	720	EI, CI		3	10·21	2	9·21	1	8·24
13	Ethyl butanoate	802	802	EI, CI	Fruity, apple-like, sweet, banana, pineapple	362	8·29	223	10·21	124	10·21
14	Isopropyl butanoate		848	EI	Fruity	3	7·39	2	11·3	4	11·28
18	3-Methylbutyl acetate	875	876	EI	Fruity, banana, candy circus peanuts	5	8·24	10	8·91	11	9·27
20	Propyl butanoate		895	EI	Sharp, rancid, sweaty, sickening	tr		tr		tr	
25	Methyl hexanoate	942	998	EI, CI	Pineapple, ether	6	9·23	4·5	9·11	5	8·23
33	Butyl butanoate		908	EI	Fruity, pineapple	6	6·72	9	8·21	15	7·95
34	Ethyl hexanoate	1002	1003	EI, CI	Fruity, wine-like, apple, banana	993	7·29	737	7·31	215	9·24
36	Isoamyl isobutyrate	1020	1021		Honey, apricot	ne		ne		ne	
37	Isopropyl hexanoate		1046	EI		14	10·10	7	6·29	14	8·31
38	3-Methylbutyl butanoate		1061	EI		9	9·31	8	8·24	2	10·22
42	Ethyl heptanoate		1083	EI	Fruity, wine-like, brandy	1	8·21	2	7·21	2	10·31
45	Methyl octanoate	1138	1138	EI, CI	Green, fruity	4	7·23	5	8·22	7	9·26
46	Isobutyl hexanoate		1149	EI	Apple	4	6·22	tr		tr	
47	Butyl hexanoate		1185	EI, CI	Fruity, wine-like	5	6·39	tr		tr	
50	Ethyl octanoate	1196	1196	EI, CI	Fruity, banana, apple-like, pineapple, brandy	214	5·29	229	6·93	37	7·19
53	2-Methylbutyl hexanoate		1247	EI		2	10·11	5	8·24	20	7·22
55	Ethyl nonanoate	1319	1320	EI, CI	Fruity, slightly fatty, oily, nutty	tr		tr		tr	
58	Ethyl decenoate		1387	EI		8	12·47	5	11·21	tr	
	Methyl decanoate (IS)			EI, CI		ne		ne		ne	
59	Ethyl decanoate	1397	1395	EI	Brandy, oily, fruity, grape-like	30	8·29	24	10·21	2	9·21
65	Ethyl dodecanoate	1590	1591	EI	Tallowy, soapy	2	7·31	2	9·23	tr	
68	Ethyl tetradecanoate		1790		Tallowy, soapy	ne		ne		ne	
	Ketones										
3	2-Pentanone		715	EI, CI	Sweet, fruity, cheesy	296	7·31	143	8·65	54	9·54
5	3-Hydroxy-2-butanone	718	720	EI	Buttery	85	8·23	70	9·98	60	8·85
12	2-Hexanone	792	793	EI, CI	Blue cheese	10	6·12	4	7·20	3	10·55
17	5-Hepten-2-one		866	EI		tr		tr		tr	
19	2-Heptanone	891	893	EI, CI	Blue cheese, spicy, cinnamon	536	7·16	320	10·54	200	7·89
31	2-Methyl-6-hepten-5-one		896			ne		ne		ne	

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Table 1. (*cont.*)

Peak no.†	Compound	Linear retention index‡ Ref.	Linear retention index‡ Obs.	Identification	Odour descriptor§	Concentration, μg/kg Ripening time 12 months $\bar{X}$	12 months CV	18 months $\bar{X}$	18 months CV	24 months $\bar{X}$	24 months CV
32	2-Octanone	999	998	EI, CI	Musty, floral, green, herbaceous	tr		tr		tr	
40	8-Nonen-2-one		1079	EI		76	8·23	20	9·41	90	8·90
41	2-Nonanone	1093	1091	EI, CI	Fruity, floral	497	7·91	172	8·29	150	9·20
49	2-Decanone		1192	EI, CI	Slightly fatty	tr		tr		tr	
54	2-Undecanone	1295	1296	EI	Citrus, rose	37	5·24	38	7·25	10	11·21
62	2-Tridecanone		1527	EI	Warm, herbaceous, slightly spicy	4	9·1	3	11·21	2	10·47
	Alcohols										
2	1-Butanol		705	EI	Medicinal	45	12·10	37	8·21	236	13·10
4	2-Pentanol		717	EI	Mild green, fusel oil	361	9·10	47	7·39	90	14·20
7	3-Methyl-3-buten-1-ol		734	EI	Herbaceous	400	7·29	430	9·31	420	11·29
8	3-Methyl-1-butanol	740	742	EI, CI	Fusel oil, whisky	260	8·25	290	7·11	280	10·31
9	2-Methyl-1-butanol	742	743	EI, CI	Fusel oil, whisky	29	9·30	3·5	10·21	40	12·12
10	1-Pentanol	764	765	EI	Alcoholic, iodoform-like	12	12·31	10	8·29	9	9·95
11	3-Methyl-2-buten-1-ol		772	EI, CI	Fresh, fruit, green, slight lavender	12	8·20	8·6	7·99	20	9·71
15	Furfuryl alcohol		857	EI	Cooked sugar	tr		tr		tr	
16	1-Hexanol	859	861	EI, CI	Slightly fruity, green	30	7·29	20	6·23	40	8·29
21	2-Heptanol		901	EI	Earthy, oily	157	8·25	94	6·91	44	9·23
28	1-Heptanol			EI, CI	Fragrant, woody, heavy, oily	3	8·51	4	8·31	4	9·21
30	1-Octen-3-ol	982	985	EI	Mushroom, musty/mouldy, earthy	13	11·85	25	10·31	12	9·23
43	2-Nonanol			EI, CI	Fatty, mild, green, melon	11	12·87	6	11·91	4	8·31
	Aldehydes										
1	3-Methyl-butanal	700	701	EI, CI	Unripe, apple-like, cheesy	6	6·21	8	7·21	8	7·31
22	3-Methyl-thiopropanal		909	EI		3	9·31	3	9·29	1	10·11
27	Benzaldehyde	965	968	EI, CI	Almond-like, nutty, fragrant	7	7·29	7	10·11	6	9·10
44	Nonanal	1104	1106	EI, CI	Floral, citrus, green, grass-like, fatty	tr		tr		6	7·91
52	Decanal	1208	1209	EI	Orange peel-like, sweet, waxy, floral	5	9·21	4	8·30	6·5	8·55
56	Undecanal		1310	EI	Fatty with orange, herbaceous	tr		tr		tr	

	Nitrogen compounds									
23	2,6-Dimethyl pyrazine	922	EI	Cocoa, roasted nuts, roast beef, coffee	22	8·24	7	9·31	56	10·22
24	2,3-Dimethyl pyrazine	927	EI	Green, nutty, coffee, peanut butter, walnut	2	7·21	2	8·20	2	7·91
35	Trimethyl pyrazine	1014	EI	Roasted nuts, cocoa, baked potato, peanut	3	8·55	2	7·91	2	6·31
39	3-Ethyl-2,5-dimethyl pyrazine	1082	EI	Coffee, roasted nuts, potato chips, burnt	tr		7	8·81	8	5·29
47	2-Methyl-3,5-diethyl pyrazine	1156	EI	Roasted, cocoa, nutty	2	6·21	4	7·59	2	7·87
	Lactones									
61	γ-Decalactone	1504	1503	EI, CI	Fruity, peach	2	8·25	2	9·11	2	11·31
63	δ-Decalactone	1510	1510	EI, CI	Creamy, coconut, peach, buttery on dilution	4	9·26	5	9·31	2	10·21
67	δ-Dodecalactone	1720	1719	EI	Fruity, peach, buttery, coconut	tr		tr		tr	
	Miscellaneous									
26	5-Methyl-dihydro(3H)-furanone			EI		tr		tr		tr	
29	3-Methyl-2-(5H)-furanone			EI		tr		tr		tr	
51	Dodecane	1200	1200	EI, CI		ne		ne		ne	
57	Nicotine	1381	1384	EI		tr		tr		tr	
64	2,6-Di-tert-butyl-*p*-cresol (BHT)			EI	Very faint musty, occasional cresylic-type odour	ne		ne		ne	
66	Phthalic acid alkyl ester			EI		ne		ne		ne	

$\bar{X}$, mean of triplicate analyses of three different extractions from different samples of the same whole cheese; CV, coefficient of variation, %; EI, electron impact mass spectrometry; CI, chemical ionization mass spectrometry using NH_3; IS, internal standard; tr, trace; ne, not evaluated since the relative peak contained more than one component.

† Peak numbers are the same as on Fig. 1.

‡ Measured using a DB-5 column as described in the text; Ref., reference values obtained by analysis of authentic compounds; Obs., observed.

§ Odour described in *Flavour & Fragrances*, Aldrich International Edition, 1996.

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Table 2. *Concentrations of volatile compounds grouped according to chemical class in neutral volatile extracts from raw bovine milk and Grana Padano cheese 12 months old*

Chemical class	Concentration μg/kg	
	Bovine milk	Grana Padano cheese
Esters	399	1671
Ketones	36	1541
Alcohols	12	1333
Aldehydes	96	21
Nitrogen compounds	41	29
Lactones	1·7	6

Mozzarella cheese (0·07 mg/kg; Moio *et al.* 1993*b*). Ethyl octanoate was more concentrated in GP cheese than in fresh bovine milk (0·08 mg/kg; Moio *et al.* 1993*a*) and bovine Mozzarella cheese (0·06 mg/kg; Moio *et al.* 1993*b*).

The high content of esters is probably linked to the esterase activity of lactic acid bacteria as previously demonstrated by Hosono *et al.* (1974). However, it does not seem to be clear whether esterification is due to spontaneous chemical or to enzymic activity (Behnke, 1980; Dumont & Adda, 1987). The distinctive fruity notes perceived during tasting of GP cheese can almost certainly be linked to the high level of ethyl esters, particularly ethyl butanoate and hexanoate. Ethyl esters of *n*-C_7, C_9 and C_{12} fatty acids were present only in trace amounts and were therefore not significant contributors to the GP aroma. Low levels of methyl, propyl and butyl esters of some short-chain fatty acids were also detected.

Ketones represent the second largest class of GP cheese volatile components accounting for ∼ 33% of neutral volatiles, similar in amount to that found in Parmesan cheese, where they are the most abundant volatiles, representing 26% of the total headspace chromatographic area (Barbieri *et al.* 1994).

The major representatives of the 2-alkanones with odd numbers of carbon atoms were 2-pentanone, 2-heptanone, 2-nonanone and 2-undecanone. These odour-active components are in Roquefort, Bleu di Causses and Bleu d'Auvergne cheese in amounts about ten times higher (Gallois & Langlois, 1990). They are also significant in other varieties of cheese, such as Swiss Gruyère (Bosset & Liardon, 1984), Gruyère de Comté (Guichard *et al.* 1987), Swiss cheese (Langler *et al.* 1967), and Camembert (Moinas *et al.* 1975; Dumont *et al.* 1976). Even-numbered carbon atom 2-alkanones, C_6, C_8 and C_{10}, were also detected in the neutral volatile extract of GP cheese. The production of 2-alkanones follows a two step process: the fatty acids are first oxidized to *β*-oxo acids, which are then decarboxylated to the corresponding 2-alkanones with one fewer carbon atom (Hawke, 1966). Both resting spore (Lawrence, 1966) and mycelium (Lawrence & Hawke, 1968) seem equally efficient in this conversion.

3-Hydroxy-2-butanone (acetoin) was detected at a level below the olfactory threshold. According to Law (1981), acetoin together with diacetyl plays an important role in the aroma of only immature soft cheeses such as Mozzarella. However, considering the perception threshold of this component (150 mg/kg in wine, Shinohara, 1984; 50 mg/kg in beer, Meilgaard, 1975) and the low level measured, 2·3 mg/kg (Moio *et al.* 1993*b*), it is questionable whether acetoin has any olfactory significance even in fresh cheese.

Alcohols represented the third class, accounting for ∼ 23% of the total neutral

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volatiles. Their presence indicates weak fermentative activity during curd and/or cheese ripening, confirmed by the fact that in fresh milk alcohols represent only 2–5% of total volatiles (Moio *et al.* 1993*a*). The formation of primary alcohols in cheese could also be due to the reduction of the corresponding aldehydes originating from the amino acids by transamination and oxidative deamination followed by decarboxylation (Behnke, 1980; Dumont & Adda, 1987).

Among the alcohols, those present in greatest quantity were 2-pentanol, 3-methyl-3-buten-1-ol, 3-methyl-1-butanol and 2-heptanol. The presence of 1-octen-3-ol should be particularly noted: it has an olfactory importance due to its low perception threshold. 1-Octen-3-ol is a key aroma compound of mushrooms and has long been recognized as an important flavour compound produced by *Penicillium roqueforti* in blue cheese (Shimp & Kinsella, 1977) and *Pen. caseicolum* in Camembert cheese. In some cases, it has been found in such high quantities that a defective metallic aroma was produced (Adda, 1984). Other alcohols occurred in GP cheese at very low levels.

Aldehydes contributed $\sim 0{\cdot}6$% to the total neutral volatiles of GP cheese (Table 2). Their very low levels indicated a regular maturation; at higher levels, they were found to give a serious 'off-flavour'.

Among heterocyclic compounds five alkylpyrazines were identified. Such compounds are normally formed during roasting and frying of a variety of foods such as potato chips, barley, coffee, peanuts, filberts, popcorn and beef (Maga, 1982) and also in a variety of cheeses such as Beaufort (Dumont & Adda, 1978), Vacherin (Dumont *et al.* 1974*b*), grated Parmesan (Dumont *et al.* 1974*a*), Comté (Dumont *et al.* 1981), Swiss Emmenthal (Sloot & Hofman, 1975), Gouda (Sloot & Harkes, 1975) and Swiss Gruyère (Liardon *et al.* 1982). Pyrazines are potent odorants characterized by roasted and nut-like notes (Maga, 1982). The presence of pyrazines in processed foods is usually associated with non-enzymic browning during heating or during prolonged storage at moderate temperature (Maga, 1982). 2,5-Dimethylpyrazine, together with other alkylpyrazines, can be generated by *Pseudomonas taetrolenus* cultures in skim milk (Morgan, 1976). Given the very low sensory threshold of these substances it is likely that they play a definite role in GP cheese aroma.

Lactones represent only 0·1% of the total neutral volatiles of GP cheese (Table 2), a figure similar to that of fresh bovine milk (Moio *et al.* 1993*a*), where almost no free lactones but high levels of lactone precursors such as γ- and δ-hydroxy fatty acids esterified in the α-position (Dimick *et al.* 1969) were found. Heating causes the hydroxy acids to lactonize (Boldingh & Taylor, 1962), and during production of both GP and Mozzarella cheese the curd is heated for different lengths of time at various temperatures. The lactone level is higher in water-buffalo Mozzarella cheese (Moio *et al.* 1993*b*), 0·105 mg/kg, where the curd is stretched at about 62–65 °C, and lower in GP, which is treated at a lower temperature. The only lactones found were key aroma components of coconut, imparting a coconut-like character to GP cheese.

Identification and relative percentage of volatile free fatty acids

The concentrated acid extract of the aqueous vacuum distillate of the GP cheese emitted a strong cheese-like odour, different from that of the fruity note exhibited by the neutral extract. This may have resulted from a high concentration of volatile FFA (VFFA) typical of other cheese varieties having a high level of FFA. Fig. 2(*a*) shows the GC profile of the acid organic extract from the crude vacuum distillate of the GP cheese. Of 50 peaks separated, 15 were identified as acid components by their electron impact mass spectra and LRI by comparison with reference compounds.

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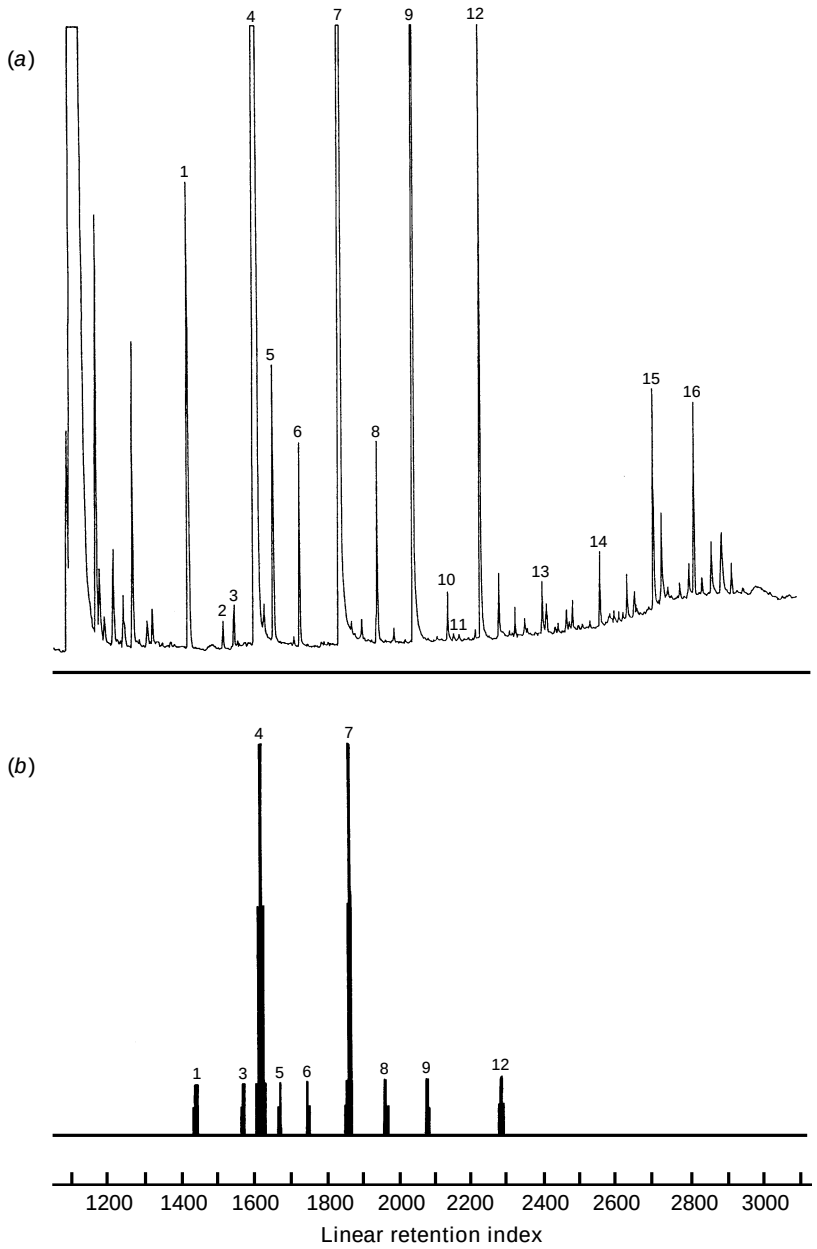


Fig. 2. Comparison of (*a*) gas chromatography–flame ionization detection and (*b*) gas chromatography–olfactory (Charm) response of combined acid organic extracts of Grana Padano cheese. Assignment and descriptions of peaks are given in Tables 3 and 5.

From the results shown in Table 3, large amounts of butanoic and hexanoic acids, both with a strong cheesy note, were present, representing 50 and 35 % of the total FFA fraction respectively. These two volatiles may be important for the background aroma of the GP cheese. Butanoic acid is produced mainly through both the butyric fermentation of lactose and the breakdown of some amino acids – threonine, glutamic acid, methionine and leucine – and by lipolysis (Ritter, 1955). A large amount of butanoic acid also occurs in other cheeses such as Cheddar, blue cheeses,

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Table 3. *Concentrations of carboxylic acids and phenols in the acidic extract of Grana Padano cheese and their olfactory properties*

Peak no.†	Compound		Linear retention index‡		Identification	Odour descriptor§	Abundance, %¶		
							Ripening time		
	IUPAC name	Trivial name	Ref.	Obs.			12 months	18 months	24 months
1	Ethanoic acid	Acetic acid	1440	1442	EI	Acetic, sour	6·3	1·2	1·2
2	Propanoic acid	Propionic acid	1533	1535	EI	Pungent, rancid	1·8	0·05	0·05
3	2-Methylpropanoic acid	Isobutyric acid	1576	1575	EI	Penetrating, rancid butter	0·4	0·16	0·09
4	Butanoic acid	Butyric acid	1623	1624	EI	Sharp, cheese, sweaty, putrid, sour	46	50	50
5	3-Methylbutanoic acid	Isovaleric acid	1680	1677	EI	Rancid, cheese, sweaty, faecal, putrid	1·6	1·36	0·6
6	Pentanoic acid	Valeric acid	1750	1749	EI	Putrid, faecal, sweaty, rancid	0·9	0·27	0·4
7	Hexanoic acid	Caproic acid	1852	1853	EI	Sickening, sweaty, rancid, sour, sharp, pungent, cheesy, fatty	30	38	39
8	Heptanoic acid	Enanthic acid	1966	1965	EI	Disagreeable rancid odour, sour sweat-like, fatty odour	0·33	0·25	0·3
9	Octanoic acid	Caprylic acid	2083	2083	EI	Unpleasant, oily, fatty odour, rancid taste	5	6·3	6·2
10	Nonanoic acid	Pelargonic acid	2178	2181	EI	Cheese, waxy	0·6	0·12	0·09
11	2-Methoxy-4-vinylphenol	Vinyl-4-guaiacol	2197	2197	EI	Spicy, apple, rum, toasted peanut	ne	ne	ne
12	Decanoic acid	Capric acid	2280	2284	EI	Fatty, unpleasant rancid odour, citrus	1	1·3	1·3
13	Benzoic acid		2423	2420	EI	Urine-like, faint balsamic	0·17	0·04	0·035
14	Dodecanoic acid	Lauric acid	2515	2516	EI	Waxy, oily	1·1	0·07	0·096
15	Tetradecanoic acid	Myristic acid	2712	2714	EI	Faint, waxy, oily	1·4	0·17	0·14
16	Hexadecanoic acid	Palmitic acid	2900	2899	EI	Virtually odourless	3·4	0·7	0·5

EI, electron impact mass spectrometry; ne, not evaluated.
† Peak numbers are the same as on Fig. 2.
‡ Measured using a DB-Wax column as described in the text: Ref, reference values obtained by analysis of authentic compounds; obs, observed.
§ Odour described in *Flavours & Fragrances*, Aldrich International Edition, 1996.
¶ Expressed as a percentage of total carboxylic acids.

Limburger, Provolone and Pecorino Romano (Day, 1967). The most probable origin of hexanoic acid is butterfat lipolysis, but the presence of straight-chain fatty acids with odd numbers of carbon atoms such as pentanoic, heptanoic and nonanoic acids suggested a partial fermentative origin. The degradation of lactose by Enterobacteriaceae such as *Escherichia coli* and *Esch. aerogenes* into lactic, formic and ethanoic acids, as well as into ethanol and carbon dioxide, can explain only a minute portion of the high level of ethanoic acid found in GP cheese. Owing to the low count of enterobacteria in GP cheese, homofermentative lactic acid bacteria such as streptococci (e.g. *Streptococcus thermophilus*) and/or *Lactococcus lactis* could also contribute to the biogenesis of ethanoic acid. Most of the ethanoic acid probably originates from early degradation of lactose by heterofermentative lactic acid bacteria such as *Lactobacillus fermenti* and *Leuconostoc* spp. (Ritter, 1955). Propionibacteria certainly generate propanoic acid in cheese: 2- and 3-methylbutanoic acids originate from isoleucine and leucine respectively (Ritter, 1955; Baraud *et al.* 1970), and 2-methylpropanoic acid from the breakdown of valine (Ritter & Hänni, 1960; Nakae & Elliott, 1965).

Finally, some aromatic compounds such as benzoic acid and 2-methoxy-4-vinylphenol were identified in the acid volatile fraction of GP cheese.

High resolution gas chromatographic–olfactometric analysis of the neutral volatile fraction

The Charm aromagram of GP cheese is shown in Fig. 1(*b*). The 23 peaks shown corresponded to the most odour-active compounds in the neutral volatile fraction of GP cheese.

The main peaks of the Charm profile in Fig. 1(*b*) corresponded to ethyl butanoate, 2-heptanol, 3-methylthiopropanal, 1-octen-3-ol, ethylhexanoate and nonanal (Table 4). The two ethyl esters, both characterized by fruity odours, together contributed $\sim 30\%$ of the total Charm response. These confer a fruity note to cheese as shown by Bills *et al.* (1965) who, spiking Cheddar cheese with 5 μg/g of each, obtained a more fruity flavour.

The contribution of the two alcohols 2-heptanol and 1-octen-3-ol, which have a green and mushroom aroma respectively, accounted for $\sim 28\%$ of the total Charm value, whereas the two aldehydes 3-methylthiopropanal and nonanal, which have a cooked milk and tallowy odour respectively, represented $\sim 20\%$ of the total Charm value.

Secondary odour components having olfactometric indices of ~ 25 were found in the following peaks: 11, 19, 39, 45, 53, U4, U5, 63 and 67. Two odours reminiscent of flowers were detected at LRI 1319 and 1361, where no peaks appeared in the FID chromatogram. This implies that the components responsible for these odours occurred at extremely low levels and had a very low aroma threshold. Owing to the low amount of these components in the organic extract of GP cheese, they were not identified. The remaining components of the Charm chromatogram were 2-pentanone, 1-pentanol, 3-methylbutyl acetate and 3,5-diethyl-2-methylpyrazine.

High resolution gas chromatographic–olfactometric analysis of the volatile fatty acid fraction

The GP cheese aromagram obtained by Charm analysis of the FFA fraction is shown in Fig. 2(*b*). The main peaks in the Charm aromagram corresponded to the two strong odorants, butanoic and hexanoic acids. It should be noted that in both the FID profile (Table 3) and the Charm profile (Table 5), these acids represented

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Table 4. *Main odorants detected in the Charm response chromatograms from neutral volatile extracts of Grana Padano cheese 12 months old*

Peak no.†	Compound‡	Linear retention index	Odour descriptor§	Charm value¶ $\bar{X}$	Charm value¶ %
3	2-Pentanone	715	Sweet, fruity	3	0·17
10	1-Pentanol	766	Balsamic	4	0·24
11	3-Methyl-2-buten-1-ol	772	Cheese, fruity, green	25	1·38
13	Ethyl butanoate	802	Fruity, green	254	14·6
18	3-Methylbutyl acetate	876	Fruity, banana	4	0·24
19	2-Heptanone	893	Fruity, fatty, spicy, herbaceous	25	1·32
21	2-Heptanol	901	Green, earthy	245	14·08
22	3-Methylthiopropanal	909	Cooked milk	182	10·46
24	2,3-Dimethyl pyrazine	927	Roasted nuts, coffee, peanut butter, walnut	21	1·4
30	1-Octen-3-ol	985	Mushroom-like, earthy	244	14·04
34	Ethyl hexanoate	1003	Fruity, unripe fruit, apple	333	19·14
U1	Unknown	1030	Burnt, roasted	4	0·24
U2	Unknown	1051	Floral	3	0·17
U3	Unknown	1063	Caramel	15	0·86
39	3-Ethyl-2,5-dimethylpyrazine	1082	Earthy, potato chips	25	1·26
44	Nonanal	1106	Green, grass-like, tallowy	172	9·89
45	Methyl octanoate	1138	Green, fruity	26	1·49
47	2-Methyl-3,5-diethylpyrazine	1156	Potato	6	0·34
53	2-Methylbutyl hexanoate	1247	Minty	33	1·89
U4	Unknown	1319	Floral, acacia	35	2·02
U5	Unknown	1361	Floral	30	1·73
63	δ-Decalactone	1510	Creamy, coconut, peach	27	1·56
67	δ-Dodecalactone	1719	Coconut powder, buttery, peach	29	1·66

$\bar{X}$, mean of triplicate analyses of three different extractions from different samples of the same whole cheese; %, relative percentage of the total Charm value.

† Numbering as in Fig. 1.

‡ Each compound was identified by comparing it with a reference substance on the basis of its linear retention index determined on a DB-5 column, mass spectrometry spectrum obtained by electron impact and chemical ionization, and odour quality perceived at the sniffing port.

§ Odour description assigned during Charm analysis.

¶ Area under the peaks in the Charm chromatogram.

Table 5. *Main odorants detected in the Charm response chromatograms from acid volatile extract of Grana Padano cheese 12 months old*

Peak no.†	Compound‡	Linear retention index	Odour descriptor§	Charm value¶ $\bar{X}$	Charm value¶ %
1	Ethanoic acid	1442	Acetic, sour	6	2·29
3	2-Methylpropanoic acid	1575	Cheese, rancid butter	6	2·29
4	Butanoic acid	1624	Cheese, rancid, sour	131	50·0
5	3-Methylbutanoic acid	1677	Cheese rind, rancid	6	2·29
6	Pentanoic acid	1749	Stable, sweaty, bedsteam, sheep, goat	4	1·53
7	Hexanoic acid	1853	Cheese rind, grated cheese, sweaty	87	33·2
8	Heptanoic acid	1965	Fatty, rancid	5	1·91
9	Octanoic acid	2083	Fatty, rancid	5	1·91
12	Decanoic acid	2284	Grassy, fatty, sour	12	4·58

$\bar{X}$, mean of triplicate analyses of three different extractions from different samples of the same whole cheese; %, relative percentage of the total Charm value.

† Numbering as in Fig. 2.

‡ Each compound was identified by comparing it with a reference substrate on the basis of its linear retention index determined on a DB-Wax column, mass spectrometry spectrum obtained by electron impact and odour quality perceived at the sniffing port.

§ Odour description assigned during Charm analysis.

¶ Area under the peaks in the Charm chromatogram.

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similar proportions of total area. This indicates that these two acids, characterized by similar and very low thresholds, were not accompanied in the VFFA extract by other secondary acid components with lower threshold values. The odour of these two components was clearly similar to that of the cheese, even at the highest dilutions, where the odour of butanoic acid was more sour and rancid, and that of hexanoic acid was similar to cheese rind and grated cheese. This result is consistent with the findings of Patton (1963) who, using reagents blocking functional groups in cheese and in distillates and a ten-member technical panel to assess the effects of these reagents on aroma, found that VFFA such as ethanoic, butanoic, hexanoic and octanoic acids constituted the backbone or *sine qua non* of Cheddar aroma. This has been confirmed by Liebich *et al*. (1970) who, in attempting to simulate cheese flavour mixtures, concluded that FFA are the basis for any cheese flavour and that the characteristic aroma of cheese is due to the ratio of VFFA to the mixture of other volatile components. Lindsay (1983) reported that butanoic and hexanoic acids are important components of the aroma quality of Pecorino Romano cheese. On the basis of previous reports and results from the present study, it can be concluded that only two VFFA, butanoic and hexanoic acids, contribute appreciably to the basic odour of GP cheese. In fact, an excessive level of these two FFA, particularly *n*-butanoic acid, produces a rancid flavour in GP cheese (Adda, 1984).

Changes in the odorants of the neutral fraction during ripening

Changes in the key neutral odorant constituents with an olfactometric index > 20 have been recorded during GP ripening. The fruity, green and apple odours generated by ethyl hexanoate, ethyl butanoate, 2-heptanol and 2-heptanone tended to decrease with age, whereas those of roasted nuts, peanut butter, coffee and baked potato (associated with 2,6-dimethylpyrazine and 2-methyl-3,5-diethylpyrazine) increased, probably as a result of bacterial activity. 2-Methyl butylhexanoate, with a minty odour, and nonanal, responsible for the tallowy fatty and waxy notes, tended to increase. No changes in the concentrations of 1-octen-3-ol, 3-methyl thiopropanal and δ-lactones (C_{10} and C_{12}) were found during ripening.

Changes in odorants of the volatile fatty acid fraction during maturation

A small change in the relative proportions of ethanoic, butanoic, hexanoic, octanoic and decanoic acids was found between 12 and 24 months, whereas there were no changes in the proportions of 3-methylbutanoic, 2-methylpropanoic and pentanoic acids.

Comparison of the Charm profiles of a genuine and an imitation Grana Padano cheese

The aroma profiles of the neutral volatile extract of an imitation GP cheese are reported in Fig. 3. The aroma profile of GP cheese (Fig. 1*b*) was more complex than that of imitation cheese. It contained 23 odorous components whereas that of imitation cheese had only 17. In addition to this, there were also quantitative differences between the corresponding components in the two aroma profiles. The main difference between the two aromagrams was the lower intensity of 1-octen-3-ol (peak 30) and nonanal (peak 44) in the imitation cheese. Moreover, the aromagram of the imitation cheese lacked the odorous peaks 3, 10, 11, U3, 45, 47 and 53, characterized by fruity, balsamic, green, caramel, green, potato and minty notes, whereas it contained an odour peak with a putrid odour characterized by LRI 977 absent from the genuine GP cheese aromagram.

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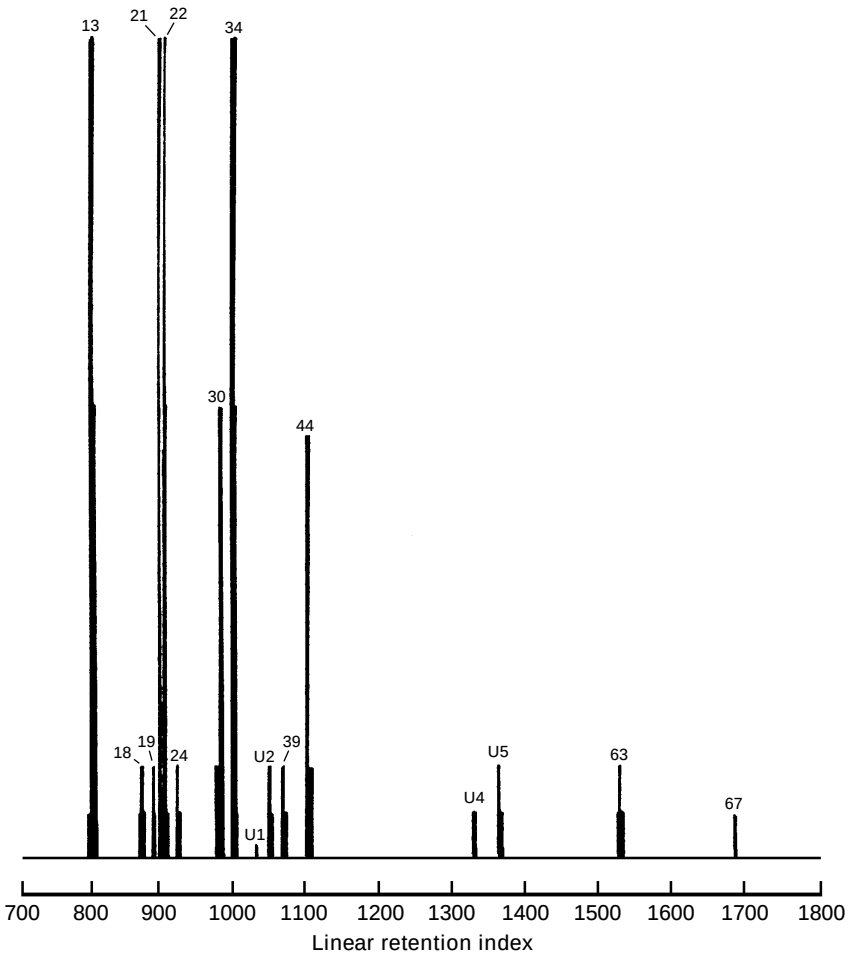


Fig. 3. Aromagrams of the neutral extracts of an imitation Grana Padano cheese.

Further study is required, however, to confirm the aromatic complexity of this Appelation d'Origine Contrôlée cheese and the changes the impact compounds undergo during the ripening.

In conclusion, the aroma of GP cheese seemed to consist of only ~ 31 components of which 27 have been identified. The notes that imparted a fruity odour in the fresh cheese seemed to be partly lost during ripening, while a more complex aroma composed of roasted nut, peanut butter, baked potato and earthy odour developed.

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