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Flavour and Off-flavour Compounds of Swiss Gruyère Cheese

Identification of Key Odorants by Quantitative Instrumental and Sensory Studies

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ABSTRACT

Potent odorants of a typical sample of Gruyère cheese and of a Gruyère exhibiting a potato-like off-flavour were quantified by isotope dilution analysis. The studies of sensory models revealed that 2-/ 3-methylbutanal, methional, dimethyltrisulfide, phenylacetaldehyde, 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, methanethiol, as well as acetic, propionic, butyric, 3-methylbutyric and phenylacetic acids comprise the typical flavour of Gruyère cheese. The potato-like character of one sample showing an aroma defect, however, was mainly attributed to a too high concentration of methional.

Key words: Flavour; Gruyère cheese; off-flavour; stable isotope dilution analysis

INTRODUCTION

Cheese aroma has already been the object of many analytical studies. Over one hundred volatile components identified in various types of cheese are currently listed in the database edited by Nijssen, Visscher, Maarse, Willemsens and Boelens (1996). The knowledge on their generation has been reviewed by Dumont and Adda (1979), Forss (1979), Behnke (1980), as well as by McSweeney and Sousa (2000). However, authentic Swiss Gruyère cheese has hardly been considered for this kind of investigation (Groux and Moinas, 1974; Liardon, Bosset & Blanc ,1982; Bosset and Liardon ,1984, 1985; Bosset, Collomb & Sieber ,1993; Bosset, Bütikofer, Gauch & Sieber ,1994; Muir, Hunter, Banks & Horne, 1995; Lavanchy and Bütikofer, 1999).

The aim of the current study is to update our knowledge on this topic by identifying key odorants of this cheese variety.

In the first part of this study (Rychlik and Bosset, 2002) we used dynamic headspace gas chromatography-mass spectrometry (DHGC/MS), aroma extract dilution analysis (AEDA) and gas chromatography-olfactometry of static headspace samples (GCO-H) to find 38 potent odorants in Gruyère cheese. Of these odorants, AEDA, on the one hand, revealed methional, 2-ethyl-3,5-dimethylpyrazine (EDMP), 2-/3-methylbutanal, dimethyltrisulfide, phenylacetaldehyde, (E)-2-nonenal, dimethyltetrasulfide, 2- and 3-methylbutyric acid, phenylacetic acid and butyric acid to be important flavour contributors. On the other hand, the results of GCO-H indicated that methanethiol, dimethylsulfide and ethyl 2-methylbutanoate might play an prominent role in Gruyère flavour.

The above mentioned odorants were identified in a high grade reference Gruyère (RG). Moreover, a Gruyère having a potato-like off-flavour (PG) was also investigated. Similar off-flavours have already been reported, first by Dumont, Roger and Adda (1975) in a French Comté cheese, whose defect seemed to be caused by 3-methoxy-2-propylpyrazine. Later, a potato-like taint of a smear coated Munster cheese was found to be associated with the occurrence of 2-methoxy-3-isopropylpyrazine (Dumont, Mourgues & Adda, 1983). Similarily, the differential DHSGC/MS and AEDA performed in part 1 of this study (Rychlik and Bosset, 2002) led us to conclude that pyrazines, namely 2-ethyl-3,5-dimethylpyrazine (EDMP) and 2,3-diethyl-5-methylpyrazine (DEMP) may account for the potato-like taint in PG. The results of these experiments, however, were not corrected for losses or discriminations during isolation and separation procedures. Consequently, accurate quantitative data and calculation of odour activity values are required to indicate the key odorants. The purpose of part 2 of the study was, therefore, to accurately quantify the concentrations of the odorants and, based on these data, to prepare sensory models in order to identify the primary flavour compounds.

MATERIALS AND METHODS

Cheeses

A sample of Gruyère cheese exhibiting a potato-like off-flavour (PG) and a high quality reference Gruyère cheese without off-flavour (RG) were supplied by a Swiss village factory as reported by Rychlik and Bosset (2002). Unripened cheese of the variety Mozzarella (UC) (Zottarella from Zott, Mertingen, Germany, dry matter 42 %) was used as a cheese base (or cheese model) for sensory trials. UC was cut into 1 cm cubes and then freeze-dried.

Chemicals

Diethylether, n-pentane and dichloromethane were purified as previously reported (Schieberle and Grosch, 1983).

Pure reference compounds listed in the different tables were purchased: no. **1**((S)-(+)-2-methylbutanal), **2-5**, **7-11, 16** (S)-(+)-2-methylbutyric acid), **17** [$^{13}C]_2$-phenylacetic acid (Aldrich, Steinheim, Germany); **12** (Merck, Darmstadt, Germany); **10**, **13-15**, **18** (Fluka, Buchs, Switzerland). The following compounds were gifts: 2-ethyl-3,5-dimethylpyrazine, (Z)-2-nonenal, [^{2}H]-dimethyltrisulfide, [^{2}H]-2-ethyl-3,5-dimethylpyrazine, [^{2}H]-methional, [^{2}H]-(E)-2-nonenal from Prof. Grosch (formerly Deutsche Forschungsanstalt für Lebensmittelchemie, Garching, Germany),[^{2}H]-3-methylbutyric acid from Dr. Guth (formerly Deutsche Forschungsanstalt für

Lebensmittelchemie, Garching, Germany), [^{2}H]-3-methylbutanal and [^{13}C]-phenylacetaldehyde from Prof. Schieberle (Deutsche Forschungsanstalt für Lebensmittelchemie, Garching, Germany).

High resolution gas chromatography (HRGC), HRGC/ Olfactometry (HRGC/O) and HRGC/ mass spectrometry (HRGC/MS) of static headspace samples

HRGC, HRGC/O and HRGC/MS were performed as detailed previously (Rychlik and Bosset, 2002).

High resolution gas chromatography/mass spectrometry (HRGC/MS) of extracts

Mass chromatography was performed with an ion trap detector ITD 800 (Finnigan MAT, Bremen, Germany) coupled to the capillary columns detailed in **Table 1** using the electron impact mode (EI-MS) or the chemical ionization mode (CI-MS) with methanol as reagent gas. Ionization energy was 70 eV.The calibration factors and quantitative data were calculated as described previously (Rychlik, 2000).

Quantitative measurements

The cheese samples were ground as detailed in the first part of the study (Rychlik and Bosset, 2002).

*Quantitation of odorants **1** and **2***. The powdered cheese (50 g) was suspended in diethyl ether (300 mL) containing 15 µg of [^{2}H]-3-methylbutanal. The suspension was stirred for 4 h, filtered and the residue was stirred for another 18 h in diethyl ether (200 ml). The combined ethereal solutions were dried over anhydrous sodium sulphate and then concentrated to a volume of 150 mL by distilling off the solvent under a Vigreux column (60 x 1 cm, Bahr, Manching, Germany).

After submitting the extract to distillation under high vacuum (Rychlik and Bosset, 2002) the distillate was extracted with aqueous sodium carbonate (0.5 mol/L, 3 x 50 mL) and then washed with a saturated aqueous solution of sodium chloride (30 mL). The distillate was dried over anhydrous sodium sulphate and concentrated to 200 µL by distillation and microdistillation (Bemelmans, 1979). The volatiles **1** and **2** were quantified in an aliquot (0.5 µL) by HRGC/MS (**Table 1**).

*Quantitation of odorants **3 to 9**.* Extraction of the cheese powder (50 g) was first performed for 4 h in a solvent mixture of water/ dichloromethane/ methanol (4+5+10, vol+vol+vol; 300 mL) containing 15 µg [^{2}H]-methional, 6 µg [^{2}H]-dimethyltrisulfide, 2 µg [^{13}C]-phenylacetaldehyde, 5 µg [^{2}H]-2-ethyl-3,5-dimethylpyrazine, 0.5 µg [^{2}H]-2,3-diethyl-5-methylpyrazine, 10 µg [^{2}H]-(E)-2-nonenal, and 40 µg [^{2}H]-δ-decalactone. After filtering the suspension the filter residue was stirred a further 18 h in dichloromethane (200 mL). The combined extracts were washed with water (3 x 300 mL), dried and distilled under vacuum as described above. The condensate was washed with aqueous sodium carbonate (0.5 mol/L, 3 x 50 mL), then with a saturated aqueous solution of sodium chloride (3 x 30 mL), dried over anhydrous sodium sulphate and concentrated to 200 µL by distillation and microdistillation. In an aliquot (0,5 µL), odorants **3** to **9** were quantified by HRGC/MS (**Table 1**).

*Quantitation of odorant **10**.* The ground cheese (5 g) was extracted for 4 h in a solvent mixture of water/ dichloromethane/ methanol (4+5+10, vol+vol+vol; 100 mL) containing 40 µg [^{13}C]-phenylacetic acid. After filtering the suspension the filter residue was stirred a further 18 h in dichloromethane (100 mL). The combined extracts were washed with water (3 x 100 mL), dried and distilled under vacuum as described above. After extracting the distillate with aqueous sodium carbonate (0.5 mol L^{-1}, 3 x 50 mL) the pH of the aqueous extract was adjusted to 3 by addition of aqueous hydrochloric acid (5 mol L^{-1}). After the release of aqueous carbon dioxide, the solution was extracted with diethyl ether (5 x 30 mL), and the combined extracts were dried over anhydrous sodium sulphate. The extracts were concentrated to 200 µL by distilling off the solvent under a Vigreux column and by microdistillation. In an aliquot (0.5 µL), **10** was quantified by HRGC/MS (**Table 1**).

*Quantitation of odorant **11**.* The frozen, ground cheese sample (5 g) was transferred to a vessel (250 mL) which was sealed with a septum. The internal standard $[^2H]_3$-methanethiol was liberated by adding aqueous sodium hydroxide to $[^2H]_3$-methyl isothiuronium iodide (Guth and Grosch, 1994) and then injected with a gas-tight syringe into the vessel. The sample was then equilibrated for 30 min at 25 °C. Then, a headspace volume of 5 mL was withdrawn and analysed.

Analysis of volatile short-chain fatty acids

Determination of volatile acids was performed as described by Kubickova and Grosch (1998b).

Analysis of free amino acids

Free amino acids and ammonia were analysed as described by Warmke, Belitz and Grosch (1996).

Cheese models

Three kinds of models were prepared: one model simulating the Gruyère cheese with potato-like off-flavour (MPG); another simulating the cheese without off-flavour (MRG); and a third being similar to MPG but not containing methional (MA), so as to test the flavour impact of the latter compound. The amounts of the chemicals used for the preparation of the models are listed in **Table 2**. Acetic acid (**12**), propionic acid (**13**), butyric acid (**15**), 3-methylbutyric acid (**17**), and phenylacetic acid (**10**), in the amounts indicated in **Table 2**, were dissolved in water (35 mL). This aqueous solution was adjusted to pH 5.6 by addition of sodium hydroxide (1 mol L^{-1}) and poured into a mortar containing the freeze-dried and pulverised unripened cheese (UC) (65 g). After mixing, UC plus acids were transferred into a beaker, which was sealed with foil, and stored overnight at 4 °C. The odorants **1** to **6, 8, 11** (amounts in **Table 2**) were dissolved in sunflower oil (1 mL). Then, 1 h before flavour profile analysis, the oil containing the odorants was mixed with UC plus acids to obtain the complete models.

Flavour score analysis

Flavour score analysis was performed as described earlier (Kubickova and Grosch, 1998a) by a panel of 5 male and 1 female trained judges aged between 25 and 35. The intensity of the odour and taste characteristics was scored on a scale ranging from 0 (quality lacking) to 3 (strong). The results obtained by the panellists were averaged (standard deviation of 0.5 on an average) and then rounded down or up to the nearest 0.5. The significance of the result was evaluated by the t-test according to O'Mahony (1986).

Microbiological analyses

The occurence of *Clostridia* (*sporogenes, butyricum and tyrobatyricum*), *Enterobacteriaceae*, *Enterococcae*, yeasts and moulds in the cheese samples was analysed as described by Bosset et al. (2001).

RESULTS AND DISCUSSION

Unlike the characteristic odour of high grade Swiss Gruyère cheese (Lavanchy and Bütikofer, 1999), the tainted Gruyère (PG) produced by a Swiss village cheese factory exhibited a sweaty and potato-like off-flavour. In contrast to this, cheese loaves of a good quality reference Gruyère (RG) produced in the same factory, but ripened in a neighbouring ripening cellar, did not show any defect.

In the first part of the study, AEDA and GCO-H were performed in order to screen for potent odorants (Rychlik and Bosset, 2002). On the basis of the results from that study, the compounds for quantification could be selected to clarify the flavour difference between the cheeses. In AEDA and GCO-H the FD-factors of 2-ethyl-3,5-dimethylpyrazine (EDMP), (E)-2-nonenal, 2/3-methylbutyric acid, phenylacetic acid, and methanethiol were found to be significantly different in PG and RG. In order to gain a better insight into the flavour of Gruyère cheese, the concentrations of odorants exhibiting high FD-Factors , i. e. 2,3-diethyl-5-methylpyrazine (DEMP), 2-/3-methylbutanal, methional, and dimethyltrisulfide, were also determined.

Quantitation using stable isotope dilution analysis

Trace odour compounds were quantified by stable isotope dilution analyses. By using the isotopomers of the odorants detailed in **Table 1** as internal standards, it was possible to correct for losses during cleanup and concentration. The quantitative data were used to calculate odour activity values (OAV, ratios of concentration and odour thresholds) in **Table 3** on the basis of their odour thresholds in oil. As Preininger, Warmke and Grosch (1996) showed in their study of Emmentaler cheese, the choice of odour thresholds in oil is suitable to calculate OAV and to limit the number of key odorants in hard cheeses. In PG and RG methanethiol showed the highest OAV, but its content in RG was nearly as twice as high as in PG. Methional exhibited the next lower OAV in PG, and its content in PG was 2.56 times that in RG. Also, the malty smelling 2- and 3-methylbutanal, the musty EDMP, DEMP and the honey-like smelling phenylacetic acid were found in higher concentrations in PG.

Volatile short-chain fatty acids were quantified by the method detailed by Kubickova and Grosch (1998a) and OAV were calculated in **Table 4** on the basis of retro-nasal odour thresholds, as determined by Warmke et al. (1996). In agreement with the results of AEDA, 2- and 3-methyl butyric acids showed the highest OAV in PG, followed by butyric and acetic acids. In particular, the first three acids showed significantly elevated OAV in PG.

Considering the differences in OAVs, methional, EDMP and DEMP were likely to be involved in producing the potato-like character while 2-/3-methylbutyric acid and butyric acid probably contributed to the sweaty off-flavour in PG. As methanethiol showed lower concentrations in PG, both off-flavours seemed not to be caused by this compound.

The calculation of OAVs, however, assumes a linear relationship between concentration and odour stimulus, which is an inadequate simplification. Furthermore, the dependance of the odour qualities on the concentrations of odorants and the interactions between the different flavour compounds are not taken into consideration, when using OAV calculations.

Model cheeses

Therefore, further sensory studies of models had to be performed. The models were prepared by adding the odorants with OAVs exceeding a value of 1 (**Table 2)** to an unripened cheese (UC), using the method of Preininger et al. (1996). Models

MPG and MRG contained odorants in amounts quantified for PG and RG, respectively. Model MA was identical to MPG except that it did not contain methional.

The panellists identified the odour qualities potato-like and sweaty in both MPG and MRG models; however, the intensity of odour for both qualities were more intense in the MPG model (**Table 5**) and the odour of MRG was described as more Gruyère like. These findings proved that the sensory models were suitable to simulate the real Gruyère cheese samples as had been noted by Preininger et al. (1996) for Emmentaler cheese.

In contrast to the flavour impression of MPG, the potato-like off-flavour was not detected in the model from which methional was missing (MA). This result clearly suggests that methional, and not the combination of methanethiol and pyrazines, was mainly responsible for this taint. The sweaty flavour was also more intense in MPG and significantly lower in the other models. As the acid concentrations in MPG were the same as in MA, it can be concluded that methional in MPG enhances not only the potato-like but also the sweaty character. It should be noted that the model without methional (MA) was described as malty. Obviously, in MPG and MRG methional masks this flavour characteristic which is caused in MA by 2- and 3-methylbutanal.

Free amino acids and free short-chain fatty acids

The concentration of methionine (the potential precursor of methional, methanethiol, and dimethyltrisulfide) and other free amino acids were higher in PG (**Table 6**). This indicates that a bacterial contamination might have caused faster degradation of proteins and, subsequently, of free amino acids to the respective aldehydes. This conclusion can be drawn from the higher amounts of methional, 2-methylbutanal and

3-methylbutanal in PG. As dimethyltrisulfide and methanethiol are not elevated to the same extent, their formation may not be related to the microorganisms encountered.

In order to evaluate the bacterial contamination, it seems reasonable also to compare our analytical data with those reported in ripening studies on Gruyère. Bütikofer and Fuchs (1997) found that the content of free amino acids in Gruyère increased during ripening, and that a high concentration of free glutamic acid was correlated with late fermentation, the so-called late blowing. Therefore, it seemed at first likely that late blowing had occurred in PG.

A comparison of the free short-chain fatty acids in PG and RG showed that PG had high concentrations of methylpropionic, butyric, 2/3-methylbutyric, and hexanoic acids, a lower level of propionic acid and a similar level of acetic acid. Consideration of the studies of Bosset et al. (1993) would suggest that the increase of some short-chain fatty acids points to a higher age of ripening for PG. The low contents of acetic and propionic acid, however, exclude a late blowing of PG, because the concentration of these compounds is typically higher in late fermented Gruyère cheeses (Steffen et al., 1980). As the potato-like off-flavour seems to occur occasionally in cheeses, a comparison with reported cases is worth considering, as well. Dumont et al. (1983) attributed a similar defect in a Munster cheese to 2-methoxy-3-isopropylpyrazine, which had been produced by strains of *Pseudomonas* in the surface of the loaves. We could neither identify this pyrazine in our extracts nor find any unusual strains such as *Clostridia* (*sporogenes, butyricum and tyrobatyricum*), *Enterobacteriaceae*, *Enterococcae*, yeasts and moulds as contaminants in these tainted loaves. Moreover, no bacterial strains could be identified after streaking out a smear preparation on casein agar. Consequently, it remains unclear, which microorganisms caused the defect .

The free amino acids are likely to account for the taste of Gruyère. Considering a taste threshold of 2 mmol kg^{-1} determined by Warmke et al. (1996), the highest taste activity value of 30 among the amino acids could be calculated for glutamic acid in RG. In order to determine the primary contributors to Gruyère taste, investigations should screen for further compounds by gel chromatography and high performance liquid chromatography (Warmke et al., 1996).

CONCLUSIONS

The sensory tests demonstrated that the quantified compounds are key odorants of Gruyère cheese. Earlier studies (Bosset and Liardon, 1984; Bosset, Collomb & Sieber, 1993; Engels and Visser, 1994; Engels, Dekker, Jong, Neeter & Visser, 1997) already suggested that acetic, propionic, butyric and isovaleric acids as well as 3-methylbutanal and dimethyltrisulfide are potent odorants in this variety of cheese. However, for the first time 2-methylbutanal, methanethiol, methional, EDMP, DEMP, and phenylacetic acid have been shown to contribute to Gruyère flavour. Compared to that of Emmentaler cheese, the flavour of Gruyère cheese is described as more intensely sweaty (Muir et al., 1995) and less caramel-like. These differences are likely to be due to the lower concentrations of caramel-like smelling furanones and the higher content of 2-/3-methyl butyric acid in Gruyère (Preininger et al., 1996). The sulphurous smelling methanethiol, which had been shown to be negligible for the flavour of Emmentaler, exhibits the highest OAV in Gruyère cheese and may contribute to its weak potato-like odour.

Unlike French fries (Wagner and Grosch, 1998) and boiled potatoes (Mutti, 2000), where methional is not essential for a potato-like flavour, this odorant was shown to have a significant impact on the potato-like character of the Gruyère cheese showing the investigated potato-like off-flavour. In addition, in Gruyère methional enhances the sweaty odour of 2-/3-methyl butyric acid and butyric acid and masks the malty odour of 2-/3-methylbutanal.

In order to elucidate the origin of the flavour defect, cheese loaves produced in the cheese factory were subjected to different ripening schemes. As mentioned before, the loaves of RG had been ripened in a neighbouring ripening cellar and showed no off-flavour. Therefore it was concluded, that the conditions of ripening and/ or brine application in the cheese factory were critical factors. On the other hand, the state of the raw milk and the method of production of loaves did not appear to have any influence on this defect. After further different ripening trials, the brine and brine bath of the factory were replaced and fresh smear brine was used. As a result, this defect no longer occurred and it was concluded that the factory was sanitised.

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Table 1. Thin film capillary columns, selected ions, and response factors for mass chromatography of neutral and alkaline odorants.

Odorant [a]	capillary column	Selected ion of odorant m/z	internal standard (IS)	selected ion of IS m/z	response factor [b]
2-Methylbutanal (**1**)	CP-wax	69	[^{2}H]-**2**	71	1.53
3-Methylbutanal (**2**)	CP-wax	69	[^{2}H]-**2**	71	0.48
Methional (**3**)	DB-5	105	[^{2}H]-**3**	108	1.00
Dimethyltrisulfide (**4**)	DB-5	127	[^{2}H]-**4**	133	1.00
Phenyl acetaldehyde (**5**)	DB-5	121	[^{13}C]-**5**	123	1.00
2-Ethyl-3,5-dimethyl-pyrazine (**6**)	DB-1701	167	[^{2}H]-**6**	170	0.85
(E)-2-Nonenal (**7**)	DB-5	141	[^{2}H]-**7**	143	0.76
2,3-Diethyl-5-methyl-pyrazine (**8**)	DB-1701	167	[^{2}H]-**8**	170	0.85
δ-Decalactone (**9**)	DB-5	171	[^{2}H]-**9**	173-176 [c]	0.92
Phenylacetic acid (**10**)	DB-FFAP	137	[^{13}C]-**10**	139	1.00
Methanethiol (**11**)	DB-5	49	[^{2}H]-**11**	52	1.00

[a] The numbers indicated in brackets refer to those listed under Materials and methods.
[b] The response factor used for quantification was determined and calculated as recently reported (Rychlik, 2000).
[c] The sum of the relative abundances of the ions was calculated.

Table 2. Amounts of flavour compounds used for the preparation of the cheese models.

Compound [a]	MPG[b]	MRG[c]	MA[d]
	Model (μg (100 g)$^{-1}$)		
2-Methylbutanal (**1**)	90	26	90
3-Methylbutanal (**2**)	34	22	34
Methional (**3**)	25	10	0
Dimethyltrisulfide (**4**)	7	14	7
Phenylacetaldehyde (**5**)	2	2	2
2-Ethyl-3,5-dimethyl pyrazine (**6**)	7	0	7
2,3-Diethyl-5-methyl pyrazine (**8**)	0.24	0.06	0.24
Methanethiol (**11**)[e]	44	70	44
Acetic acid (**12**)	30000	30000	30000
Propionic acid (**13**)	3000	8700	3000
Butyric acid (**15**)	27000	9700	27000
3-Methylbutyric acid (**17**)	48000	8000	48000
Phenylacetic acid (**10**)	1580	720	1580

[a] The numbers indicated in brackets refer to those listed under Materials and methods.

[b] MPG: model simulating the Gruyère cheese with potato-like off-flavour.

[c] MRG: model simulating the Gruyère cheese without off-flavour.

[d] MA: model similar to MPG without containing methional.

[e] the weights of 44 µg and 70 µg correspond to 19 µL and 33 µL gaseous methanethiol, respectively.

Table 3. Concentrations, odour thresholds and corresponding odour activity values of odorants in Gruyère cheeses with a potato-like off-flavour (PG) and without an off-flavour (RG).

Odorant [a]	Concentrations (µg kg^{-1}) PG	Concentrations (µg kg^{-1}) RG	nasal odour threshold in oil [b] (µg kg^{-1})	Odour activity values PG	Odour activity values RG
2-Methylbutanal (**1**)	897	255	10	90	26
3-Methylbutanal (**2**)	343	219	13	26	17
Methional (**3**)	253	99	0.2	1270	495
Dimethyltrisulfide (**4**)	67	136	2.5	27	54
Phenylacetaldehyde (**5**)	24	20	25	1	1
2-Ethyl –3,5-dimethyl-pyrazine (**6**)	73	0.7	2.2	33	0.3
(E)-2-Nonenal (**7**)	174	323	900	0.2	0.4
2,3-Diethyl-5-methyl-pyrazine (**8**)	2.4	0.6	0.5	4.8	1
δ-Decalactone (**9**)	1420	1690	400	3.6	4.2
Phenylacetic acid (**10**)	15800	7270	186	85	39
Methanethiol (**11**)	436	700	0.06	7270	11300

[a] The numbers indicated in brackets refer to those listed under Materials and methods.

[a] The nasal thresholds for odorants **1**, **6**, **8** in sunflower oil were obtained from Wagner and Grosch (1998), those for **2**, **3**, **9** from Preininger and Grosch (1994). The thresholds for odorants **4**, **11** were determined by Kubickova and Grosch (1998b), for **5** by Pfnür (1998), for **7** by Guth and Grosch (1990), and for **10** by Kerscher (2000).

Table 4. Concentrations, retronasal thresholds and odour activity values of short-chain fatty acids in Gruyère cheeses with a potato-like off-flavour (PG) and without an off-flavour (RG).

Compound [a]	retronasal threshold [b]	Concentration PG	Concentration RG	Odour activity value PG	Odour activity value RG
	(mg kg $^{-1}$)				
Acetic acid (**12**)	54	301	298	5.6	5.5
Propionic acid (**13**)	30	31	87	1.0	2.9
Methylpropionic acid (**14**)	88	99	41	1.1	0.5
Butyric acid (**15**)	18	271	97	15.1	5.4
2-/3-Methylbutyric acid (**16**/**17**)	10	480	81	48	8.1
Hexanoic acid (**18**)	81	116	28	1.4	0.3

[a] The numbers indicated in brackets refer to those listed under Materials and methods.

[b] determined by Warmke et al. (1996).

Table 5. Flavour scores [a] of the models representing the Gruyère cheese with a potato-like off-flavour (MPG), without an potato-like off-flavour (MRG), and model MA which is similar to MPG but without methional.

Flavour descriptor	Model		
	MPG	MRG	MA
Sweaty	2.5[b]	1.5	1.5
Potato-like	2[b]	1.5[c]	0.5
Buttery	1.5	1.5	1.5
Sweet/ fruity	1	1	1.5
Pungent	1	1	0.5
Malty	1.5	1	2[d]

[a] Scored on a three point scale ranging from 0 (not detectable) to 3 (strong).

[b] Significant difference ($p<0.05$) to the corresponding attribute of Model MRG.

[c] Significant difference ($p<0.05$) to the corresponding attribute of Model MA.

[d] Significant difference ($p<0.05$) to the corresponding attribute of Model MPG and MRG.

Table 6. Concentrations of free amino acids and ammonia in Gruyère cheeses with a potato-like off-flavour (PG) and without an off-flavour (RG).

Amino acid In mmol kg $^{-1}$	PG	RG
Aspartic acid	16.1	9.6
Threonine	5.5	7.8
Serine	20.9	19.5
Glutamic acid	79.6	60.7
Proline	45.1	36.3
Glycine	13.1	9.1
Alanine	11.7	8.5
Valine	31.4	25.7
Methionine	11.8	9.4
Isoleucine	20.0	16.4
Leucine	34.3	27.0
Tyrosine	7.1	4.5
Phenylalanine	17.9	13.7
Histidine	9.4	10.0
Lysine	45.7	31.4
Ammonia	59.0	37.2