

Published in:

International Dairy Journal 11 (2001) 895–901

The final publication is available at Elsevier via [https://doi.org/10.1016/S0958-6946\(01\)00108-X](https://doi.org/10.1016/S0958-6946(01)00108-X)

4 Flavour and Off-flavour Compounds of Swiss Gruyère

5 Cheese

6 Evaluation of Potent Odorants

7
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14 *Key words:* Aroma extract dilution analysis; flavour; Gruyère cheese; off-
15 flavour

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1 ABSTRACT

2 The flavour of a typical sample of Gruyère cheese and that of a Gruyère exhibiting a
3 potato-like off-flavour was examined by instrumental and sensory analyses. Based
4 on the results of dynamic headspace gas chromatography-mass spectrometry
5 (DHGC/MS), aroma extract dilution analysis (AEDA) and gas chromatography-
6 olfactometry of static headspace samples (GCO-H), 2-/ 3-methylbutanal, methional,
7 dimethyltrisulfide, phenylacetaldehyde, 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-
8 methylpyrazine, methanethiol, as well as butyric, 2-/3-methylbutyric and phenylacetic
9 acid form the typical flavour of Gruyère cheese. The potato-like character of the
10 sample showing an aroma defect, however, could not be attributed definitively to one
11 of these compounds. Considering the results of DHGC/MS and AEDA, 2-ethyl-3,5-
12 dimethylpyrazine and 2,3-diethyl-5-methylpyrazine could be possible causes of the
13 off-flavour.

14

15

16 INTRODUCTION

17 Swiss Gruyère cheese is a hard cheese exhibiting a characteristic flavour that is
18 described as rancid, sulfury and animal-like (Muir, Hunter, Banks & Horne, 1995).

19 Some years ago, a Swiss village cheese factory reported that a lot of Gruyère loaves
20 had to be discarded because they showed a potato-like off-flavour.

21 A perusal of the literature indicated two reports of similar flavour defects in cheeses.

22 The first was detected in a French Comté cheese by Dumont, Roger and Adda
23 (1975), who attributed it tentatively to 3-methoxy-2-propylpyrazine. As no systematic
24 sensory studies were performed, the contribution of this compound to the flavour
25 defect could not be confirmed. The second study dealt with a smear coated Munster

1 cheese, whose flavour compounds were assessed by gas chromatography coupled
2 to olfactometry and to mass spectrometry (Dumont, Morgues & Adda, 1983). In the
3 alkaline fraction 2-methoxy-3-isopropylpyrazine produced a potato-like odour and
4 was therefore held responsible for the off-flavour.

5 The flavour composition of typical Gruyère cheese has been the subject of
6 several recent studies. Liardon, Bosset and Blanc (1982), Bosset and Liardon (1984,
7 1985) and Bosset, Collomb and Sieber (1993) followed the concentration changes of
8 alkaline, neutral and acidic volatiles during ripening. More recently, Engels and
9 Visser (1994) examined the water-soluble fraction of different cheeses and
10 considered butyric acid to be a potent odorant of Gruyère cheese.

11 However, as there has been no systematic approach to screening for important
12 flavour contributors, the true composition of Gruyère flavour has so far remained a
13 matter of speculation. The purpose of the present study was therefore (i) to
14 investigate potent odorants of Gruyère cheese and (ii) to determine those substances
15 responsible for the above-mentioned potato-like off-flavour. In a similar way as we
16 reported the flavour of Swiss Emmentaler cheese (Preininger, Rychlik & Grosch,
17 1994, Preininger, Warmke & Grosch, 1996; Preininger and Grosch, 1994), the aim of
18 the first part of this study was to conduct an aroma extract dilution analysis (AEDA)
19 and to undertake several assays in order to characterise the headspace volatiles.
20 After screening for potent odorants, quantification of these compounds as well as
21 identifications of those, which are responsible for the off-flavour were performed in
22 the second part (Rychlik and Bosset, 2002).

23

24

25 MATERIALS AND METHODS

1 *Cheeses*

2 For a period of several months, a Swiss village factory had serious problems
3 due to the production of Gruyère cheese loaves exhibiting a potato-like off-flavour .
4 One of them was used for the current study (PG = potato-like Gruyère). A reference
5 Gruyère cheese (RG) without this flavour defect was obtained by ripening other
6 cheese loaves from this manufacture in a neighbouring village manufacture.

7

8 *Chemicals*

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

11 The pure reference compounds listed in the various tables were purchased: no.
12 **1**((S)-(+)-2-methylbutanal), **2-5, 7, 9, 10, 12, 14-17, 19-21, 26** ((S)-(+)-2-methylbutyric
13 acid), **27, 29, 30-34, 37, 38** (Aldrich, Steinheim, Germany); **34**, (Merck, Darmstadt,
14 Germany); **10, 23-25, 28, 35, 36** (Fluka, Buchs, Switzerland). The following
15 compounds were gifts: 2-acetyl-1-pyrroline, dimethyltetrasulfide, (Z)-6-dodecen- γ -
16 lactone, 2-ethyl-3,5-dimethylpyrazine, (Z)-2-nonenal from Prof. Grosch (formerly
17 Deutsche Forschungsanstalt für Lebensmittelchemie, Garching, Germany) and (Z)-4-
18 heptenal from Haarmann & Reimer (Holzminden, Germany).

19

20 *Steam distillation of cheeses and dynamic headspace gas chromatography-mass* 21 *spectrometry*

22 After addition of boiled (15 min) Milli-Q water (100 mL), the cheese samples (50
23 g) were grated and homogenized for 2 min by means of a Polytron dispersion
24 aggregat PT-DA 3020/2 with rotor 30/2M (Kinematica, Littau/Luzern, Switzerland)

1 and adjusted to pH 8.5 using aqueous sodium hydroxide (5 mol L^{-1} , 1 mL). The
2 suspension was then distilled at $60 \text{ }^{\circ}\text{C}$ into a flask containing sulphuric acid (30 %, 10
3 mL) using the modified rotavapor equipment reported by Imhof and Bosset (1989).
4 After concentrating in a rotavapor, the distillate was adjusted to pH 11 by addition of
5 sodium hydroxide and subjected to dynamic headspace GC/MS, as detailed
6 previously (Bosset, Bütikofer, Gauch & Sieber, 1997).

7

8 *Isolation of the volatile fraction for AEDA; separation into neutral/alkaline and acidic*
9 *volatiles*

10 Each cheese sample (200 g) was frozen in liquid nitrogen, then broken into
11 smaller pieces and ground in a Waring blender (Privileg, Quelle, Fürth, Germany).
12 The powder was then suspended in diethyl ether (500 mL), stirred for 4 h, and the
13 suspension was filtered. The filtrate was dried over anhydrous sodium sulphate and
14 concentrated to about 150 mL by distilling off the solvent under a Vigreux column (60
15 x 1 cm, Bahr, Manching, Germany). The solution of the volatiles was distilled off from
16 the non-volatile materials under high vacuum (5 to 6 mPa) in an apparatus described
17 earlier (Jung et al., 1992). When a pressure of 5 to 6 mPa was reached, the extract
18 was slowly allowed to drop over 1.5 h into the distillation flask, which was heated to
19 $40 \text{ }^{\circ}\text{C}$. Then, the distillation was continued for 90 min at $60 \text{ }^{\circ}\text{C}$. The trapped
20 condensate was extracted twice with aqueous sodium carbonate (total volume 200
21 mL, 0.5 mol L^{-1}) and washed with a saturated aqueous solution of sodium chloride
22 (200 mL). Then, the ethereal solution containing the neutral and alkaline components
23 was dried over anhydrous sodium sulphate and finally concentrated to 0.2 mL under
24 a Vigreux column (40 x 1 cm) and by microdistillation (Bemelmans, 1979).

1 To isolate the acidic volatiles, the aqueous alkaline solution was acidified to pH 3
2 with concentrated hydrochloric acid and extracted with diethylether (2 x 100 mL). The
3 ether solution was washed with a saturated aqueous solution of sodium chloride (200
4 mL), then dried over anhydrous sodium sulphate and finally concentrated to 0.2 mL.

5

6 *Separation of the alkaline volatiles and column chromatography (CC) of the neutral*
7 *volatiles for identification experiments*

8 The neutral/alkaline fraction (NAF) was separated into fractions containing neutral or
9 alkaline compounds by extraction with aqueous hydrochloric acid (0.1 mol/L, 2 x 50
10 mL). The pH of the aqueous extract containing the alkaline compounds was adjusted
11 to 12 by addition of sodium hydroxide (20 %), and the mixture was then extracted
12 with diethyl ether (3 x 50 mL). After concentration to 0.2 mL, the alkaline volatiles in
13 the ethereal extract were analysed by high resolution gas chromatography coupled to
14 mass spectrometry (HRGC/MS).

15 The organic layer remaining after extracting NAF with hydrochloric acid contained
16 the neutral compounds and was furthermore fractionated at 10 - 12 °C on a water-
17 cooled column (30 x 1,6 cm), packed with a slurry of silica gel 60 (Merck, Darmstadt,
18 Germany, purified as described by Esterbauer (1968)) in pentane/ diethylether (95/5,
19 mL (100 mL)⁻¹). Stepwise elution for each column was performed with the following
20 pentane/diethylether mixtures: 25 mL (95+5, vol+vol, fraction 1), 50 mL (85+15,
21 vol+vol, fraction 2), 45 mL (7+3, vol+vol, fraction 3), 75 mL (2+8, vol+vol, fraction 4)
22 and finally 50 mL pure diethylether (fraction 5).

23

24

1 *High resolution gas chromatography (HRGC)*

2 High resolution gas chromatography (HRGC) was performed on a type 5300
3 gas chromatograph (Carlo Erba, Hofheim, Germany) using the following fused silica
4 capillary columns: capillary DB-5 (30 m x 0.32 mm, $d_f = 0.25 \mu\text{m}$), capillary DB-1701
5 (30 m x 0.32 mm, $d_f = 0.25 \mu\text{m}$), and capillary DB-FFAP (30 m x 0.32 mm, $d_f = 0.25$
6 μm). The three capillary columns were supplied by Fisons Instruments, Mainz,
7 Germany.

8 The samples were applied by the cold on-column technique at 35 °C. Two min
9 after injecting 0.5 μL of the sample the temperature of the oven was raised to 60 °C
10 (50 °C for DB-1701) at a rate of 40 °C/min, held for 2 min isothermally and then
11 raised at 4 °C/min (5 °C/min for DB-FFAP) to 240 °C. The flow rate of the carrier gas,
12 helium, was 2 mL/min.

13

14 *Aroma extract dilution analysis (AEDA)*

15 The FD (flavour dilution)-values and odour descriptions of the compounds in the
16 neutral/alkaline and the acidic fractions of the cheeses were determined by AEDA
17 (Ullrich and Grosch, 1987). An aliquot of the respective fraction was stepwise diluted
18 with diethyl ether (1+1, vol+vol) and the diluted solutions (0.5 μL) were separated on
19 the capillary columns DB-5, DB-1701, and DB-FFAP. The effluent was split to a flame
20 ionization detector (FID) and a sniffing port (1+1, vol+vol) and evaluated by two
21 assessors. Retention data of the compounds are presented as retention indices (RI)
22 calculated according to Van den Dool and Kratz (1963).

23

1

2 *HRGC/ Olfactometry (HRGC/O) and HRGC/ mass spectrometry (HRGC/MS) of static*
3 *headspace samples*

4 Static headspace analysis was performed with a CP-9001 gas chromatograph
5 interfaced to the purge and trap system TC/PTI 4001 (Chrompack, Frankfurt,
6 Germany), as previously reported (Rychlik und Grosch, 1996). The glass tube in the
7 desorption heating block of the purge and trap facility was empty and deactivated.

8 A ground sample of frozen cheese (5 g) was filled into a water-jacket vessel (250 mL)
9 sealed with a septum. After tempering for 30 min at 25 °C, headspace samples
10 (0.625 ml - 25 mL) were drawn by a gastight syringe and injected with a velocity of 3
11 mL min⁻¹ into the purge system which operated in the desorption mode for 10 min at
12 250 °C. The carrier gas, helium (flow 20 mL min⁻¹), swept these headspace samples
13 into the trap (40 cm x 0.53 mm fused-silica capillary coated with CP-sil 8 CP, d_f = 5 μ
14 m; Chrompack, Frankfurt, Germany) which was precooled with liquid nitrogen to -
15 110 °C for 5 min. The trap was heated up very rapidly to 200 °C to start HRGC/O.
16 This temperature was held for 1 min and the sample was flushed by the helium (flow
17 rate 3 mL min⁻¹) into the gas chromatograph. The thin film capillary used for static
18 headspace GC/O was RTX-5 (30m x 0.53 mm fused silica capillary, d_f = 1.5 μm,
19 Amchro, Sulzbach/Taunus, Germany). The temperature of the oven was held at 0 °C
20 for 0 min and then programmed at a rate of 6 °C min⁻¹ to 230 °C. At the exit of the
21 capillary, the effluent was split 1+1 (vol+vol) into an FID and the sniffing port using
22 deactivated fused-silica capillaries (30 cm x 0.15 mm). The FID and the sniffing port
23 were held at 250 °C. Nitrogen (20 mL min⁻¹) was used as make-up gas for the FID.

1 After each GC/O run the purge system was automatically cleaned (clean-up flow, 50
2 mL min⁻¹ helium; clean-up temperature, 275 °C).

3 MS-analyses were performed with an INCOS XL (Finnigan MAT, Bremen,
4 Germany) in tandem with capillary RTX-5. Mass spectra in the electron impact mode
5 (MS-EI) were generated at 70 eV and in the chemical ionization mode (MS-CI) at 115
6 eV with isobutane as reactant gas.

7

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

9 For identification of the odorants, MS analyses were performed with a MAT 95 S
10 (Finnigan, Bremen, Germany) in tandem with the capillaries DB-5, DB-1701, and DB-
11 FFAP. Mass spectra in the electron impact mode (MS-EI) were generated at 70 eV
12 and in the chemical ionization mode (MS-CI) at 115 eV with isobutane as reagent
13 gas.

14

15 RESULTS AND DISCUSSION

16 High grade Swiss Gruyère cheese exhibits a slightly milky (smear, rind),
17 vegetable- and animal-like (meat broth), and spicy (pepper) flavour with a white
18 vinegar note (Lavanchy and Bütikofer, 1999). In contrast to the odour of cheese
19 loaves of a high quality reference Gruyère (RG), the tainted Gruyère (PG) produced
20 by a Swiss village cheese factory smelled intensively sweaty and cooked potato-like.

21 In order to get a first insight into the nature of the defect, dynamic headspace GC/MS
22 (DHSGC/MS) analyses of alkaline extracts (obtained with the enriched off-flavour
23 components) of the two cheese samples were performed. The results in **Table 1**
24 indicate that the cheese with the potato-like off-flavour had much higher

1 concentrations of tetramethylpyrazine, 2,6-dimethylpyrazine, 1-methyl-[1H]-pyrrole, 5-
2 ethyl-2-methylpyridine. As the odour of pyrazines is described as potato-like (Rychlik
3 et al., 1998), these compounds were at first suspected of causing the off-flavour.

4 As DHSGC-MS does not provide any sensory information, the cheeses were
5 then subjected to aroma extract dilution analysis (AEDA). This method has been
6 shown to be a powerful means for evaluating the potent odorants in cheeses, e. g. in
7 Swiss Emmentaler (Preininger et al., 1994), Camembert (Kubickova and Grosch,
8 1997), or Cheddar cheese (Milo and Reineccius, 1997). Moreover, AEDA is suitable
9 for identifying the substances responsible for off-flavours, as Heiler and Schieberle
10 (1996) reported for buttermilk. Therefore, PG and RG were both extracted with
11 diethylether and the extracts distilled under vacuum to obtain the volatile fraction.
12 The volatiles were then separated into neutral/alkaline (NAF) and acidic (AF)
13 fractions, which were both subjected to AEDA. Gas chromatography-olfactometry of
14 NAF revealed 21 and 25 odorous compounds in PG and RG, respectively (**Table 2**).
15 The overwhelming majority of odorants were identified by mass spectrometry, after
16 NAF was cleaned up by extracting the alkaline volatiles, and by column
17 chromatography. Of these, the potato-like smelling methional showed the highest
18 flavour dilution (FD) factor of 512 in both cheeses. In PG, the next lower FD factors
19 were found for 2-ethyl-3,5-dimethylpyrazine (EDMP), 2-/3-methylbutanal and an
20 unknown compound with a sweet odour quality. In contrast to this, AEDA of RG
21 revealed dimethyltrisulfide, phenylacetaldehyde, (E)-2-nonenal and
22 dimethyltetrasulfide to be further important odorants in this sample with FD-factors
23 exceeding 128.

24 In the acidic phases of both cheeses the 12 compounds listed in **Table 3** showed FD-
25 factors higher than 4. In both samples, the sweaty smelling mixture of 2- and 3-

1 methylbutyric acid had the highest FD-factor followed by phenylacetic acid and
2 butyric acid.

3 It was not surprising that methional exhibits such a high FD factor in PG, because of
4 its potato-like odour. But this odorant had the same high FD in RG, which did not
5 have a potato-like off-flavour; hence, AEDA could not prove the impact of methional
6 on this off-flavour. However, methional is not essential for a potato-like flavour, e. g.
7 in French fries (Wagner and Grosch, 1998) or in boiled potatoes (Mutti, 2000). Both
8 studies attributed this odour quality to a combination of methanethiol and pyrazines.
9 As highly volatile compounds such as the former are underestimated in AEDA
10 because of losses during distilling steps, gas chromatography-olfactometry of static
11 headspace samples (GCO-H) was subsequently performed. As is apparent from
12 **Table 4**, methanethiol in RG showed the highest FD-factor of 32 corresponding to a
13 minimal headspace volume of 0.6 mL. Further important odorants in the headspace
14 of RG were dimethylsulfide, dimethyltrisulfide and ethyl 2-methylbutanoate. By
15 contrast, in PG dimethylsulfide had the highest FD of 16 followed by methanethiol, 3-
16 methylbutanal and dimethyltrisulfide. Since the FD of methanethiol in PG was lower
17 than in RG, this compound seemed not to be responsible for the flavour defect.

18 In summary, methanethiol as well as the neutral compounds methional, EDMP,
19 2-/3-methylbutanal, dimethyltrisulfide, phenylacetaldehyde, (E)-2-nonenal,
20 dimethyltetrasulfide as well as the acidic volatiles 2- and 3-methylbutyric,
21 phenylacetic and butyric acids were found to be potent odorants in Swiss Gruyère
22 cheese. Of these compounds, butyric and isovaleric acids have already been
23 previously suggested as being contributors to Gruyère flavour (Bosset et al., 1993;
24 Engels and Visser, 1994). Similarly, our results confirmed earlier reports that 3-
25 methylbutanal (Bosset and Liardon, 1984; Engels et al., 1997) as well as

1 dimethyltrisulfide (Engels et al., 1997) play an important role in the odour of this
2 variety of cheese.

3

4 CONCLUSIONS

5 Based on the results of AEDA of acidic volatiles, 2-/3-methyl butyric and butyric
6 acids seem to be responsible for the sweaty odour of PG. However, the cause of its
7 potato-like taint is still not clear. Unlike in French fries (Wagner and Grosch, 1998)
8 and in boiled potatoes (Mutti, 2000), GCO-H gives no indication that methanethiol is
9 the causal substance. The results of AEDA, however, lead us to conclude, that, due
10 to their higher FD in PG, EDMP and 2,3-diethyl-5-methylpyrazine (DEMP) may evoke
11 the defect. But both AEDA and GCO-H suffer from different limitations, the lack of
12 precision and possible discriminations of these olfactory methods being the decisive
13 ones (Grosch, 1993). Furthermore, the latter methods do not consider synergistic or
14 suppressive effects of different odorants in a flavour mixture, since the compounds
15 are evaluated separately after gas chromatography. The lacking precision and
16 possible discriminations of the olfactory methods can be overcome by accurate
17 quantifications. By subsequent sensory analyses of models simulating the cheeses
18 with and without off-flavour, the interactions of the odorants can be considered, and
19 those compounds can be identified, which are responsible for the flavour defect.
20 These investigations will be reported in the second part of this study (Rychlik and
21 Bosset, 2002).

22

1 Acknowledgement

2 We are grateful to Mrs. D. Fottner (Garching) and Mr. R. Gauch (Liebefeld / Bern) for
3 their excellent technical assistance, and Dr J.-P.-Dumont (INRA Nantes, France) as
4 well as Mrs G. Urbach (South Caulfield, Australia) for their valuable reviewing of this
5 paper.

6

7 REFERENCES

- 8 Bemelmans, J.M.H. (1979). Review of isolation and concentration techniques. In D.
9 G. Land, & H. E. Nursten, *Progress in Flavour Research* (pp. 79-98). London:
10 Applied Science.
- 11 Bosset, J.O., & Liardon, R. (1984). The aroma composition of Swiss Gruyere cheese.
12 II. The neutral volatile components. *Lebensmittel-Wissenschaft und –*
13 *Technologie*, 17, 359-362.
- 14 Bosset, J.O., & Liardon, R. (1985). The aroma composition of Swiss Gruyere cheese.
15 III. Relative changes in the content of alkaline and neutral volatile components
16 during ripening. *Lebensmittel-Wissenschaft und –Technologie*, 18, 178-185.
- 17 Bosset, J.O., Bütikofer, U., Gauch, R., & Sieber, R. (1997). Reifungsverlauf von in
18 Folien verpacktem Emmentaler Käse mit und ohne Zusatz von *Lactobacillus*
19 *casei* subsp *casei*. II. Gaschromatographische Untersuchung einiger flüchtiger,
20 neutraler Verbindungen mit Hilfe einer dynamischen Dampfmanalyse.
21 *Lebensmittel-Wissenschaft und –Technologie*, 30, 771.

- 1 Bosset, J.O., Collomb, M., & Sieber, R. (1993). The aroma composition of Swiss
2 Gruyere cheese. IV. The acidic volatile components and their changes in
3 content during ripening. *Lebensmittel-Wissenschaft und -Technologie*, 26, 581-
4 592.
- 5 Dumont, J. P., Mourgues, R. & Adda, J. (1983). Potato-like off flavour in smear
6 coated cheese: a defect induced by bacteria. In A. A. Williams, & R. K. Atkin,
7 *Sensory Quality in Foods and Beverages: Definition, Measurement and Control*
8 (pp. 424-428). London: Ellis Horwood.
- 9 Dumont, J.P., Roger, S., & Adda, J. (1975). Mise en évidence d'un composé à
10 hétérocycle azoté responsable d'un défaut d'arôme dans le Gruyère de Comté.
11 *Lait*, 55, 479-487.
- 12 Engels, W.J.M., & Visser, S. (1994). Isolation and comparative characterization of
13 components that contribute to the flavour of different types of cheese.
14 *Netherlands Milk and Dairy Journal*, 48, 127-140.
- 15 Engels, W.J.M., Dekker, R., Jong C, de, Neeter, R., & Visser, S. (1997). A
16 comparative study of volatile compounds in the water-soluble fraction of various
17 types of ripened cheese. *International Dairy Journal*, 7, 255-263.
- 18 Esterbauer, H. (1968). Über die Autoxidation von Linolsäure-methylester in Wasser
19 III: Chromatographische Auftrennung der wasserlöslichen Reaktionsprodukte.
20 *Fette, Seifen, Anstrichmittel*, 70, 1-4.
- 21 Grosch, W. (1993). Detection of potent odorants in foods by aroma extract dilution
22 analysis. *Trends in Food Science and Technology*, 4, 68-73.
- 23 Heiler, C., & Schieberle, P. (1996). Studies on the metallic off-flavour in buttermilk:
24 Identification of potent aroma compounds. *Lebensmittel-Wissenschaft und –*
25 *Technologie*, 29, 460-464.

- 1 Imhof, R., & Bosset, J. O. (1989). Einfache quantitative photometrische Bestimmung
2 von "Gesamt"-Carbonylkomponenten in biologischen Medien. *Mitteilungen aus*
3 *dem Gebiete der Lebensmitteluntersuchung und Hygiene*, 88, 409-419
- 4 Jung, H.P., Sen, A., & Grosch, W. (1992). Evaluation of potent odorants in parsley
5 leaves [*Petroselinum crispum* (Mill.) Nym. ssp. *crispum*] by aroma extract
6 dilution analysis. *Lebensmittel-Wissenschaft und –Technologie*, 25, 55-60.
- 7 Kubickova, J., & Grosch, W. (1997). Evaluation of potent odorants of Camembert
8 cheese by dilution and concentration techniques. *International Dairy Journal*, 7,
9 65-70.
- 10 Lavanchy, P. & Bütikofer, U. (1999). Caractérisation sensorielle de fromages à pâte
11 dure ou mi-dure fabriqués en Suisse. *Mitteilungen aus dem Gebiete der*
12 *Lebensmitteluntersuchung und Hygiene*, 90, 670-683.
- 13 Liardon, R., Bosset, J.O., & Blanc, B. (1982). The aroma composition of Swiss
14 Gruyere cheese. I. The Alkaline Volatile Components. *Lebensmittel-*
15 *Wissenschaft und –Technologie*, 15, 143-147.
- 16 Milo, C., & Reineccius, G.A. (1997). Identification and quantification of potent
17 odorants in regular fat and low-fat mild cheddar cheese. *Journal of Agricultural*
18 *and Food Chemistry*, 45, 3590-3594.
- 19 Muir, D.D., Hunter, E.A., Banks, J.M., & Horne, D.S. (1995). Sensory properties of
20 hard cheese: identification of key attributes. *International Dairy Journal*, 5, 157-
21 177.
- 22 Mutti, B. (2000). *Aroma frisch gekochter Kartoffeln – Einfluß von Sorte und Lagerung*
23 *sowie Vergleich mit dem Aroma getrockneter Kartoffeln*. Ph D thesis, Neufahrn:
24 Dr. Hut, ISBN 3-934767-12-5.

- 1 Preininger, M., & Grosch, W. (1994). Evaluation of key odorants of the neutral
2 volatiles of Emmentaler cheese by the calculation of odour activity values.
3 *Lebensmittel-Wissenschaft und –Technologie*, 27, 237-244.
- 4 Preininger, M., Rychlik, M., & Grosch, W. (1994). Potent odorants of the neutral
5 volatile fraction of Swiss cheese (Emmentaler). In H. Maarse, & D. G. van der
6 Heij, *Trends in Flavour Research* (pp. 267-270). Amsterdam: Elsevier Science
7 Publishers.
- 8 Preininger, M., Warmke, R., & Grosch, W. (1996). Identification of the character
9 impact flavour compounds of Swiss cheese by sensory studies of models.
10 *Zeitschrift für Lebensmittel-Untersuchung und –Forschung*, 202, 30-34.
- 11 Rychlik, M. & Bosset, J. O. (2002). Flavour and off-flavour compounds of Swiss
12 Gruyère cheese. 2. Identification of key odorants by quantitative and sensory
13 studies. *International Dairy Journal*, submitted.
- 14 Rychlik, M., & Grosch, W. (1996). Identification and Quantification of Potent Odorants
15 Formed by Toasting of Wheat Bread. *Lebensmittel-Wissenschaft und –*
16 *Technologie*, 29, 515-525.
- 17 Rychlik, M., Schieberle, P., & Grosch, W.(1998). *Compilation of odor thresholds, odor*
18 *qualities and retention indices of key food odorants*. Garching: Deutsche
19 Forschungsanstalt für Lebensmittelchemie, Lichtenbergstr. 4, ISBN 3-9803426-
20 5-4.
- 21 Schieberle, P., & Grosch, W. (1983). Identifizierung von Aromastoffen aus der Kruste
22 von Roggenbrot. *Zeitschrift für Lebensmittel-Untersuchung und –Forschung*,
23 177, 173-180.
- 24 Ullrich, F., & Grosch, W. (1987). Identification of the most intense volatile flavour
25 compounds formed during autoxidation of linoleic acid. *Zeitschrift für*
26 *Lebensmittel-Untersuchung und –Forschung*, 184, 277-282.

- 1 Van den Dool, H., & Kratz, P.D. (1963). A generalization of the retention index
- 2 system including linear temperature programmed gas-liquid partition
- 3 chromatography. *Journal of Chromatography*, 11, 463-471.
- 4 Wagner, R.K., & Grosch, W. (1998). Key odorants of French fries. *Journal of the*
- 5 *American Oil Chemists Society*, 75, 1385-1392.

Table 1. Comparison of dynamic headspace gas chromatography – mass spectrometry of alkaline extracts from Gruyère cheeses with a potato-like off-flavour (PG) and without an off-flavour (RG)

Compound ^a	ratio PG/RG ^b
Pyridine	3
1-Methyl-[1H]-pyrrole	11
2-Methylpyridine	4
3-Methylpyridine	3
2,4-Dimethylpyridine	3
2,6-Dimethylpyrazine	10
Dimethylpyridine	3
2-Ethyl-6-methylpyrazine	2
Trimethylpyrazine	2
5-Ethyl-2-methylthiazole	4
5-Ethyl-2-methylpyridine	8
5-Ethenyl-2-methylpyridine	4
2-Ethyl-3,5-dimethylpyrazine	4
Tetramethylpyrazine	12
3-Ethyl-2,6-dimethylpyridine	7

^a The compounds were identified by comparing their mass spectra with those of reference substances.

^b Ratio of peakheights in the extract of PG to that of RG.

Table 2. Neutral/alkaline odorants of Gruyère cheeses with a potato-like off-flavour (PG) and without an off-flavour (RG)

Odorant ^a	odor quality ^b	sub-fraction ^c	RI ^d		FD ^e	
			DB-5 ^f	DB-1701 ^f	PG	RG
2-/3-Methylbutanal (1/2)	malty	2	649	724	128	64
Methyl 2-methylbutanoate (3)	sweet	1	777	841	1	16
Ethyl 2-methylbutanoate (4)	sweet	1	848	903	4	64
2-Heptanone (5)	green	2	884	976	16	64
(Z)-4-Heptenal ^g (6)	biscuit-like	2	897	986	-	32
Methional (7)	boiled potato	3	905	1035	512	512
2-Acetyl-1-pyrroline ^g (8)	roasty	AL	921	1010	16	32
Dimethyltrisulfide (9)	cabbage-like	1	964	1030	64	128
Unknown	fatty	2	1024		32	32
Phenylacetaldehyde (10)	honey-like	2	1041	1169	64	128
2-Ethyl-3,5-dimethylpyrazine (11)	earthy	AL	1082	1148	256	32
Unknown	sweet	1	1091	1192	8	16
2-Phenylethanol (12)	honey-like	2	1114	1267	32	-
(Z)-2-Nonenal (13)	green	2	1150	1254	8	32
2,3-Diethyl-5-methylpyrazine (14)	earthy	AL	1159	1211	64	16
(E)-2-Nonenal (15)	green	2	1161	1269	8	128
Unknown	seasoning-like	1	1172	1249	64	32
Ethyl octanoate (16)	fatty, fruity	1	1199	1259	2	32
(E,E)-2,4-Nonadienal ^g (17)	deep-fried	2	1212	1340	-	32
Dimethyltetrasulfide (18)	cabbage-like	1	1209	1297	64	128
Indole (19)	mothball-like	3	1293	1540	32	16
(E,E)-2,4-Decadienal (20)	deep-fried	2	1313	1445	-	16

Unknown	sweet, honey-like	1	1349	1495	128	32
Unknown	raspberry-like	1	1472	1589	-	32
δ -Decalactone (21)	sweet, coconut-like	3	1494	1713	64	64
(Z)-6-Dodecen- γ -lactone ^g (22)	sweet	3	1657	1878	64	32

The numbers indicated in brackets refer to those listed under Materials and Methods.

^a The compound was identified by comparing it with the reference compound on the basis of the following criteria: RI on the two capillaries detailed in the Table, odour quality at the sniffing port, and mass spectra in the electron impact mode and the chemical ionization mode.

^b Odour description perceived at the sniffing port.

^c Fractions in which the compound was identified; AL alkaline fraction; No.: fraction of column chromatography (see Materials and methods).

^d Retention index.

^e Flavour dilution factor.

^f different capillary columns (see Materials and methods).

^g The mass spectra were too weak for an unequivocal interpretation. The compound was tentatively identified on the basis of the resting criteria detailed in footnote ^a.

Table 3. Acidic odorants of Gruyère cheeses with a potato-like off-flavour (PG) and without an off-flavour (RG)

No.	Odorant ^a	Odor quality ^b	RI (DB-FFAP) ^c	FD ^d	
				PG	RG
	Propionic acid (23)	fruity, pungent	1524	4	16
	Methylpropanoic acid (24)	sweaty, rancid	1562	32	8
	Butyric acid (25)	sweaty	1622	256	128
	2- and 3-Methylbutanoic acid (26/27)	sweaty	1662	8196	512
	Unknown	sweet	1802	16	8
	Hexanoic acid (28)	goat-like	1841	32	16
	Unknown	sweet	1912	16	8
	4-Hydroxy-2,5-dimethyl- 3-(2H)furanone (29)	strawberry-like	2033	64	32
	2-Ethyl-4-hydroxy-5-methyl 3-(2H)furanone (30)	strawberry-like	2061	32	32
	3-Hydroxy-4,5-dimethyl- 2-(5H)furanone ^e (31)	seasoning-like	2199	32	16
	Phenylacetic acid (32)	honey-like	2569	4096	512
	Phenylpropanoic acid (33)	flowery	2638	256	128

^a The compound was identified by comparing it with the reference compound on the basis of the following criteria: retention index on capillary DB-FFAP, odour quality at the sniffing port, and mass spectra in the electron impact mode and the chemical ionization mode.

^b Odour description perceived at the sniffing port.

^c Retention index on capillary DB-FFAP.

^d Flavour dilution factor.

^e The mass spectra were too weak for an unequivocal interpretation. The compound was tentatively identified on the basis of the resting criteria detailed in footnote ^a.

Table 4. Results of the static headspace analysis of Gruyère cheeses with a potato-like off-flavour (PG) and without an off-flavour (RG)

Odorant ^a	Odour quality ^b	RI (RTX-5) ^c	PG	RG
			FD-Factor ^d	
Acetaldehyd (34)	green	<500	2	2
Methanethiol (35)	sulfurous	<500	8	32
Dimethylsulfide (36)	sulfurous	505	16	16
2-Methylpropanal (37)	malty	556	2	1
3-Methylbutanal (2)	malty	654	8	8
2-Methylbutanal (1)	malty	664	4	1
Methyl 2-methylbutanoate (3)	sweet	777	1	1
Dimethyldisulfide (38)	sulfurous	741	1	2
Ethyl 2-methylbutanoate ^e (4)	sweet	848	4	8
Methional ^e (7)	boiled potato	909	1	1
Dimethyltrisulfide (9)	cabbage-like, sulfurous	968	8	16

^a The compound was identified by comparing it with the reference compound on the basis of RI on capillary RTX-5, odour quality at the sniffing port, and mass spectra in EI-Mode.

^b Odour description perceived at the sniffing port.

^c Retention index on capillary RTX-5.

^d A headspace volume of 20 mL was equated to a flavour dilution (FD) factor of 1. The FD factor values of the other odorants were calculated on this basis.

^e The mass spectra were too weak for an unequivocal interpretation. The compound was tentatively identified on the basis of the resting criteria detailed in footnote ^a.