JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2008,** *56,* 5299–5307 **5299**

Quantitative Studies and Taste Re-engineering Experiments toward the Decoding of the Nonvolatile Sensometabolome of Gouda Cheese

SIMONE TOELSTEDE[†] AND THOMAS HOFMANN*[,#]

Institut für Lebensmittelchemie, Universität Münster, Corrensstrasse 45, D-48149 Münster, Germany, and Lehrstuhl für Lebensmittelchemie and Molekulare Sensorik, Technische Universität München, Lise-Meitner-Strasse 34, D-85354 Freising-Weihenstephan, Germany

The first comprehensive quantitative determination of 49 putative taste-active metabolites and mineral salts in 4- and 44-week-ripened Gouda cheese, respectively, has been performed; the ranking of these compounds in their sensory impact based on dose-over-threshold (DoT) factors, followed by the confirmation of their sensory relevance by taste reconstruction and omission experiments enabled the decoding of the nonvolatile sensometabolome of Gouda cheese. The bitterness of the cheese matured for 44 weeks was found to be induced by $CaCl_2$ and $MgCl_2$, as well as various bitter-tasting free amino acids, whereas bitter peptides were found to influence more the bitterness quality rather than the bitter intensity of the cheese. The DoT factors determined for the individual bitter peptides gave strong evidence that their sensory contribution is mainly due to the decapeptide YPFPGPIHNS and the nonapeptides YPFPGPIPN and YPFPGPIHN, assigned to the casein sequences β-CN(60−69) and β-CN(60−68), respectively, as well as the tetrapeptide LPQE released from α_{s1}-CN(11−14). Lactic acid and hydrogen phosphate were identified to play the key role for the sourness of Gouda cheese, whereas umami taste was found to be due to monosodium L-glutamate and sodium lactate. Moreover, saltiness was induced by sodium chloride and sodium phosphate and was demonstrated to be significantly enhanced by L-arginine.

KEYWORDS: Gouda cheese; sensometabolome; taste compounds; bitter peptides; umami; salt enhancer; arginine

INTRODUCTION

Gouda cheese is a highly desirable dairy product appreciated by consumers all over the world. Whereas multiple studies performed in the past 30 years have been performed to identify and quantify the odor-active volatiles in cheese, knowledge on the nonvolatile, taste-active metabolites is still rather fragmentary. Although literature studies suggest lactic acid, sodium chloride, and free amino acids, as well as bitter peptides of various structures, as the key compounds imparting the typical taste profile of cheese (*1*), the data published on saltiness-inducing and, in particular, the bitterness-inducing molecules are somehow contradictory (*2*–*8*).

The comprehensive spectrum of sensory active, low molecular weight compounds, coined "sensometabolome" (*9*), reflects the sensory phenotype and triggers the typical smell and taste of food products. The goal of sensometabolomics is to catalogue, quantify, and evaluate the sensory activity of metabolites that are present in raw materials and/or are produced upon food processing such as thermal or high-hydrostatic pressure treatment and fermentation, respectively.

Sensory-directed fractionation of a matured Gouda cheese recently enabled the comprehensive mapping of single bitter-tasting fractions and revealed the identification of various bitter peptides formed by proteolysis of caseins (*9*). Eleven peptides were aligned to β-casein, among which six peptides were released from the N-terminal part of the protein within the sequence β-CN(57−69), and two peptides originated from the C-terminal part, β-CN(198−206). Other peptides were liberated from miscellaneous regions of β-casein such as β-CN(22−28), β-CN(74−86), β-CN(74−77), and β-CN(135−138), respectively, and from α_{s1}-casein, namely, the sequences α_{s1}-CN(11−14), α_{s1}-CN (56−60), α_{s1}-CN(135−136), α_{s1}-CN(70/71−74), and α_{s1}-CN(110/111−114), respectively. Sensory evaluation of the synthesized and highly purified peptides revealed that 12 of these peptides showed pronounced bitter taste with recognition thresholds between 0.05 and 6.0 mmol/L, with the decapeptide YPFPGPIHNS showing the lowest threshold and originating from the protein sequence β-CN(60−69) (*9*).

To the best of our knowledge, no comprehensive quantitative study on the entire sensometabolome of young and ripened

* Author to whom correspondence should be addressed (telephone +49-8161/71-2902; fax +49-8161/71-2949; e-mail thomas.hofmann@wzw.tum.de).

[†] Universität Münster.

[#] Technische Universität München.

10.1021/jf800552n CCC: $40.75
Published on Web 06/17/2008

Gouda cheese and, in particular, on single bitter peptides has been performed so far. Therefore, the objective of this work was to catalogue and quantify putative taste-active metabolites and mineral salts, to rank them in their sensory impact on the basis of dose/activity considerations, and to prove their sensory relevance by re-engineering the nonvolatile sensometabolome of Gouda cheese.

MATERIALS AND METHODS

Chemicals. All chemicals used were purchased from Merck (Darmstadt, Germany) and Fluka (Neu-Ulm, Germany), respectively. Solvents were of HPLC grade (Merck). Deionized water used for chromatography was purified by means of a MilliQ-water Gradient A 10 system (Millipore, Billerica, MA). Synthetic bitter peptides were purchased from EZBiolab (Westfield, IN) and purified as described recently (*9*). Gouda cheese samples ripened under controlled standard conditions for 4 (GC4) and 44 weeks (GC44), respectively, were selected by a professional cheese sensory panel in the Dutch food industry and were confirmed to represent the characteristic flavor profiles of a young and a matured Gouda cheese, respectively. Both cheese samples were freshly cut from cheese wheels, sealed in rindless portions (100 g) in nitrogen-flushed packages, and stored at −20 °C until use. Formic acid, which is GRAS listed as a flavoring agent for food and feed applications, was used to adjust the pH value of solutions to be sensorially evaluated; trace amounts (<0.002%) of this acid do not influence the sensory profile of the test solution.

Preparation of the Water-Soluble Extract (WSE). Samples (60 g) of the young (4 weeks) and matured Gouda cheese (44 weeks), cut into small pieces, and deionized water (240 mL) were placed into a centrifuge beaker, homogenized for 5 min by means of an Ultra-Turrax T 25 basic (Ika Labortechnik, Germany), and then centrifuged at 10000 rpm for 20 min at 4 °C by means of a Varifuge 20 RS (Kendro, Langenselbold, Germany). The upper solid fat layer as well as the protein pellet formed was removed to afford the liquid layer including the cheese water-solubles (pH 5.7). The protein pellet and fat layer were re-extracted with deionized water (240 mL) as detailed above, the aqueous layers were pooled, and soluble casein was precipitated upon adjustment of the pH value to 4.6 by the addition of formic acid (1%, v/v; in water). After centrifugation at 10000 rpm at 4 °C for 20 min, followed by paper filtration (Macherey-Nagel, 615-1/4) and freeze-drying, a casein-free water-soluble extract (WSE4, WSE44) was obtained, which was stored at −20 °C until further analysis.

Quantitative Analysis of Bitter Peptides. For quantitation of bitter peptides, an aliquot (5 μL) of an aqueous solution of WSE4 (3 mg/mL) and WSE44 (2 mg/mL), respectively, was analyzed by means of HPLC-MS/MS on a Fusion-RP80, 150 × 2.0 mm i.d., 5 μm column (Phenomenex, Aschaffenburg, Germany) equipped with a guard column of the same type. Using a flow rate of 0.2 mL/min, chromatography was performed starting with a 1% aqueous solution of formic acid for 5 min, then increasing the content of acetonitrile (containing 1% formic acid) to 50% within 45 min and, finally, to 100% within 5 min. As shown in **Figure 1**, the following peptides were analyzed using the mass transitions given in parentheses: VRGPFP (*m*/*z* 336.8→70.0), YPFPGPIPN (*m*/*z* 501.42→70.0), MI (*m*/*z* 263.3→136.0), VYPFPGPIPN (*m*/*z* 551.0→70.0), YPFPGPIHNS (*m*/*z* 565.1→70.0), GPVRGPFP (*m*/*z* 414.0→70.0), DIKQM (*m*/*z* 634.4→84.1), YPFPGPIHN (*m*/*z* 521.3→110.3), EIVPN (*m*/*z* 571.3→230.1), LPQE (*m*/*z* 486.2→70.1), LVYPFPGPIHN (*m*/*z* 627.5→110.0), and SLVYPFPGPIHNS (*m*/*z* 714.6→173.1). Quantitative analysis was performed in triplicates by comparing the peak areas obtained for the corresponding mass traces with those of defined standard solutions of each reference compound.

Quantitative Analyses of Organic Acids, Amino Acids, Soluble Carbohydrates, and Minerals. A defined amount of lyophilized WSE4 and WSE44, respectively, was dissolved in deionized water and, after membrane filtration, an aliquot (10 μL) was analyzed by means of an ICS 2500 ion chromatography system (Dionex, Idstein, Germany) equipped with an AS 50 thermal compartment, a GS 50 gradient pump, an ED 50 electrochemical detector, an AS 50 A autosampler, and a GM-4 gradient mixer. Quantitation was performed using an external standard calibration with standard solutions ranging from 0.1 to 100 mg/L (six-point calibration). System control and data acquisition were accomplished using Dionex Chromeleon version 6.60 software.

Anions. Anions were analyzed on an Ion Pac AS 11-HC, 250 × 2.0 mm i.d. column connected with an Ion Pac AG 11-HC, 50 × 2.0 mm guard column and an anion self-regenerating suppressor ASRS Ultra II 2.0 mm, current 76 mA (Dionex), installed between the column and the ED50-type conductivity detector. Chromatography was performed at 30 °C with a flow rate of 0.38 mL/min using the following gradient consisting of water (solvent A), an aqueous 5 mmol/L NaOH (solvent B), and an aqueous 100 mol/L NaOH (solvent C): A/B/C (80:20:0, v/v/v) for 8 min, to A/B/C (85:0:15, v/v/v) within 10 min, then to A/B/C (70:0:30, v/v/v) within 10 min and, finally, to A/B/C (40:0:60, v/v/v) within 10 min.

Cations. Cations were analyzed on an Ion Pac CS 16, 250 × 3.0 mm i.d. column equipped with an Ion Pac CG 16, 50 × 3.0 mm i.d. guard column and a cation self-regenerating suppressor CSRS Ultra II 2.0 mm, current 37 mA (Dionex), installed between the column and the conductivity detector. Chromatography was performed at 40 °C with a mixture (70:30, v/v) of water and aqueous 100 mol/L methanesulfonic acid with a flow rate of 0.36 mL/min.

Amino Acids. For ion chromatography of amino acids an Amino Pac PA-10, 250 × 2.0 mm i.d. column equipped with an Amino Pac PA-10, 50 × 2.0 mm guard column in combination with the ED50-type pulsed amperometric detector was used. Chromatography was performed at 30 °C at a flow rate of 0.25 mL/min using the following gradient of water (eluent A), an aqueous 200 mmol/L NaOH solution (eluent B), and a 1.0 mmol/L sodium acetate solution (eluent C): A/B/C (76:24:0, v/v/v) for 8 min, to A/B/C (40:20:40, v/v/v) within 10 min, then to A/B/C (44:16:40, v/v/v) within 3 min and, finally, to A/B/C (14:16:70, v/v/v) within 2 min.

Carbohydrates. Carbohydrates were separated on a Carbo Pac PA-10, 250 × 2.0 mm i.d. column equipped with a 50 × 2.0 mm guard column of the same type and detected by the ED50-type pulsed amperometric detector. Chromatography was performed at 30 °C at a flow rate of 0.25 mL/min using the following gradient of water (eluent A) and an aqueous 250 mmol/L NaOH solution (eluent B): A/B (91:9, v/v) for 20 min, then to A/B (0:100, v/v) within 20 min and, finally, held isocratically for 10 min.

Fatty Acids. Free fatty acids were analyzed following the procedure reported in the literature (*10*).

Biogenic Amines. Biogenic amines were analyzed as their dansyl derivatives by means of RP-HPLC following the procedure reported in the literature (*11, 12*).

Dry Matter Content. The dry matter content was determined in a vacuum drying oven kept at 65 °C as reported earlier (*13*).

Fat Content. The total fat content was determined using the gravimetric micromethod reported in the literature (*14*).

Sensory Analyses. *General Conditions, Panel Training*. To familiarize the subjects with the taste language used by our sensory group and to get them trained in recognizing and distinguishing different qualities of oral sensations in analytical sensory experiments, 11 assessors (4 women and 7 men, 23−39 years of age), who gave informed consent to participate in the sensory tests of the present investigation and have no history of known taste disorders, participated for at least two years in weekly training sessions. For example, the subjects were trained to evaluate the taste of aqueous solutions (2 mL; pH 5.7) of the following standard taste compounds in bottled water (Evian, low mineralization = 500 mg/L) using the sip-and-spit method: NaCl (20 mmol/L) for salty taste, lactose (50 mmol/L) for sweet taste, lactic acid (20 mmol/L) for sour taste, and monosodium L-glutamate (3 mmol/L) for umami taste. For training of bitter taste, solutions of $MgSO_4$ (166 mmol/L) representing a short-lasting, metallic bitter taste quality perceived mainly at the anterior part of the tongue, salicin (1.4 mmol/L), imparting a long-lasting bitter taste sensation perceived mainly at the back of the tongue as well as in the throat, and caffeine (8.0 mmol/L), providing a long-lasting bitterness perceived throughout the oral cavity, were used as references. For the training of viscosity, a gelatin solution (0.5% in water) was used; for the training of the activity of mouthfeel enhancement and complexity increase, coined kokumi activity, the panel was asked to compare the gustatory impact of a blank model chicken

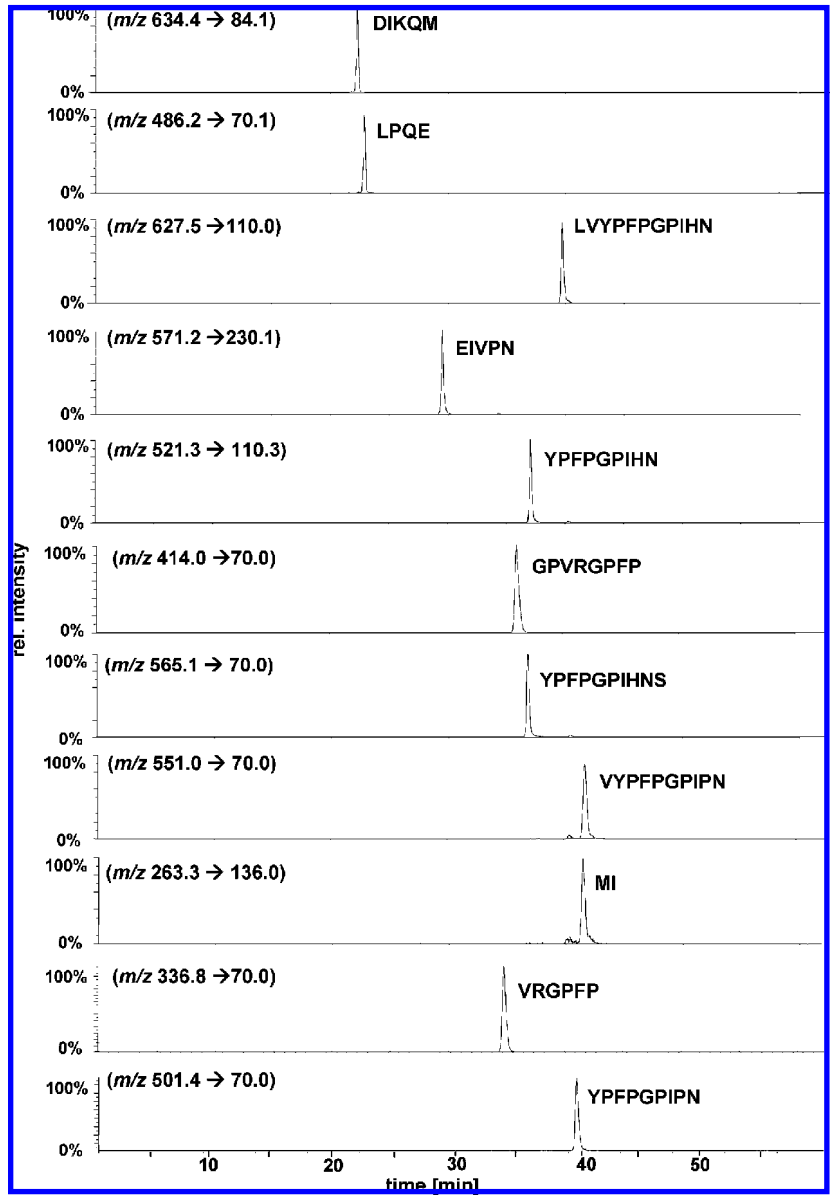

Figure 1. HPLC-MS/MS (MRS) analysis of bitter peptides in the water-soluble extract (WSE) of 44-week-aged Gouda cheese (GC44).

Table 1. Sensory Evaluation of the Water-Soluble Extract (WSE) of Gouda Cheese (GC) Matured for 4 and 44 Weeks, Respectively

	intensities for individual taste qualities[a]			
taste quality	WSE4	GC4	WSE44	GC44
sour	1.0	1.0	2.0	2.0
bitter	1.0	0.5	2.5	3.0
umami	1.0	1.0	2.0	2.0
salty	2.0	1.5	4.5	4.0
sweet	0.5	1.0	0.0	0.5

[a] Intensities were judged on a scale from 0 (not detectable) to 5 (strongly detectable) by 11 trained panelists. The data are given as the mean of triplicates.

broth (control) with a solution of reduced glutathione (5 mmol/L) in chicken broth (both at pH 6.5). The sensory sessions were performed at 21 °C in an air-conditioned room with separated booths in three independent sessions. To prevent cross-modal interactions with odorants, the panelists used nose clips.

Taste Recognition Threshold Concentrations. Threshold concentrations of purified compounds were determined in bottled water adjusted to pH 5.7 with trace amounts of formic acid (1% in water) using triangle tests with ascending concentrations of the stimulus following the procedure reported previously (*9*).

Taste Profile Analysis. Cheese samples GC4 and GC44, cut into cubic pieces (1 × 1 cm), were presented to the trained sensory panel to evaluate the taste qualities bitter, sour, sweet, salty, and umami on an intensity scale from 0 (not detectable) to 5 (strongly detectable). For taste profile analysis of the water-soluble cheese extract, the WSE lyophilisate was dissolved in bottled water in "natural" cheese concentration and the pH value was adjusted to that of the cheese matured for 4 (pH 5.6) and 44 weeks (pH 5.7), respectively, by adding trace amounts of a 1% aqueous solution of formic acid. An aqueous 1:4 dilution of this stock solution was then presented to the sensory panelists, who were asked to rate the intensity of the individual taste qualities on a scale from 0 (not detectable) to 5 (strongly detectable).

Re-engineering of the Nonvolatile Sensometabolome. To reconstitute the sensometabolome of WSE4 and WSE44, respectively, the "natural" concentrations of the tastants summarized in groups II−V (**Table 2**) were taken up in bottled water and the pH value of that solution was adjusted to 5.7 by the addition of trace amounts of a 1% aqueous formic acid solution. The overall taste profile of these basic taste recombinants, rWSE4 and rWSE44, was evaluated by means of taste profile analysis using nose clips. In addition, the extended taste recombinants, $rWSE4^{+}$ and $rESW44^{+}$, were prepared by adding the bitter peptides (group I in **Table 2**) to the corresponding basic taste recombinants.

Taste Omission Experiments. To evaluate the individual taste contribution of distinct taste compounds, partial taste recombinants were

Table 2. Taste Qualities, Taste Recognition Thresholds, Concentrations, and Dose-over-Threshold (DoT) Factors of Nonvolatile Sensometabolites and Mineral Salts in Gouda Cheese

taste compound[a]	TC (μmol/kg)[b]	concn (μmol/kg[c]) (DoT[d]) WSE4	WSE44	WSE44/WSE4 ratio
		Group I: Bitter Peptides		
YPFPGPIHNS	50	5.73 (0.1)	54.52 (1.1)	9.5
YPFPGPIPN	230	23.32 (0.1)	203.91 (0.9)	8.8
YPFPGPIHN	100	3.59 (<0.1)	61.97 (0.6)	17.3
LPQE	600	32.42 (<0.1)	389.41 (0.6)	12.0
DIKQM	600	50.53 (<0.1)	246.17 (0.4)	4.9
VYPFPGPIPN	170	31.39 (0.2)	36.83 (0.2)	1.2
EIVPN	430	9.10 (<0.1)	100.18 (0.2)	11.0
VRGPFP	420	4.16 (<0.1)	52.51 (0.1)	12.6
GPVRGPFP	1180	14.97 (<0.1)	87.89 (0.1)	5.9
SLVYPFPGPIHNS	60	0.93 (<0.1)	1.71 (<0.1)	1.8
LVYPFPGPIHN	80	2.70 (<0.1)	3.38 (<0.1)	1.3
MI	420	nd[s] (nd)	<0.1 (nd)	nd
		Group II: Bitter-Tasting Minerals and Amino Acids		
calcium	6200[e,p]	83730 (13.5)	92754 (15.0)	1.1
magnesium	6400[e,p]	9741 (1.5)	9935 (1.6)	1.0
L-leucine	11000[k]	13104 (1.2)	47473 (4.3)	3.6
L-tyrosine	4000[k]	3393 (0.9)	11393 (2.9)	3.4
L-isoleucine	10000[k]	4314 (0.4)	22848 (203)	5.3
L-tryptophan	4000[k]	638 (0.2)	7494 (1.9)	11.7
L-lysine	80000[k]	13828 (0.2)	56744 (0.7)	4.1
L-valine	30000	3859 (0.1)	17423 (0.6)	4.5
L-phenylalanine	45000[k]	5252 (0.1)	15503 (0.3)	2.9
L-arginine	75000[k]	5405 (<0.1)	13699 (0.2)	2.5
L-histidine	45000[k]	1209 (<0.1)	2722 (<0.1)	2.2
		Group III: Umami-like Compounds		
L-glutamic acid	1100	10392 (9.4)	41466 (37.7)	3.9
L-aspartic acid	600	1225 (2.1)	4698 (7.8)	3.8
L-glutamine	50000	7456 (0.1)	14022 (0.3)	1.9
L-asparagine	50000	9941 (0.2)	22528 (0.5)	2.3
		Group IV: Sour/Salty Compounds		
sodium	3900[p]	170006 (43.6)	361862 (92.8)	2.1
potassium	13000[p]	14223 (1.1)	16242 (1.2)	1.1
chloride	3900[q]	176423 (45.1)	387117 (99.2)	1.2
lactate	11890[f,q]	(4.7)	(5.7)	
	23770[g,q]	(4.7)	(5.7)	
phosphate	5000[h,q]	22368 (4.5)	19927 (4.0)	0.9
acetate	3100[l]	15993 (5.2)	22037 (7.1)	1.4
		Group V: Sweet-Tasting Compounds		
L-methionine	5000[j]	1848 (0.4)	10513 (2.1)	5.7
L-alanine	12000[j]	2949 (0.2)	12335 (1.0)	4.2
L-serine	25000[j]	2248 (0.1)	10645 (0.4)	4.7
glycine	25000[j]	1459 (0.1)	7217 (0.3)	4.9
L-proline	25000[j]	1991 (0.1)	7286 (0.3)	3.7
L-threonine	35000[j]	1757 (0.1)	9214 (0.3)	5.2
		Group VI: Fatty Acids[r]		
butyric acid	4000[l,m]	131 (<0.1)	670 (<0.1	5.1
caproic acid	3400[l,m]	23 (<0.1)	109 (<0.1)	4.7
caprylic acid	5200[l,n]	24 (<0.1)	45 (<0.1)	1.9
capric acid	15500[l,n]	98 (<0.1)	136 (<0.1)	1.4
lauric acid	nd	130 (nd)	178 (nd)	1.3
myristic acid	nd	256 (nd)	391 (nd)	1.5
palmitic acid	nd	523 (nd)	829 (nd)	1.6
stearic acid	nd	202 (nd)	266 (nd)	1.3
oleic acid	670[l,n]/2650[l,o]	474 (0.7/<0.1)	728 (1.1/<0.1)	1.5

[a] Taste-active compounds were determined in the water-soluble extract (WSE), if not stated otherwise. [b] Taste threshold concentrations (TC) were determined in bottled water by means of a triangle test and are given as the mean of triplicates or taken from the literature. [c] Concentration (μmol/kg) in cheese. [d] Dose-over-threshold (DoT) factor is calculated as the ratio of concentration and taste threshold. [e] Taste threshold concentration for bitter taste. [f] Taste threshold concentration for sour/salty taste. [g] Taste threshold concentration for umami taste. [h] Value taken from ref *21*. [i] Value taken from ref *26*. [j] Value taken from ref *27*. [k] Value taken from ref *28*. [l] Taste threshold determined in the emulsifier Emultop (0.02% in water). [m] Taste threshold for sourness. [n] Threshold for astringent mouthfeel. [o] Threshold for fatty mouth-coating. [p] Threshold concentration determined for the corresponding chloride salt. [q] Threshold concentration determined for the corresponding sodium salt. [r] Fatty acids determination in cheese. [s] nd, not determined.

prepared by omitting either individual tastant groups or single taste compounds from the total taste recombinant, WSE44. Each of the partial recombinants was presented to the panelists in comparison with the total taste recombinant using a triangle test. Panelists were asked to evaluate whether the solutions were identical in the overall taste or not. Those panelists who detected the odd sample correctly were asked to rate the intensity of the given taste descriptors of that sample on a scale from 0 (not detectable) to 5 (strongly detectable).

Tastant Spiking of Cheese Sample GC4. The young cheese sample, GC4, was spiked with the taste compounds in amounts required to reach

the concentrations determined in the matured cheese sample, GC44. To achieve this, a tastant cocktail containing the difference in the concentration of each compound between samples GC4 and GC44 (**Table 1**) was suspended with water (1.0 mL) and intimately mixed with a sample (20 g) of GC4. After homogenization in a mortar, the resulting solid cheese mixture was wrapped in cling film, pressed into shape, and physically matured overnight at 6 °C. This spiked cheese sample, $GC4^+$, and, in addition, the 4- and 44-week authentic cheeses, both treated in the same way as described above without any addition of tastants, were then evaluated by means of the taste profile analysis.

Liquid Chromatography−Mass Spectrometry (LC-MS/MS). LC-MS/MS measurements were acquired on an API 4000 Q-Trap LC-MS/MS system (Applied Biosystems Sciex Instruments, Darmstadt, Germany) connected to an Agilent 1100 HPLC system operating in the positive electrospray ionization mode. Ion spray voltage was set at 5500 eV, source temperature was 300 °C, and nitrogen served as curtain gas (20 psi).

RESULTS AND DISCUSSION

To gain a first insight into the influence of the degree of maturation on the taste of Gouda cheese, the cheese samples GC4 and GC44 matured for 4 and 44 weeks, respectively, were presented to 11 trained sensory panelists who were asked to judge the intensity of the taste qualities salty, sour, bitter, umami, and sweet on a linear scale from 0 (not detectable) to 5 (strongly detectable). As given in **Table 1**, the sourness and umami taste and, in particular, saltiness and bitterness were evaluated with significantly higher intensities in the GC44 sample when compared to GC4. Sweetness of GC4 and GC44 was evaluated with an intensity of 1.0 and 0.5, respectively.

As the taste-active compounds in Gouda cheese have been recently shown to be water-soluble (*2*), the nonvolatile sensometabolome of the cheese samples was comprehensively extracted from the Gouda cheese matrix and separated from a protein fraction as well as the milk fat. The water-soluble extracts WSE4 and WSE44 obtained from the cheese samples GC4 and GC44, respectively, were taken up in bottled water at the same concentration (on mass basis) as present in the cheese, adjusted to the pH value of the cheese with trace amounts of aqueous formic acid. The extracts were then diluted 1:4 with water prior to taste profile analysis because they were otherwise too strong for a sensory evaluation. The highest intensity were found for saltiness (4.5), followed by bitter taste (2.5), umami taste (2.0), and sourness (2.0) in WSE44, whereas the taste intensities of the WSE4 solution were comparatively weak (**Table 1**). The matrix differences between the cheeses samples and their aqueous extracts did not allow a direct comparison of the taste intensities, but the observation that bitterness was perceived as weaker and saltiness as stronger in the water-soluble extracts than in the cheese confirms previous data published in the literature (*15, 16*).

The following experiments were performed to investigate the sensory contribution of proteinogenic amino acids, organic acids, fatty acids, soluble carbohydrates, biogenic amines, and minerals, as well as the recently identified bitter peptides (*9*) to the taste of Gouda cheese. To demonstrate a correlation between single taste compounds and individual taste qualities, we aimed at re-engineering the nonvolatile cheese sensometabolome by preparing a cocktail containing all of these putative taste compounds in their "natural" concentrations and to compare the taste profile of this biomimetic taste recombinant to that of the WSE prepared from authentic cheese. To achieve this, first, the whole spectrum of basic taste compounds as well as the bitter peptides was quantified in the cheese samples and the taste recognition thresholds were determined. Organic acids, amino acids, carbohydrates, and minerals were determined by means of ion chromatography, and peptides were analyzed by means of HPLC-MS/MS using the multiple monitoring mode as exemplified in **Figure 1** for the sample GC44. To consider the decrease of the water content during cheese maturation and to perform the taste re-engineering experiments, dry matter contents of 57.3 g/100 g (GC4) and 64.1 g/100 g (GC44) as well as fat contents of 29.5% (GC4) and 32.5% (GC44) were determined.

Concentrations and Dose-over-Threshold (DoT) Factors of Nonvolatile Sensometabolites. Free amino acids, soluble carbohydrates, and the cations of sodium, calcium, potassium, and magnesium, as well as the anions lactate, phosphate, and chloride, were identified and quantified in WSE4 and WSE44 by means of ion chromatography. Free fatty acids were determined in cheese samples by means of gas chromatography, and biogenic amines were determined as their corresponding dansyl derivatives by means of HPLC (**Table 2**). In addition, 12 bitter-tasting peptides (**Table 2**), recently identified in Gouda cheese (*9*), were quantitatively determined in WSE4 and WSE44, respectively. From these putative taste compounds 19 amino acids, 4 cations, 4 anions, and 9 fatty acids were identified and quantified, whereas not even trace amounts of biogenic amines and soluble carbohydrates were detectable. In addition, the taste recognition threshold concentrations of these compounds were determined, and a the DoT factor was calculated for each compound from the ratio of the concentration and the threshold concentration (*17*). As we aimed to elucidate the key metabolites for each individual taste quality, the single taste compounds were grouped into six classes differing in their taste qualities (**Table 2**).

The bitter peptides were classified into the bitter-tasting group I (**Table 2**). Among these, the concentrations of YPFPGPIHN, VRGPFP, LPQE, and EIVPN were >11 times higher in WSE44 than in WSE4, thus demonstrating that the increase of the maturation time from 4 to 44 weeks induced a strong release of these bitter peptides from caseins. However, only the concentrations of YPFPGPIHNS and YPFPGPIPN in WSE44 were found to be close to their recognition thresholds, followed by YPFPGPIHN, LPQE, and DIKQM, the concentrations of which were 2−3 times below their taste threshold concentration. All of the other bitter peptides were evaluated with DoT factors of <0.2 in WSE44. In WSE4, not even a single peptide exceeded its threshold, thus giving strong evidence that peptides do not play any role for the typical taste of a young 4-week-matured Gouda cheese (**Table 2**).

The bitter tasting group II consisted of the divalent cations magnesium and calcium as well as the amino acids L-leucine, L-tyrosine, L-isoleucine, L-tryptophan, L-lysine, L-valine, L-phenylalanine, L-arginine, and L-histidine (**Table 2**). By far the highest DoT factor of 15.0 was calculated for calcium chloride in WSE44, closely followed by a value of 13.5 found for the WSE4 sample. Among the bitter amino acids, L-leucine, L-tyrosine, L-isoleucine, and L-tryptophan were evaluated with the highest DoT factors of 4.3, 2.9, 2.3, and 1.9, respectively, in WSE44, whereas in the WSE4 sample only L-leucine reached its threshold concentration. Independent of the cheese sample, all of the other bitter amino acids were significantly below their threshold concentrations. Comparison of the concentrations of bitter amino acids in both samples confirmed that the concentration of these bitter compounds is increasing during cheese maturation; for example, 11.7 and 5.3 times higher amounts of L-tryptophan and L-isoleucine were found in WSE44 when compared to WSE4.

The umami-like-tasting group III consisted of the amino acids L-glutamic acid, L-aspartic acid, L-glutamine, and L-asparagine

(**Table 2**). Whereas the concentrations of L-glutamic acid and L-aspartic acid were about 4-fold higher in WSE44 than in WSE4, the amounts of the corresponding amides increased only by a factor of 2 upon cheese ripening. Calculation of DoT factors revealed high values of 37.7 and 9.4 for L-glutamate in WSE44 and WSE4, respectively, whereas the corresponding amides were present below their threshold concentrations.

Group IV, summarizing the salty and sour compounds, contains the cations sodium and potassium, as well as the anions lactate, phosphate, chloride, and acetate (**Table 2**). Quantitative analyses of WSE44 revealed by far the highest concentrations of 447 and 387 mmol/kg for sodium and chloride, respectively, whereas the sample WSE4 contained about 50% of the sodium chloride but the same amount of potassium and phosphate as found for WSE44. This might be explained by the migration of NaCl from the rind into the cheese during ripening. High DoT factors of 92.8 and 43.6 were calculated for sodium, evaluated as chloride, in WSE44 and WSE4, respectively. Also, lactate, acetate, and phosphate were significantly above their threshold concentrations in WSE4 and WSE44 and can be expected to contribute to the typical taste of both cheese samples.

The sweet-tasting group V consisted of the amino acids L-methionine, L-alanine, L-serine, glycine, L-proline, and L-threonine (**Table 2**). In WSE44, only L-methionine and L-alanine were present in concentrations above or at their taste thresholds and might contribute to the sweetness of the 44-week-ripened cheese. In comparison, none of these amino acids were evaluated with DoT factors above 1 in the WSE4 sample.

The fatty acids C_4-C_{18} were combined in group VI (**Table 2**). Taste thresholds were determined from five of the nine fatty acids in an aqueous emulsion at room temperature. Due to their low solubility, lauric acid, myristic acid, palmitic acid, and stearic acid were not evaluated in single solutions. Oleic acid was found to induce an astringent mouthfeel above 670 μmol/kg as well as a fatty mouth-coating above 2650 μmol/kg. Butyric and caproic acid elicit sourness, whereas capric and caprylic acid showed a rough astringent sensation. All of the fatty acids tested exhibited rather high taste thresholds, being far above their concentrations in Gouda cheese. As an emulsion containing the fatty acids C_4-C_{18} in their "natural" cheese concentrations did not impart any taste sensation, it was concluded that these fatty acids do not play any role for the Gouda cheese taste and, as a consequence, the fatty acids were not considered any more in the following taste re-engineering experiments.

Re-engineering of the Water-Soluble, Nonvolatile Sensometabolome of Gouda Cheese. To confirm the results of the instrumental analysis and to check whether the compounds already identified can create the typical taste of the WSE and the cheese, respectively, taste re-engineering experiments were performed in the following.

First, aqueous basic taste recombinants (rWSE4/44) of the water-soluble extract of both cheeses were prepared containing all of the compounds summarized in the groups II−V (**Table 2**), each at the concentration determined in WSE, and the taste profiles of these basic taste recombinants were compared with that of the WSEs. To achieve this, the taste compounds were dissolved in bottled water in their "natural" concentrations, and the pH value was adjusted to that of the WSE by adding some trace amounts of formic acid. After 1:4 dilution of the recombinants as well as the WSEs with bottled water, the trained sensory panel was then asked to evaluate the taste profile of these samples by scoring the taste descriptors, given in **Table 3**, on a scale from 0 (not detectable) to 5 (strongly detectable).

Sensory evaluation of the basic taste recombinant rWSE4 as well as the authentic WSE4 revealed the highest intensity for saltiness evaluated with a score of 1.9 or 2.0, respectively (**Table 3**). Also, sourness and bitterness of the recombinant, judged with an intensity of 0.9, were rather close to that of the WSE4 evaluated with a score of 1.0, and umami and sweet taste matched completely. The taste profile of rWSE4 was very close to that of the WSE4, and the trained panelists concluded that the typical taste of the freshly prepared WSE of the 4-week-matured cheese could be completely reconstituted by the blend of the compounds present in groups II−V (**Table 2**).

Table 3. Sensory Evaluation of Water-Soluble Extract (WSE), Basic Recombinants (rWSE), and Basic Recombinants Including Bitter Peptides (rWSE$^+$) of Gouda Cheese Matured for 4 and 44 Weeks, Respectively

	intensities for individual taste qualities[a]					
taste quality	WSE4	rWSE4	rWSE4$^+$	WSE44	rWSE44	rWSE44$^+$
sour	1.0	0.9	0.9	2.0	1.6	1.6
bitter	1.0	0.9	0.9	2.5	2.4	2.5[b]
umami	1.0	1.0	1.0	2.0	1.7	1.7
salty	2.0	1.9	1.9	4.5	4.4	4.4
sweet	0.5	0.5	0.5	0	0	0

[a] Intensities were judged by the panel on a scale from 0 (not detectable) to 5 (strongly detectable) and are given as the mean of triplicates. The values of the individual panelists determined in three independent sessions differed more than ±0.3 unit. [b] The bitterness was perceived as more long-lasting and was perceived predominantly at the back of the tongue.

The basic recombinant rWSE44 was evaluated with high intensities for saltiness (4.4) and bitterness (2.4), being rather close to the scores 4.5 and 2.5, respectively, found for the authentic extract WSE44 (**Table 3**). Whereas the sour as well as the umami taste quality showed an intensity of 2.0 in WSE44, the corresponding recombinant rWSE44 was judged with values of 1.6 and 1.7 in slightly lower intensities. The sensory panel reported that, in particular, the quality and impact of the sour sensation perceived in WSE44 was not fully reconstituted in rWSE44. This acidic punch might possibly be due to sour-tasting peptides (*18, 19*) and will be investigated in future work. In addition, the sensory panelists described some differences in body and mouthfeel between authentic WSE and corresponding recombinants. Studies on the molecules inducing mouthfeel in cheese will be published separately.

To investigate the sensory impact of the recently identified bitter peptides (*9*), the amounts of the 12 peptides determined in WSE4 and WSE44 were added to the taste recombinants to give the total recombinants rWSE4$^+$ and rWSE44$^+$. Although the addition of the peptide mix to rWSE4 could not be detected by the sensory panel in a triangle test, the higher amounts of these peptides present in rWSE44$^+$ were detectable by the panel when compared to rWSE44, which lacked tastant group I (**Table 3**). Although the taste profile analysis did not reveal any significant increase of bitter intensity in rWSE44$^+$ (2.4→2.5), the sensory panel reported that the bitterness quality was influenced by the peptides, shifting the bitterness perception more to the back of the tongue and being more long-lasting (**Table 3**).

Spiking Experiments in Cheese. To further confirm the importance of the identified taste molecules for the typical taste profile of the 44-week-matured cheese and to investigate the influence of the cheese matrix in taste perception, the aim of the following experiment was to perform a taste re-engineering experiment in the cheese matrix. Because incorporation of the taste compounds in an artificial cheese matrix produced either by homogenizing the protein pellet and the milk fat obtained during cheese extraction with water (*20*) or by mixing water, fat, and freeze-dried mozzarella as reported in the literature (*21*)

Table 4. Sensory Evaluation of 4-(GC4) and 44-Week-Matured Cheese (GC44) as well as $GC4^+$ Spiked with the Difference in the Concentration of Each Taste Compounds between GC44 and GC4

	intensities for individual taste qualities[a]		
taste quality	GC4	$GC4^+$	GC44
sour	1.0	1.8	2.0
bitter	0.5	2.9	3.0
umami	1.0	2.0	2.0
salty	1.5	3.8	4.0
sweet	1.0	0.5	0.5

[a] Intensities were judged by the panel on a scale from 0 (not detectable) to 5 (strongly detectable) and are given as the mean of triplicates. The values of the individual panelists determined in three independent sessions differed more than ±0.3 unit.

did not give an acceptable imitate of the Gouda cheese matrix, the 4-week-matured Gouda cheese (GC4) was used as a suitable matrix. The difference in the concentration of each tastant between the GC4 and GC44 samples was calculated, and a cocktail of these compounds, dissolved in a small amount of water, was added to a defined portion of GC4. After homogenization, this spiked cheese sample, $GC4^+$, was compressed in cling foil, physically matured for 24 h at 6 °C, and then sensorially evaluated in comparison to GC4 and GC44, which were treated in the same way but without the addition of the tastant cocktail (**Table 4**). The taste profile of the spiked Gouda sample $GC4^+$ matched rather well with the authentic cheese matured for 44 weeks (GC44), and the sensory panel reported that $GC4^+$ exhibited the typical taste profile of a matured Gouda cheese. Whereas the intensities of the umami taste (2.0) and sweetness (0.5) were identical in $GC4^+$ and GC44, bitterness (2.9), saltiness (3.8), and sourness (1.8) were perceived in slightly lower intensities than the authentic cheese sample GC44 (**Table 4**). On the basis of these findings, it can be concluded that the basic taste compounds driving the taste of matured Gouda cheese have been successfully characterized. Whereas $GC4^+$ and GC44 were rather similar in their basic taste qualities, the sensory panel picked up a difference in mouthfeel, body, and complexity as already found for the aqueous solutions of WSE44 and $rWSE^+$. Studies on the components responsible for that effect will be published separately.

Taste Omission Experiments. The above sensory studies on the recombinants rWSE44 and $rWSE44^+$ revealed that the bitter peptides do not significantly increase the bitter intensity of the cheese, but do influence the bitter taste quality perceived (**Table 3**). To investigate the taste contribution and relevance of the other taste compounds as well as interactions between different tastant groups, taste omission experiments were performed with rWSE44. To achieve this, individual taste recombinants, lacking either in one tastant group or in one or more individual taste compounds, were evaluated by means of triangle tests using two samples of the complete taste recombinant (rWSE44) as the control. Those panelists who detected any difference in the taste profile were asked to rate the intensity of the key taste descriptors salty, umami, bitter, sour, and sweet on a five-point scale.

First, single-tastant groups were omitted from the basic recombinant rWSE44, and the sensory impact of this tastant omission was evaluated by a taste profile analysis (**Table 5**). The omission of group II (**Table 2**), containing the bitter amino acids as well as $MgCl_2$ and $CaCl_2$, resulted in a complete loss of bitterness as well as a strong decrease of the intensity of saltiness (4.4→3.4). In addition, the umami note and sourness were evaluated with 0.2 unit lower intensity when compared to rWSE44. Additional partial recombinants were prepared, lacking in either the bitter-tasting amino acids or the inorganic salts. Omission of $MgCl_2$ and $CaCl_2$ induced a stronger decrease of bitterness (2.4→0.2) than the omission of the bitter amino acids (2.4→1.9), thus demonstrating these inorganic salts and, in particular, calcium ions as key drivers for bitterness. The importance of the counterion chloride for the taste impact of calcium and magnesium ions could be excluded by another experiment in which hydrochloric acid was added to compensate for the losses of chloride when $MgCl_2$ and $CaCl_2$ were omitted. This omission of calcium and magnesium ions did induce a strong decrease of bitterness (2.4→0.2), although the concentration of chloride was kept constant (**Table 5**). These data confirm earlier reports (*7*) showing a correlation of the bitterness of goat cheese with the amounts of calcium and magnesium ions. To a minor extent, the amino acids also contributed to Gouda bitterness, because the omission of all nine bitter-tasting amino acids induced a decrease of bitterness from 2.4 to 1.9 and a change in bitter quality shifting to an unpleasant, metallic type of bitterness. Interestingly, also the omission of the amino acids evaluated with DoT factors below 1.0 (**Table 2**) was sensorially detectable and lowered the bitterness of the recombinant by 0.3 unit, thus indicating an additive effect of these amino acids. As the omission of these amino acids induced also a slight decrease of saltiness, and because L-arginine can function as a salt enhancer and ENaC activator (*22*), the single amino acid, L-arginine, was omitted from the recombinant prior to sensory analysis. Six of seven panelists detected the recombinant lacking L-arginine in a triangle test and evaluated this sample by 0.6 unit less salty when compared to the recombinant (rWSE44) containing the basic amino acid (**Table 5**). These data clearly demonstrate that L-arginine enhances the salty taste of the cheese.

In a second set of experiments, the umami-like-tasting amino acids of tastant group III were omitted from rWSE44 (**Table 5**). The panelists judged the partial recombinant without all components of group III only with a very low umami intensity (0.2) and an increased bitterness (2.4→3.0), with a shift toward a metallic bitter quality. The same effects were observed when only the L-glutamate was omitted, thus being well in line with the suppressive effect of L-glutamate on bitterness reported earlier (*23*). Omission of L-glutamine and L-asparagine induced just a minor drop of the umami intensity from 1.7 to 1.3 and a slight increase of bitterness by 0.2 unit. These data clearly demonstrate L-glutamic acid as the key umami contributor in Gouda cheese.

In a third set of experiments, the sour- and salty-tasting compounds of tastant group IV were omitted from rWSE44 (**Table 5**). The saltiness of the partial recombinant without all components of group IV was perceived with 3.5 units lower intensity when compared to rWSE44. The remaining saltiness (0.9) can be expected to be due to components present in the bitter group II, namely, $CaCl_2$ and $MgCl_2$, as well as L-arginine, recently shown to function as a salt enhancer and ENaC activator (*22*). In addition, the intensity of sourness and umami taste was lowered from 1.6 to 0.3 and from 1.7 to 0.7, respectively. The decrease in umami intensity by the omission of sodium ions in the recombinant is well in line with the long-known fact that the monosodium salt of L-glutamate shows higher umami activity than the corresponding free amino acid. Collaborating with the bitterness suppression of monosodium L-glutamate (*23*), the decrease in umami intensity induced by the omission of sodium ions also resulted in increased bitterness (2.4→3.3). Similar effects were found when only NaCl was omitted from

Table 5. Influence of the Omission of Tastant Groups or Individual Taste Compounds on the Taste Profile of the Recombinant rWSE44

omission of	no.[a]	description of taste difference [b] (change in intensity)
group II (bitter)[c]	7	loss of bitterness (2.4→0.0), slightly less umami (1.7→1.5) and sour (1.6→1.4), less salty (4.4→3.4)
$MgCl_2$, $CaCl_2$	7	loss of bitterness (2.4→0.2), slightly increased saltiness (4.4→4.9), less sour (1.6→1.0)
bitter amino acids	6	less bitter (2.4→1.9), early bitterness lacking, metallic bitter quality more umami (1.7→1.8) and sour (1.6→1.8)
bitter amino acids (DoT < 1.0)	5	less bitter (2.4→2.1), less salty (4.4→4.1)
L-arginine	6	less salty (4.4→3.8)
group III (umami)[c]	7	loss of umami taste (1.7→0.2), increased bitterness (2.4→3.0) and increase in metallic bitter quality, less salty (4.4→3.8) and sour (1.6→1.3)
L-glutamic acid	7	loss of umami taste (1.7→0.2), increased bitterness (2.4→3.0) and increase in metallic bitter quality, less salty (4.4→4.0) and sour (1.6→1.4)
L-glutamine, L-asparagine	6	less umami (1.7→1.3), more bitter (2.4→2.6)
group IV (sour/salty)[d]	7	decrease of saltiness (4.4→0.9), sourness (1.6→0.3), and umami (1.7→0.7), increased bitterness (2.4→3.3)
sodium chloride	7	decreased saltiness (4.4→0.9), increased bitterness (2.4→3.0) and sourness (1.6→2.0), less umami (1.7→0.5)
sodium phosphate	6	less salty (4.4→3.9) and sour (1.6→1.4)
sodium lactate	6	less sour (1.6→1.2) and umami (1.7→1.5), slightly more bitter (2.4→2.5)
group V (sweet)[d]	6	slightly more bitter (2.4→2.5) and sour (1.6→1.7), less body and mouthfeel
sweet amino acids (Dot <1.0)	5	less mouthfeel

[a] Number of individuals of seven panelists detecting the recombinant lacking certain tastants by means of a triangle test. [b] Partial recombinants lacking certain tastants were presented to the panel by means of a triangle test. If sample was correctly chosen, differences in taste intensities were evaluated on a scale from 0 (not detectable) to 5 (strongly detectable) and are given as the mean of triplicates. The values of the individual panelists determined in three independent sessions differed more than ±0.4 unit. [c] Components of each group are listed ion **Table 2**. [d] To compensate for the loss of chloride when $MgCl_2$ and $CaCl_2$ were omitted, chloride ions were introduced by the addition of hydrochloric acid.

rWSE44, thus confirming literature data on the bitter suppressive activity of NaCl (*24, 25*). Interestingly, the extent of the bitterness increase (2.4→3.0) was lower than that observed upon omission of the entire group IV, thus suggesting another bitterness suppressor to be present in group IV. Whereas the omission of sodium phosphate induced a decrease in saltiness (4.4→3.9), the omission of sodium lactate resulted in less sourness, lower umami intensity, and a slightly increased bitterness (**Table 5**). The omission of NaCl intensified the perception of sourness, thus indicating that the sour taste of the cheese is due to lactic acid and hydrogen phosphate and that NaCl exhibits a suppressive effect on sourness perception.

In the last set of omission experiments, the partial recombinants were lacking the sweet-tasting amino acids of group V (**Table 5**). Six of seven panelists were able to pick up a difference between the partial recombinant without all components of group V and rWSE44. The panel described that difference with a slight increase in sourness and bitterness as well as a decrease in body and mouthfeel. The omission of only the sweet-tasting amino acids evaluated with a DoT factor <1.0 induced a decrease in mouthfeel versus rWSE44, thus demonstrating that these sweet-tasting amino acids contribute to the overall taste perception of the cheese, even at their subthreshold concentrations.

In conclusion, quantitative studies, followed by taste reengineering and omission experiments, on the nonvolatile sensometabolome of Gouda cheese revealed that its bitterness is induced by $CaCl_2$ and $MgCl_2$, as well as by bitter-tasting free amino acids. In addition, bitter peptides were found to influence more the bitterness quality rather than the bitterness intensity of Gouda cheese. The DoT factors determined for the individual bitter peptides gave strong evidence that their sensory contribution is mainly due to YPFPGPIHNS, YPFPGPIPN, and YPFPGPIHN, assigned to the casein sequences β-CN(60−69) and β-CN(60−68), respectively, as well as LPQE released from α_{s1}-CN(11−14) (*9*). Umami taste was found to be due to the key metabolites monosodium L-glutamate and sodium lactate. Saltiness was induced by sodium chloride and sodium phosphate and was shown to be significantly enhanced by L-arginine. In addition, lactic acid and hydrogen phosphate play key roles in the sourness of Gouda cheese. After the basic taste qualities had been defined on a molecular level, studies on the sensometabolites contributing to mouthfeel differences between the 4- and 44-week-matured Gouda cheese are currently under investigation and will be published separately.

ACKNOWLEDGMENT

We thank Friesland Foods for providing cheese samples and for financial support.

LITERATURE CITED

(1) Fox, P. F.; McSweeney, P. L.; Cogan, T. M.; Guinee, T. P. Sensory character of cheese and its evaluation. In *Cheese—Chemistry, Physics and Microbiology—General Aspects*, 3rd ed.; Elsevier Academic Press: London, U.K., 2004; pp 455−487.

(2) Biede, S. L.; Hammond, E. G. Swiss cheese flavor: II. Organoleptic analysis. *J. Dairy Sci.* **1979**, *62*, 238–248.

(3) Aston, J. W.; Creamer, L. K. Contribution of the components of the water-soluble fraction to the flavor of Cheddar cheese. *N. Z. J. Dairy Sci. Technol.* **1986**, *21*, 229–248.

(4) Roudot-Algaron, F.; Kerhoas, L.; Le Bars, D.; Einhorn, J.; Gripon, J. C. Isolation of γ-glutamyl peptides from Comté cheese. *J. Dairy Sci* **1994**, *77*, 1161–1166.

(5) Sommerer, N.; Garem, A.; Molle, D.; Septier, C.; Le Quere, J. L.; Salles, C. Isolation of a peptidic fraction from the goat cheese water-soluble extract by nanofiltration for sensory evaluation studies. *Dev. Food Sci.* **1998**, *40*, 207–217.

(6) Molina, E.; Ramos, M.; Alonso, L.; Lopez-Fandino, R. Contribution of low molecular weight water soluble compounds to the taste of cheeses made of cows', ewes' and goats' milk. *Int. Dairy J.* **1999**, *9*, 613–621.

(7) Engel, E.; Nicklaus, S.; Garem, A.; Septier, C.; Salles, C.; Le Quere, J. L. Taste active compounds in a goat cheese water-soluble extract. 1. Development and sensory validation of a model water-soluble extract. *J. Agric. Food Chem.* **2000**, *48*, 4252–4259.

(8) McSweeney, P. L. H. The flavor of milk and dairy products: III. Cheese: taste. *Int. J. Dairy Technol.* **1997**, *50*, 123–128.

(9) Tolestede, S.; Hofmann, T. Sensomics mapping and identification of the key bitter metabolites in Gouda cheese. *J. Agric. Food Chem.* **2008**, *56*, 2795–2804.
(10) Collomb, M.; Malke, P.; Spahni, M.; Sieber, R.; Bütikofer, U. Gas chromatographic determination of free fatty acids in cheese: precision of the method and influence of seasons on lipolysis in different Swiss cheeses. *Mitt. Gebiete Lebensm. Hyg.* **2003**, *94*, 212–229.
(11) Bütikofer, U.; Fuchs, D.; Hurni, D.; Bosset, J. O. On the determination of biogenic amines in cheese (in German). *Mitt. Gebiete Lebensm. Hyg.* **1990**, *81*, 120–133.
(12) Moret, S.; Lanfranco, S. C. High-performance liquid chromatographic evaluation of biogenic amines in foods—an analysis of different methods of sample preparation in relation to food characteristics. *J. Chromatogr., A* **1996**, *729*, 363–369.
(13) Analysis of foods—determination of the dry weight of cheese (reference method). Official collection of analysis methods § 64 LFGB (in German), 1984, L 03.00-9.
(14) Schulte, E. Micromethod for the rapid gravimetric analysis of the fat content in food after acidic hydrolysis (in German). *Dtsch. Lebensm.-Rundsch.* **2001**, *97*, 85–89.
(15) Engel, E.; Tournier, C.; Salles, C.; Le Quéré, J. L. Evolution of the composition of a selected bitter camembert cheese during ripening: release and migration of taste-active compounds. *J. Agric. Food Chem.* **2001**, *49*, 2940–2947.
(16) Engel, E.; Septier, C.; Leconte, N.; Salles, C.; Le Quere, J.-L. Determination of taste-active compounds of a bitter Camembert cheese by omission tests. *J. Dairy Res.* **2001**, *68*, 675–688.
(17) Scharbert, S.; Hofmann, T. Molecular definition of black tea taste by means of quantitative studies, taste reconstitution, and omission experiments. *J. Agric. Food Chem.* **2005**, *53*, 5377–5384.
(18) Roudot-Algaron, F. The taste of amino acids, peptides and proteins: examples of tasty peptides in casein hydrolyzates. *Lait* **1996**, *76*, 313–348.
(19) Nishimura, T.; Kato, H. Taste of free amino acids and peptides. *Food Rev. Int.* **1988**, *4*, 39–58.
(20) Engel, E.; Nicklaus, S.; Septier, C.; Salles, C.; Le Quéré, J. L. Evolution of the taste of a bitter Camembert cheese during ripening: characterization of a matrix effect. *J. Agric. Food Chem.* **2001**, *49*, 2930–2939.
(21) Warmke, R. Identification of taste compounds in Emmental cheese and determination of concentration changes during ripening (in German). Ph.D. thesis, Technische Universität München, 1997.
(22) Stähler, F.; Riedel, K.; Demgensky, S.; Neumann, K.; Dunkel, A.; Täubert, A.; Raab, B.; Beherens, M.; Raguse, J.-D.; Hofmann, T.; Meyerhof, W. A role of the epithelial sodium channel in human salt taste transduction? *Chemosens. Percept.* **2008**, in press.
(23) Warmke, R.; Belitz, H. D. Influence of glutamic acid on the bitter taste of various compounds. *Z. Lebensm.-Unters. Forsch.* **1993**, *197*, 132–133.
(24) Breslin, P. A. S.; Beauchamp, G. K. Suppression of bitterness by sodium: variation among bitter taste stimuli. *Chem. Sens.* **1995**, *20*, 609–623.
(25) Engel, E.; Nicklaus, S.; Septier, C.; Salles, C.; Le Quere, J. L. Taste active compounds in a goat cheese water-soluble extract. 2. Determination of the relative impact of water-soluble extract components on its taste using omission tests. *J. Agric. Food Chem.* **2000**, *48*, 4260–4267.
(26) Wieser, H.; Jugel, H.; Belitz, H. D. Relationships between structure and sweet taste of amino acids. *Z. Lebensm.-Unters.-Forsch.* **1977**, *164*, 277–282.
(27) Warendorf, T. Taste-active compounds in bouillon (in German). Ph.D. thesis, Technische Universität München, 1991.
(28) Wieser, H.; Belitz, H. D. Relations between structure and bitter taste of amino acids and peptides. I. Amino acids and related compounds. *Z. Lebensm.-Unters.-Forsch.* **1975**, *159*, 65–72.

Received for review February 22, 2008. Revised manuscript received April 7, 2008. Accepted April 7, 2008. We thank Friesland Foods for financial support.

JF800552N