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Sensory-Directed Identification of β-Alanyl Dipeptides as Contributors to the Thick-Sour and White-Meaty Orosensation Induced by Chicken Broth

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Sensory-directed fractionation of a double-boiled chicken broth using ultrafiltration, gel permeation chromatography, PFPP-HPLC, and HILIC combined with analytical sensory techniques led to the identification of β-alanyl-*N*-methyl-L-histidine, β-alanyl-L-histidine, and the previously unreported β-alanylglycine as the key contributors to the thick-sour orosensation and typical white-meaty character of chicken broth. Quantitative analysis, followed by taste recombination and omission experiments, revealed for the first time that, when present together with L-glutamic acid and sodium and/or potassium ions, subthreshold concentrations of these β-alanyl peptides enhance the typical thick-sour orosensation and white-meaty character known for poultry meat, although these taste-modulatory peptides exhibited only a faint sour and slightly astringent intrinsic taste when tasted individually.

KEYWORDS: Chicken broth; taste; taste modulation; β-alanyl peptides; β-alanylglycine; carnosine; anserine; taste dilution analysis

INTRODUCTION

Due to its desirable white-meaty and thick-sour taste profile, chicken broth is highly appreciated as a sapid base for savory dishes all over the world. The knowledge on the odorous volatiles contributing to the typical aroma of chicken broth is rather comprehensive and more than 450 odor-active compounds were reported in the literature (*1*, *2*). In contrast, only fragmentary data are available on the nonvolatile constituents contributing to the attractive orosensory sensation induced by a freshly prepared chicken broth. Most literature studies report only on quantitative data of basic taste compounds such as carbohydrates, amino acids, nucleotides, organic acids, and minerals (*3*−*9*). Moreover, bitter-tasting 2,5-diketopiperazines were identified in chicken essence (*10*), but the amounts of these heterocycles in the chicken essence are far below their taste threshold concentrations (*11*). However, neither comprehensive taste reconstitution experiments nor sensory-directed identification experiments targeting the thick-sour, white-meaty orosensation induced by chicken broth have been reported so far.

Although mouthfulness is a very important driver of the taste quality of a product, only a rather limited number of studies successfully identified molecules imparting a mouthfulness enhancing effect in savory foods. For example, *N*-(1-methyl-4-hydroxy-3-imidazolin-2,2-ylidene)alanine was identified in beef broth (*12*, *13*), although its proposed thick-sour and brothy taste was not yet confirmed by means of taste recombination experiments. In addition, sulfur-containing amino acids and peptides such as L-glutathione, *S*-allyl-L-cysteine sulfoxide, γ-glutamyl-*trans*-*S*-propenyl-L-cysteine sulfoxide, and 3-(*S*)-methyl-1,4-thiazane-5-(*R*)-carboxylic acid (*S*)-oxide have been identified in *Allium* species and shown to induce mouthfulness, thickness, and a long-lasting taste sensation, coined kokumi (*14*−*16*). Moreover, a series of γ-glutamyl di- and tripeptides from *Phaseolus vulgaris* L. were demonstrated to enhance the kokumi flavor of savory products (*17*).

In the past decade, sensory-directed fractionation of food extracts by involving various liquid chromatography techniques as well as analytical sensory tools enabled the identification of taste-active lead molecules in foods such as black tea infusions (*18*), dried morel mushrooms (*19*), red wine (*20*), Swiss cheese (*21*), aged Gouda cheese (*22*), boiled snow crab (*23*), and stewed beef juice (*24*). In addition, the discovery of taste-modulating molecules such as the umami- and sweet-enhancing Maillard reaction product (*S*)-alapyridaine in beef broth (*25*) as well as kokumi-active γ-glutamyl dipeptides in common beans (*17*) and matured Gouda cheese (*26*) was achieved.

However, a sensory-guided fractionation approach has not previously been applied to target the molecules inducing the typical thick-sour, white-meaty orosensation of chicken broth. As the so-called double-boiled chicken (DBC) broth, prepared by heating a chicken in a water-filled clay pot for at least 24 h at about 90 °C, is well appreciated in southeastern Asia for its strong sour-thick mouthfeel and white-meaty flavor, the objective of the present study was to identify the lead molecules responsible for the white-meaty and sour-thick orosensation of a double-boiled chicken broth by means of sensory-directed fractionation.

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MATERIALS AND METHODS

Chemicals. All chemicals were purchased from Aldrich (Steinheim, Germany), peptides were obtained from Bachem (Weil am Rhein, Germany), and ultrapure water used for chromatography was prepared by means of a Milli-Q Gradient A 10 system (Millipore, Schwalbach, Germany). Anserine nitrate (Bachem) was separated from the nitrate ion and was isolated as pure anserine by preparative high-performance liquid chromatography (HPLC) on a 250 × 21.2 mm i.d., 5 μm, Monochrom MS column (Varian, Darmstadt, Germany) equipped with a 50 × 21.2 mm i.d., 5 μm guard column (Varian). Solvents were of HPLC grade (Fisher Scientific, Schwerte, Germany), and deuterated solvents were supplied by Euriso-Top (Gif-Sur-Yvette, France). The chickens were obtained from the German food industry.

Preparation of DBC Broth. A piece of chicken (1 kg) including meat, bones, and skin was placed into the clay pot of a KZB50-B-type slow cooker (Purple Clay Technology PTE Ltd., Singapore, China), water (1 L) was added, and the clay pot was sealed with a clay lid and placed into the water bath of the slow cooker. After the chicken had been heated in the clay pot for 24 h at 89−93 °C, the solid parts of the chicken were removed using filtration; the resulting DBC broth (pH 6.5) was cooled to room temperature and, finally, stored at −21 °C until use.

Solvent Extraction of DBC Broth. An aliquot (500 g) of the chicken broth was defatted by extraction with *n*-pentane (5 × 300 mL). The combined organic layers were separated from solvent under vacuum to yield lipid fraction A (6.6 g). The residual aqueous layer was lyophilized to give the aqueous fraction B (11.1 g), which was used for sensory evaluation and chemical analysis.

Ultrafiltration. *Tangential Flow Ultrafiltration*. An aliquot (10 g) of fraction B was dissolved in water (500 mL) and separated by means of tangential flow ultrafiltration using a Vivaflow 200 filtration unit (Sartorius, Goettingen, Germany) equipped with a polyethersulfone (PES) membrane (5 kDa cutoff). The sample solution was pumped through the filtration unit using a Masterflex L/S Economy Drive peristaltic pump (Cole-Parmer, Vernon Hills, IL) equipped with an Easy-Load Masterflex L/S pump head (Cole-Parmer), the flow rate was adjusted to achieve a pressure of 0.25 MPa above the membrane, and filtration was performed until the high molecular weight retentate (fraction B1) was concentrated to a final volume of 25 mL. Freeze-drying of the low molecular weight filtrate and the high molecular weight retentate afforded fraction B1 (< 5 kDa; 52.8% in yield) and fraction B2 (≥ 5 kDa; 47.2% in yield), respectively, as amorphous powders, which were kept at −21 °C until use.

Stirred-Cell Ultrafiltration. An aliquot (2 g) of the low molecular weight fraction B1 was dissolved in water (250 mL) and separated by using an Amicon 8400-type ultrafiltration cell (Amicon, Witten, Germany) equipped with a YM1-type cellulose filter (1 kDa cutoff; Millipore, Bedford, MA) at a nitrogen pressure of 0.35 MPa. Lyophilization of the filtrate containing the low molecular weight compounds afforded fraction B1-1 (< 1 kDa; 87.3% in yield), whereas freeze-drying of the retentate containing molecules with high molecular weights yielded fraction B1-2 (1−5 kDa; 12.7% in yield).

Gel Permeation Chromatography (GPC). Fraction B1-1 (1.5 g) was dissolved in water (10 mL) and applied onto the top of a 100 × 5 cm XK 50/100 glass column (GE Healthcare, Munich, Germany) filled with a slurry of Sephadex G-15 (GE Healthcare), which was conditioned with water adjusted to pH 4.0 with aqueous formic acid (1 g/100 g). Chromatographic separation was performed using the same mobile phase at a flow rate of 2 mL/min for 20 h. Monitoring the effluent at 220 nm by means of an L-7420-type UV−vis detector (Merck) allowed individual fractions to be collected every 10 min by means of an Ultrarac Fraction Collector 7000 (LKB Produkter, Bromma, Sweden) and combined to give seven GPC fractions, namely, I−VII (**Figure 1**). The fractions were then individually freeze-dried and used for isolation and identification of the taste-active compounds and taste modulators by means of TDA and cTDA, respectively.

Subfractionation of GPC Fraction III. An aliquot (2 g) of GPC fraction III was dissolved in water (20 mL) using an ultrasonic bath, the solution was membrane-filtered (0.45 μm), and portions of 500 μL were separated by means of preparative HPLC on a 250 × 21.2 mm i.d., 5 μm, Monochrom MS column (Varian, Darmstadt, Germany) equipped with a 50 × 21.2 mm i.d., 5 μm guard column (Varian). Monitoring the effluent by means of an evaporative light scattering detector (ELSD), chromatography was performed at a flow rate of 18 mL/min using isocratic conditions with aqueous trifluoroacetic acid (0.1% in water) as solvent. Over a run time of 20 min, the effluent was collected into eight HPLC fractions (III-1−III-8), which were diluted with water and freeze-dried. The lyophilized materials were dissolved in the basic taste recombinant (bRec) and evaluated by means of cTDA using the unspiked bRec as control.

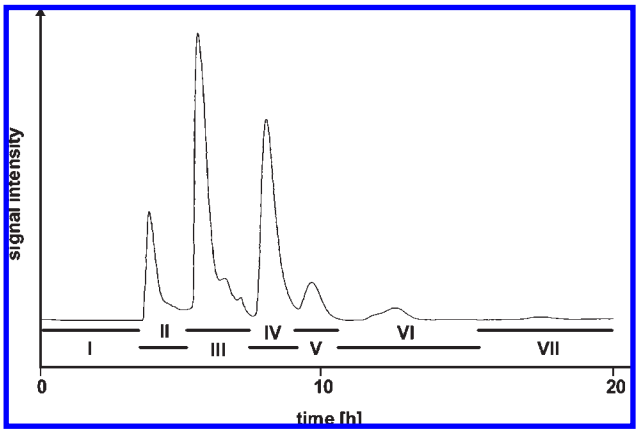

Figure 1. GPC chromatogram (220 nm) of the low molecular weight fraction B1-1 prepared from chicken broth.

Quantitative Analysis of Basic Taste Compounds by Means of High-Performance Ion Chromatography (HPIC). A defined volume of the defatted chicken broth (fraction B) was membrane filtered (0.45 μm) and used directly for the analysis of carbohydrates and polyols or diluted 1:500 with water prior to the analysis of anions, cations, amino acids, and organic acids. Aliquots (5−25 μL) were injected into an ICS-2500 ion chromatography system (Dionex, Idstein, Germany) consisting of a GS 50 gradient pump, an AS 50 autosampler, an AS 50 thermal compartment, and an ED 50 electrochemical detector operating in conductivity and pulsed amperometric mode, respectively. System control and data processing were performed using Chromeleon software (version 6.60, Dionex). For quantitative analysis, external standard calibration with standard solutions ranging from 0.5 to 100 mg/L (six-point calibration) was performed.

Anions. Anions were analyzed on an IonPac AS11-HC analytical column (250 × 2 mm, Dionex) connected with an IonPac AG11-HC guard column (50 × 2 mm, Dionex) and a self-regenerating anion suppressor ASRS Ultra II (2 mm, Dionex), which was installed between the analytical column and the conductivity measuring cell and operated in the autosuppression recycle mode at 76 mA. Chromatography was performed at 30 °C with a flow rate of 0.38 mL/min using a gradient consisting of water (solvent A), an aqueous 5 mmol/L NaOH (solvent B), and an aqueous 100 mmol/L NaOH (solvent C). Starting with a mixture of 80% A and 20% B for 8 min, the NaOH concentration was increased successively to 70% A and 30% C within 10 min and, finally, to 40% A and 60% C within 10 min. The quantitative data are given as the mean of triplicates (relative standard deviation (RSD) for each data point < ±10.0%).

Cations. Cations were analyzed on an IonPac CS16 column (250 × 3 mm, Dionex) connected with an IonPac CG16 guard column (50 × 3 mm, Dionex) and a self-regenerating cation suppressor CSRS Ultra II (2 mm, Dionex), which was installed between the column and the conductivity detector and operated in the autosuppression recycle mode at 37 mA. Chromatography was performed at 40 °C with isocratic elution using aqueous 30 mmol/L methane sulfonic acid as the eluent at a flow rate of 0.36 mL/min. The quantitative data are given as the mean of triplicates (RSD for each data point < ±8.0%).

Carbohydrates. Carbohydrates were analyzed by means of anion exchange chromatography on a CarboPac PA-10 column (250 × 2 mm, Dionex) connected with a CarboPac PA-10 guard column (50 × 2 mm, Dionex). Detection was performed by means of a pulsed amperometric detector, which was equipped with a gold working electrode operating with a standard quadruple waveform. Chromatography was performed at

30 °C at a flow rate of 0.25 mL/min starting with a mixture (80:20, v/v) of water (eluent A) and an aqueous 200 mmol/L NaOH solution (eluent B) for 20 min, then increasing eluent B to 100% within 20 min, and, finally, held isocratically for 10 min. The quantitative data are given as the mean of four replicates (RSD for each data point < ±10.0%).

Polyols. Polyols were analyzed on a CarboPac MA1 column (4 × 250 mm, Dionex) equipped with a CarboPac MA1 guard column (4 × 50 mm, Dionex). Detection was performed by means of a pulsed amperometric detector, which was equipped with a gold working electrode operating with a standard quadruple waveform. Chromatography was performed at 30 °C with isocratic elution using aqueous NaOH (480 mmol/L) as the eluent with a flow rate of 0.4 mL/min for 70 min. The quantitative data are given as the mean of triplicates (RSD for each data point < ±6.0%).

Organic Acids. Low molecular weight organic acids were analyzed by means of high-performance ion exclusion chromatography on an IonPac ICE-AS6 column (9 × 250 mm, Dionex). Prior to conductivity detection, the conductivity of the mobile phase was reduced by chemical suppression by means of an AMMS-ICE II suppressor (Dionex) using tetrabutylammonium hydroxide (5 mmol/L) as the regenerant (3 mL/min). Separation of the analytes was achieved at 20 °C by isocratic elution with 0.4 mM heptafluorobutyric acid at a flow rate of 1 mL/min for 30 min. The quantitative data are given as the mean of triplicates (RSD for each data point < ±8.0%).

Amino Acids. Analysis of amino acids was performed on an AminoPac PA-10 column (250 × 2 mm, Dionex) connected with an AminoPac PA-10 guard column (50 × 2 mm, Dionex) and equipped with a pulsed amperometric detector using a gold working electrode, a pH/Ag/AgCl reference electrode, and the waveform suggested by the manufacturer. Chromatography was performed at 30 °C at a flow rate of 0.25 mL/min using the following gradient composed of water (eluent A), aqueous 200 mmol/L NaOH (eluent B), and aqueous 1 mol/L sodium acetate (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. The quantitative data are given as the mean of four replicates (RSD for each data point < ±12.0%).

Quantitative Analysis of Nucleotides and Nucleosides. An aliquot (1000 μL) of the defatted DBC broth (fraction B) was membrane filtered (0.45 μm) and diluted 1:100 with water prior to analysis. Aliquots (10 μL) were injected into the HPLC-MS/MS system equipped with a 300 × 7.8 mm i.d., 5 μm TSKgel Amide-80 column (Tosoh Bioscience). Using acetonitrile containing 1% formic acid as solvent A and aqueous formic acid (1% in water) as solvent B, chromatography was performed at a flow rate of 1 mL/min with an initial mixture of 75% solvent A and 25% solvent B for 10 min. Thereafter, the content of solvent B was increased within 30 min from 25 to 50% and then to 100% within another 10 min. After chromatographic separation, the effluent was split in a ratio of 1:5 to reduce the effluent entering the mass spectrometer. The following nucleotides and nucleosides were analyzed using the mass transitions given in parentheses: 5′-AMP (*m*/*z* 346.1→134.0), 5′-UMP (*m*/*z* 322.9→96.9), 5′-GMP (*m*/*z* 361.9→210.8), 5′-XMP (*m*/*z* 362.9→210.8), 5′-CMP (*m*/*z* 321.9→96.7), 5′-IMP (*m*/*z* 346.9→135.0), xanthine (*m*/*z* 150.9→107.8), hypoxanthine (*m*/*z* 134.9→91.9), inosine (*m*/*z* 267.0→134.9), guanosine (*m*/*z* 281.9→149.8), adenosine (*m*/*z* 266.0→134.0), and 3′:5′-cAMP (*m*/*z* 327.9→133.8), respectively. 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. The quantitative data are given as the mean of triplicates (RSD for each data point < ±10.0%).

Quantitative Determination of the Gelatin Content. The gelatin content of 8900 mg/L was analyzed in fraction B after acidic hydrolysis and derivatization by means of photometric determination of the released 4-hydroxyproline (8.5 mmol/L) (*27*). The quantitative data are given as the mean of triplicates (RSD for each data point < ±13.0%).

Analytical Sensory Experiments. *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, eight assessors (four males, four females, ages 22−39 years), who had given consent to participate in the sensory tests of the present investigation and had no history of known taste disorders, participated for at least 2 years in weekly training sessions. For the training of the individual gustatory modalities, aqueous solutions (2 mL each) of the following standard taste compounds in bottled water (pH 6.0) were used by means of the sip-and-spit method: sucrose (50 mmol/L) for sweet taste, lactic acid (20 mmol/L) for sour taste, NaCl (20 mmol/L) for salty taste, caffeine (1 mmol/L) for bitter taste, monosodium L-glutamate (3 mmol/L) for umami taste, tannic acid (0.05%) for puckering astringency, and quercitin-3-*O*-β-D-glucopyranoside (0.01 mmol/L) for a velvety astringent, mouth-drying oral sensation. For training on viscosity, a gelatin solution (0.5% in water) was used; for training of the activity of mouthfulness enhancement and complexity increase, coined kokumi activity (*17*), the panel was asked to compare the gustatory impact of an aqueous solution (pH 6.5) consisting of sodium chloride (30 mmol/L) and L-glutamic acid (10 mmol/L) as the control with a solution of reduced glutathione (5 mmol/L) in the same tastant solution (pH 6.5). The training of the activity of thick-sour mouthfeel enhancement was done by comparing the orosensation induced by a model broth in the absence and presence of *N*-(1-methyl-4-hydroxy-3-imidazolin-2,2-ylidene) alanine (10.0 mmol/L) (*12*). The sensory sessions were performed at 19−22 °C in three independent sessions. To prevent cross-modal interactions with odorants, the panelists used nose clips.

Table 1. Taste Profile Analysis of Double-Boiled Chicken (DBC) Broth as well as of Ultrafiltration Fractions B1-1, B1-2, and B2 Obtained from DBC Broth

	intensities for individual taste qualities[a]						
sample	sweet	bitter	salty	umami	sour	thick-sour mouthfeel	viscosity
DBC broth	0	0	2.0	4.1	3.5	4.0	3.5
B2 (>5 kDa)	0	0	0.2	0	0.1	0	3.3
B1-2 (1−5 kDa)	0	0	0	0.1	0	0	0.3
B1-1 (<1 kDa)	0	0	2.0	4.1	3.3	3.8	0

[a] Intensities were judged on a linear scale from 0 (not detectable) to 5 (strongly detectable) by trained panelists. The data are given as the mean of triplicates (RSD for each data point < ±0.3 scale point).

Pretreatment of Fractions. Prior to sensory analysis, the fractions or isolated compounds were suspended in water, and, after removal of the volatiles in high vacuum (< 5 mPa), were freeze-dried twice. GC-MS and ion chromatographic analysis revealed that food fractions treated by that procedure are essentially free of solvents and buffer compounds used. The pH value of all samples was adjusted to 6.5 with trace amounts of either aqueous formic acid (1 g/100 g) or potassium hydroxide (0.1 mol/L). 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, because trace amounts of this acid do not influence the sensory profile of the test solution. To minimize the uptake of any toxic compound to the best of our knowledge, all of the sensory analyses were performed by using the sip-and-spit method, which means the test materials were not swallowed but expectorated.

Taste Dilution Analysis (TDA) and Comparative Taste Dilution Analysis (cTDA). The lyophilized GPC fractions were dissolved in (i) water (5.0 mL) to perform the TDA or (ii) in an aqueous basic taste recombinant (bRec, 5.0 mL) to perform the cTDA and adjusted to pH 6.5 by adding trace amounts of aqueous formic acid (0.1 mmol/L) or aqueous KOH (0.1 mmol/L). These stock solutions were sequentially diluted 1:2 with water (pH 6.5; for TDA) or bRec (for cTDA), respectively. The serial dilutions of each fraction were randomly presented to the sensory panel in order of increasing concentration. By means of a duo test with one sample as the blank (water or bRec), panelists were asked to determine the dilution step at which a difference between sample and blank water could be detected. This so-called taste dilution (TD) factor (*28*, *29*) determined by the sensory subjects in three separate sessions were averaged. The values between individuals and sessions differed by not more than plus or minus one dilution step.

Comparative Taste Profile Analysis. The lyophilized ultrafiltration fractions B1, B2, B1-1, and B1-2 (**Table 1**) and the HPLC fractions III-1−III-8, as well as the hydrophilic interaction liquid chromatography (HILIC) fractions III-8/1−III-8/8, were dissolved in their "natural" concentrations, which means the amounts determined for each substance in the chicken broth, in 5.0 mL of either water or the bRec solution, and the pH value was adjusted to 6.5 by adding trace amounts of aqueous formic

Table 2. Taste Qualities, Taste Thresholds, Concentrations, and Dose-over-Threshold (DoT) Factors of Nonvolatile Taste Compounds in Double-Boiled Chicken Broth

taste compound	TC[a] (μmol/L)	concn[b] (μmol/L)	DoT[c]
Group I: Bitter-Tasting Amino Acids and Nucleosides			
L-leucine[d]	12000	440	<0.1
L-tyrosine[d]	5000	220	<0.1
L-isoleucine[d]	11000	460	<0.1
L-tryptophan[d]	5000	50	<0.1
L-lysine[d]	85000	2470	<0.1
L-valine[d]	35000	190	<0.1
L-phenylalanine[d]	58000	270	<0.1
L-arginine[d]	75000	520	<0.1
L-histidine[d]	48000	850	<0.1
taurine	150000	6600	<0.1
xanthine	60000	70	<0.1
hypoxanthine	44000	760	<0.1
inosine	96000	780	<0.1
guanosine	63000	3	<0.1
adenosine	77000	4	<0.1
3′:5′-cAMP	100000	3	<0.1
Group II: Umami-like Compounds			
L-glutamic acid	1100	1830	1.7
L-aspartic acid	4000	640	0.2
L-glutamine	50000	580	<0.1
L-asparagine	50000	220	<0.1
succinate	900	1300	1.4
5′-AMP	4000	120	<0.1
5′-UMP	17000	10	<0.1
5′-GMP	30000	20	<0.1
5′-XMP	35000	20	<0.1
5′-CMP	40000	10	<0.1
5′-IMP	5000	350	<0.1
Group III: Salty Compounds			
sodium[e]	7500	8700	1.3
potassium[e]	15000	29470	2.0
ammonium[e]	5000	8500	1.7
calcium[e,f]	7500	140	<0.1
magnesium[e,f]	4000	400	0.1
chloride[g]	7500	20390	2.7
phosphate[g]	7500	15340	2.0
Group IV: Sweet-Tasting Compounds			
glucose	80000	27	<0.1
fructose	40000	61	<0.1
sucrose	10000	16	<0.1
lactose	72000	5	<0.1
inositol[h]	17700	210	<0.1
xylitol	20200	690	<0.1
ethylene glycol	65000	1720	<0.1
ribitol[h]	45300	5	<0.1
L-alanine	8000	760	<0.1
L-cysteine	2000	130	<0.1
glycine	25000	600	<0.1
L-methionine	5000	170	<0.1
L-proline	26000	190	<0.1
L-serine	30000	560	<0.1
L-threonine	40000	300	<0.1
Group V: Sour-Tasting Compounds			
L-lactate	14000	22600	1.7
L-malate	3700	600	0.2
citrate	2600	140	<0.1
acetate	2000	300	0.2
Group VI: β-Alanyl Dipeptides			
L-anserine	45000	5583	0.1
L-carnosine	57000	2920	<0.01
β-alanylglycine	63000	2968	<0.01

[a] Taste threshold concentrations were determined in bottled water by means of a triangle test or taken from the literature. [b] Concentrations were determined in fraction B1-1. [c] Dose-over-threshold (DoT) factor is calculated as the ratio of concentration and taste threshold. [d] Value taken from the literature (*42*). [e] Threshold concentration determined for the corresponding chloride salt. [f] Taste threshold concentration for bitter taste. [g] Threshold concentration determined for the corresponding sodium salt. [h] Value taken from the literature (*39*).

acid (0.1 mmol/L) or aqueous KOH (0.1 mmol/L). These solutions were then presented to the trained sensory panel, and the intensities of the descriptors bitter, sweet, sour, salty, umami, viscosity, and thick-sour mouthfeel were rated on a scale from 0 (not detectable) to 5 (intensely perceived) in comparison to an unspiked recombinant (control).

Taste Recognition Threshold Concentrations. The taste threshold concentrations of the purified peptides were determined in bottled water adjusted to pH 6.5 with trace amounts of aqueous formic acid (0.1 mmol/L) using a three-alternative forced-choice test with ascending concentrations of the stimulus following the procedure reported previously (*30*). The threshold value of the sensory group was approximated by averaging the threshold values of the individuals in three independent sessions. Values between individuals and separate sessions differed by not more than plus or minus one dilution step; as a result, a threshold value of 45.0 mmol/L for anserine represents a range of 22.5−90.0 mmol/L.

Preparation of Taste Recombinants. To prepare a basic taste recombinant (bRec), the tastants summarized in groups I−V (**Table 2**) were dissolved in their "natural" concentrations in bottled water and, after solubilizing gelatin (8900 mg/L), the pH value of this solution was adjusted to 6.5 by the addition of trace amounts of an aqueous KOH solution (1 mol/L). For the preparation of the total taste recombinant (tRec), the bRec solution was spiked with the β-alanyl dipeptides in their "natural" concentrations as given in group VI in **Table 2**. In addition, a reduced taste recombinant solution (rRec) was prepared from L-glutamic acid (1830 μmol/L), 5′-AMP (120 μmol/L), and 5′-IMP (350 μmol/L), the sour-tasting lactic acid (22600 μmol/L), chlorides and phosphates of sodium (8700 μmol/L), potassium (29470 μmol/L), and ammonium (8500 μmol/L), and gelatin (8900 mg/L), as well as the thick-sour mouthfeel-enhancing peptides L-anserine (5583 μmol/L), L-carnosine (2920 μmol/L), and β-alanylglycine (2968 μmol/L). The taste profiles of the solutions bRec, tRec, and rRec were evaluated by means of taste profile analysis using nose-clips.

Taste Omission Experiments. To evaluate the individual taste contribution of distinct compounds or compound groups, partial taste recombinants were prepared by omitting either single taste compounds or individual tastant groups from the total recombinant tRec. 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 taste difference correctly were asked to rate the intensity of the given taste descriptors on a scale from 0 (not detectable) to 5 (strongly detectable).

Identification of Taste-Modulating β-Alanyl Peptides in Fraction III-8. HPLC fraction III-8 was dissolved in a mixture (1:1, v/v; 5 mL) of acetonitrile and water and, after membrane filtration, was fractionated by semipreparative hydrophilic liquid interaction chromatography (HILIC) on a 300 × 21.5 mm i.d., 10 μm, TSKgel Amide-80 column (Tosoh Bioscience, Stuttgart, Germany) equipped with a 75 × 21.5 mm i.d., 10 μm guard column (Tosoh Bioscience). Using a flow rate of 6 mL/min, chromatography was performed with a mixture (95:5; v/v) of acetonitrile and aqueous ammonium acetate (10 mmol/L) adjusted to pH 6.5 with formic acid (1% in water) as solvent A and aqueous ammonium acetate (10 mmol/L, pH 6.5) as solvent B. Starting with 80% solvent A and 20%

solvent B for 10 min and increasing the solvent B to 45% within 70 min and then to 100% within 20 min, the effluent was separated into eight HILIC subfractions (III-8/1−III-8/8), which were collected separately, freed from solvent under vacuum, and lyophilized. Sensory evaluation of the single HILIC fractions in the bRec solution identified only fraction III-8/5 as taste-active. This HILIC fraction was further purified by rechromatography on the same stationary phase using water as solvent A and acetonitrile as solvent B. Chromatography was performed isocratically with 50% solvent A for 10 min, followed by a linear gradient to 60% solvent B within 15 min and, then, maintained isocratically for 5 min. The compounds eluting in HILIC fractions III-8/5a and III-8/5b were collected, separated from solvent in vacuum, and freeze-dried to afford the isolates as white amorphous powders. LC-MS/MS and NMR experiments led to the identification of the taste modulators in fraction III-8/5a as β-alanyl-3-methyl-L-histidine (anserine) and β-alanyl-L-histidine (carnosine) and in fraction III-8/5b as β-alanylglycine. Comparison of chromatographic and spectroscopic data with those obtained for the corresponding commercial reference compounds confirmed the identity of these peptides.

β-Alanyl-3-methyl-L-histidine (*anserine*): LC-MS (ESI^+), *m/z* 241 (100, $[M + H]^+$); ^1H NMR (400 MHz, D_2O; COSY), δ 2.58 [m, 2H, H−C(2)], 3.06 [m, 1H, H−C(6_a)], 3.12 [m, 2H, H−C(1)], 3.22 [m, 1H, H−C(6_b)], 3.73 [m, 3H, H−C(10)], 4.65 [m, 1H, H−C(4)], 7.17 [m, 1H, H−C(8)], 8.51 [m, 1H, H−C(9)]; ^{13}C NMR (100 MHz, D_2O; HMQC, HMBC), δ 29.2 [C(6)], 32.8 [C(1)], 33.5 [C(10)], 36.0 [C(2)], 56.1 [C(4)], 116.9 [C(8)], 132.4 [C(7)], 135.4 [C(9)], 172.0 [C(3)], 178.5 [C(5)].

β-Alanyl-L-histidine (*carnosine*): LC-MS (ESI^+), *m/z* 227 (100, $[M + H]^+$); ^1H NMR (400 MHz, D_2O; COSY), δ 2.58 [m, 2H, H−C(2)], 3.06 [m, 1H, H−C(6_a)], 3.12 [m, 2H, H−C(1)], 3.22 [m, 1H, H−C(6_b)], 4.65 [m, 1H, H−C(4)], 7.17 [m, 1H, H−C(8)], 8.51 [m, 1H, H−C(9)]; ^{13}C NMR (100 MHz, D_2O; HMQC, HMBC), δ 29.2 [C(6)], 32.8 [C(1)], 36.0 [C(2)], 56.1 [C(4)], 116.9 [C(8)], 132.4 [C(7)], 135.4 [C(9)], 172.0 [C(3)], 178.5 [C(5)].

β-Alanylglycine: LC-MS (ESI^+), *m/z* 146 (100, $[M + H]^+$); ^1H NMR (400 MHz, D_2O; COSY), δ 2.59 [t, 2H, J = 6.5 Hz, H−C(2)], 3.15 [t, 2H, J = 6.5 Hz, H−C(1)], 3.63 [s, 2H, H−C(4)]; ^{13}C NMR (100 MHz, D_2O; HMQC, HMBC), δ 31.9 [C(2)], 35.7 [C(1)], 43.2 [C(4)], 172.1 [C(3)], 176.8 [C(5)].

Quantitative Analysis of β-Alanyl Peptides. An aliquot (1 mL) of the DBC broth was made up with water to 100 mL and membrane-filtered (0.45 μm), and, then, aliquots (10 μL) were injected into the HPLC-MS/MS system equipped with a 300 × 7.8 mm i.d., 5 μm TSKgel Amide-80 column (Tosoh Bioscience). Chromatography was performed at a flow rate of 1 mL/min using acetonitrile containing 1% formic acid as solvent A and aqueous formic acid (1% in water) as solvent B. Starting with a mixture of 70% A and 30% B for 10 min, the amount of solvent B was increased successively to 60% within 40 min and, finally, to 100% within 5 min. After chromatographic separation, the effluent was split in a ratio of 1:5 to reduce the effluent entering the mass spectrometer. After successful identification of the individual peptides on the basis of identical mass transition, retention times, and spiking experiments with the corresponding reference, quantification was performed by external standard calibration. The β-alanyl peptides were analyzed using the following mass transitions given in parentheses: β-alanyl-3-methyl-L-histidine (*m/z* 241.0→109.0), β-alanyl-L-histidine (*m/z* 227.0→110.0), and β-alanylglycine (*m/z* 147.0→76.2), respectively. Quantitative analysis was performed in triplicates using defined solutions of reference compounds (RSD for each compound < ±8.0%).

HPLC. The HPLC apparatus (Gilson International B.V., Bad Camberg, Germany) consisted of a type 321 HPLC pump, a type 7725i Rheodyne injector (500 μL loop), and a prepELS type evaporative light scattering detector (ELSD), which was operated at 40 °C with air as operating gas (3.5 bar). Chromatographic separations were performed in an analytical scale with a 300 × 7.8 mm i.d., 5 μm, HILIC column containing carbamoyl-derivatized silica gel (TSKgel Amide-80, Tosoh Bioscience, Stuttgart, Germany) operated at a flow rate of 1.0 mL/min). For preparative fractionation, the effluent was split after chromatographic separation on a 300 × 21.5 mm i.d., 10 μm, TSKgel Amide-80 column (Tosoh Bioscience) in a ratio of 1:5 ratio or after separation on a 250 × 21.2 mm i.d., 5 μm, Monochrom MS column (Varian) in a ratio of 1:20. In each case, the smaller aliquot of the effluent was channeled into the ELSD and the major aliquot was collected.

Liquid Chromatography−Mass Spectrometry (LC-MS/MS). Spectra were aquired in electrospray ionization (ESI) mode on an API 4000 Q-Trap LC-MS/MS system (AB Sciex Instruments, Darmstadt, Germany) connected to a 1100 series HPLC system from Agilent (Waldbronn, Germany). The ion spray voltage was set at 5500 V in the ESI^+ mode, nitrogen served as curtain gas (20 psi), and the declustering potential was set at +25 V. The mass spectrometer was operated in the full scan mode monitoring positive ions; fragmentation of $[M + H]^+$ molecular ions into specific product ions was induced by collision with nitrogen (4×10^{-5} torr) and a collision energy of +25 V. Detection of β-alanyl peptides was performed in multiple-reaction monitoring (MRM) mode using the transitions described above.

Nuclear Magnetic Resonance Spectroscopy (NMR). ^1H, ^{13}C, COSY, HMQC, and HMBC experiments were performed on a Bruker DPX 400 spectrometer (Bruker, Rheinstetten, Germany). Data processing was performed by using Mestre-C software (version 4.8.6; Mestrelab Research, Santiago de Compostella, Spain). D_2O was used as solvent and tetramethylsilane as the internal standard.

RESULTS AND DISCUSSION

To identify the compounds responsible for the typical taste of DBC broth, a broth was freshly prepared by heating a whole chicken for 24 h at 90 °C in the clay pot of a double-boiler pot. To gain a first insight into the taste profile of the DBC broth (pH 6.5), the trained sensory panel was asked to rate the intensity of the taste descriptors salty, sour, sweet, umami, bitter, and the thick-sour and white-meaty mouthfeel, as well as the viscosity using a linear scale from 0 (not detectable) to 5 (strongly detectable). Umami (4.0), thick-sour mouthfeel (4.0), sourness (3.5), and viscosity (3.5) were rated with the highest intensities, followed by a salty taste evaluated with a score of 2.0 (**Table 1**).

Solvent Extraction and Molecular Weight Fractionation. To study the polarity of the taste compounds, several aliquots of a DBC broth were pooled and repeatedly extracted with *n*-pentane and, after removal of the organic solvent in vacuum, the aqueous layer as well as the pentane isolate were freeze-dried to yield the lipid fraction A (37.4 g/100 g of dm) as well as the polar fraction B (62.6 g/100 g of dm). Both fractions were taken up in water in their "natural" concentrations, which means 1.32 g of fraction A and 2.20 g of fraction B were solubilized in 100 mL of water and evaluated by the sensory panel. Whereas the emulsion of fraction A was entirely tasteless, the hydrophilic fraction B exhibited a taste profile matching that of the authentic chicken broth (data not shown).

To separate the taste compounds on the basis of molecular weight differences, fraction B was separated by means of tangential flow ultrafiltration using a polyethersulfone membrane with a 5 kDa cutoff to obtain fraction B1 (< 5 kDa) and fraction B2 (≥ 5 kDa) after freeze-drying as amorphous powders. As preliminary sensory studies demonstrated that the entire taste profile was represented by the compounds present in fraction B1, this fraction was further subfractionated by stirred-cell ultrafiltration equipped with a YM1-type regenerated cellulose filter with a 1 kDa cutoff to give fractions B1-1 (< 1 kDa) and B1-2 (1−5 kDa), which were freeze-dried. Fractions B1-1, B1-2, and B2 were dissolved in bottled water each in its "natural" concentration, which means 1.014 g of fraction B1-1, 0.148 g of fraction B1-2, and 1.038 g of fraction B2 were solubilized in 100 mL of water, and these solutions were then again evaluated by means of a taste profile analysis. Whereas the high molecular weight fractions B1-2 and B2 imparted only a very faint salty, sour, and umami taste, the salty, sour, and umami taste as well as the thick-sour, white-meaty orosensation induced by low molecular weight fraction B1-1 was very close to the intensities found in the DBC broth (**Table 1**). Only the viscosity, most likely caused by gelatin, was much lower in fraction B1-1 and was present predominantly

Table 3. Taste Dilution Analysis (TDA) and Comparative Taste Dilution Analysis (cTDA) of Solutions of Individual GPC Fractions in Water and the Basic Taste Recombinant (bRec), Respectively

	TDA in water[b]		cTDA in bRec[c]	
fraction[a]	TD factor	taste quality	cTD factor	taste-modifying effect
I	<1	nd	<1	nd
II	8	umami, salty	8	enhanced umami and salty taste
III	32	umami, slightly salty	16	increased thick-sour, white-meaty mouthfeel, and enhanced complexity
IV	32	salty, slightly umami	<1	nd
V	4	bitter, astringent	<1	nd
VI	2	bitter, sour	<1	nd
VII	<1	nd	<1	nd

[a] Number of GPC fractions refers to **Figure 1**. [b] Taste dilution analysis (TDA) was performed after dissolving the individual GPC fractions in water (pH 6.5) in their "natural" concentration ratios. [c] Comparative taste dilution analysis (cTDA) performed after dissolving the individual GPC fractions in the basic taste recombinant solution (bRec, pH 6.5) containing all tastant groups I−V in the concentrations given in **Table 2**. The bRec solution lacking any GPC fractions was used as the control. nd, not detectable.

in the high molecular weight fraction B2 (**Table 1**). As these data clearly demonstrated that the most important taste compounds are present in the low molecular weight fraction, fraction B1-1 was used for further analysis.

Quantitation of Basic Taste Compounds and Taste Re-engineering. To gain insight into the impact of basic taste compounds on the taste profile of DBC broth, the concentrations of amino acids, mono- and disaccharides, organic acids, cations, alditols, and inorganic anions were determined by means of HPIC. In addition, purine nucleotides were quantified by HILIC-MS/MS. Among these compounds, 25 amino acids, 5 carbohydrates, 12 nucleotides and nucleosides, 5 organic acids, 5 cations, and 2 inorganic anions were quantitatively determined in fraction B1-1 (**Table 2**).

To check whether the compounds already identified can create the typical taste of the DBC broth, an aqueous taste reconstitute, containing the 54 basic taste compounds as well as gelatin, each in the concentration given in **Table 2**, was prepared, and the taste profile of that basic taste recombinant (bRec) was compared with that of the authentic DBC broth (**Table 5**). Comparative taste profile analysis revealed that the intensities of sourness, sweetness, bitterness, saltiness, and umami taste, as well as viscosity perceived for the bRec solution, were close to those of the DBC broth. Although the bRec solution contained gelatin to provide viscosity (*31*), the characteristic thick-sour, white-meaty mouthfeel was judged to be significantly lower (3.8→1.5) in the bRec solution, thus indicating that the basic taste recombinant is lacking compounds modulating the thick-sour mouthfeel. As the bRec solution contained all of the molecules responsible for the basic taste qualities, this recombinant was used in the following fractionation experiments as the matrix solution for the localization of the modulators of the thick-sour mouthfeel.

Sensory-Guided Fractionation of Fraction B1-1. To gain first insight into the molecules imparting the taste modulating activity, fraction B1-1 was separated by means of GPC using Sephadex G-15 as the stationary phase and water (pH 4.0) as the mobile phase. Monitoring the effluent by means of a UV−vis detector, fraction B1-1 was separated into the seven GPC fractions I−VII (**Figure 1**), which were individually freeze-dried.

An aliquot of each individual GPC fraction was taken up in its "natural" concentration, which means in the amounts obtained from the GPC column, in bottled water and used for TDA. The highest umami impact was detected in GPC fraction III judged with a TD factor of 32, followed by GPC fractions II and IV evaluated with TD factors of 8 and 4, respectively (**Table 3**). Besides umami taste, GPC fraction IV and, with a somewhat lower impact, also fractions II and III imparted a salty taste impression, and GPC fractions V and VI exhibited a bitter taste. In comparison, GPC fractions I and VII were entirely tasteless (**Table 3**).

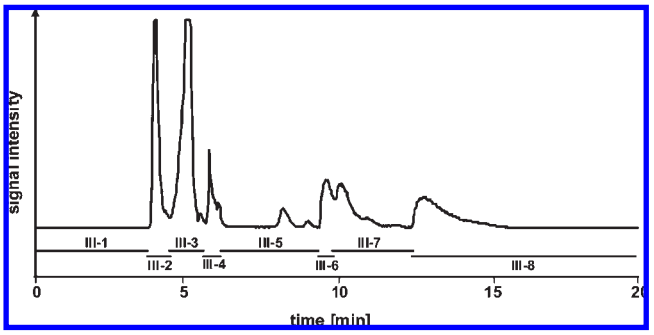

Figure 2. PFPP HPLC-ELSD chromatogram of GPC fraction III, isolated from chicken broth (preparative scale).

In addition, aliquots of the seven GPC fractions were added to a solution of the basic taste recombinant (bRec) in their "natural" concentration ratios to investigate their taste modifying activity by means of a cTDA using the blank bRec solution as the control. The data, given in **Table 3**, show that GPC fraction II increased the salt as well as the umami intensity of the bRec solution due to the intrinsic taste of this fraction. Interestingly, the addition of GPC fraction III to the bRec solution induced a thick-sour and white-meaty mouthfeel, judged with a TD factor of 16, which was absent in the blank bRec solution (control) and absent in fraction III alone.

To identify the molecules responsible for the thick-sour and white-meaty orosensation, GPC fraction III was separated by means of HPLC using a pentafluorophenylpropyl (PFPP) stationary phase, developed for the analysis for basic drugs (*32*−*34*), to give the eight subfractions III-1−III-8 (**Figure 2**). After lyophilization, these fractions were evaluated sensorially in the bRec solution. As given in **Table 4**, all HPLC fractions showed taste activity with the exception of fraction III-1; however, only the late eluting fraction III-8 was found to induce the typical thick-sour and white-meaty mouthfeel judged with an intensity of 3.9 when compared to bRec (2.6). Further subfractionation of fraction III-8 by means of semipreparative HILIC using a neutral mobile phase system containing an ammonium acetate buffer again afforded eight subfractions, namely, III-8/1−III-8/8 (**Figure 3**). These subfractions were again dissolved in the bRec solution and analyzed for their sensory properties in comparison to the blank bRec solution (control). Fraction III-8/5 was detected to exhibit the thick-sour and white-meaty mouthfeel with an intensity of 3.8, whereas all of the other fractions showed only faint taste activity or were entirely inactive. Rechromatography using the HILIC phase and nonbuffered acetonitrile/water as the eluent allowed the final separation fraction III-8/5 into two subfractions, namely, III-8/5a and III-8/5b (**Figure 4**).

Table 4. Influence of HPLC Fractions III-1−III-8 on the Sensory Profile of the Basic Taste Recombinant Solution (bRec)

fraction[a]	influence on the sensory profile of the bRec solution[b]
III-1	nd
III-2	slightly increased umami taste, low mouthfeel
III-3	slightly metallic and bitter
III-4	slightly increased umami and bitter taste
III-5	slightly increased umami and bitter taste
III-6	bitter
III-7	bitter
III-8	thick-sour and white-meaty mouthfeel[c]

[a] Number of HPLC fractions refers to **Figure 2**. [b] The fractions were dissolved in the basic taste recombinant solution (bRec, pH 6.5), containing all tastant groups I−V in the concentrations given in **Table 2** and were sensorially compared to the bRec solution lacking any additive (control) by means of a duo test. nd, no difference detectable. [c] On a 5-point linear scale, this sensation was judged with an intensity of 3.9 when compared to bRec (2.8) used as control.

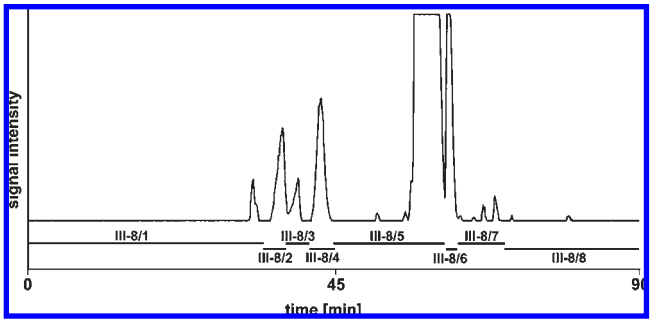

Figure 3. HILIC-ELSD chromatogram of fraction III-8, isolated from chicken broth.

The two collected fractions were assessed in terms of their sensory activity after the addition of aliquots in the bRec solution and further analyzed by means of LC-MS/MS as well as NMR experiments.

LC-MS (ESI^+) analysis of the compound isolated from fraction III-8/5b showed m/z 147 as the pseudomolecular ion ($[M + H]^+$) as well as sodium and potassium adducts with m/z 169 ($[M + Na]^+$) and m/z 185 ($[M + K)^+$), respectively, thus indicating a molecular mass of 146 Da. The daughter ion spectrum resulting from fragmentation of the $[M + H]^+$ ion indicated the target compound as an alanyl peptide exhibiting the b_1 fragment ion m/z 72 as well as the immonium ion m/z 44 (**Figure 5**). In addition, the y ion m/z 76 gave strong evidence for the presence of a C-terminal glycine residue. The presence of an alanylglycine peptide is well in line with the molecular mass of 146 Da.

To distinguish between an α- and a β-alanyl moiety in the peptide, ^{1}H NMR spectroscopic experiments were performed. The ^{1}H NMR spectrum of the isolated compound showed three resonance signals integrating for two protons each. As no signals of methyl protons were observed in the high-field above 2 ppm, α-alanine could be excluded as part of the peptide. The proton signals resonating at 3.15 and 2.59 ppm as well as their coupling patterns undoubtedly indicated the presence of the methylene groups in β-alanine, thus leading to the identification of β-alanylglycine as a peptide contributing to the thick-sour and white-meaty mouthfeel of the chicken broth.

LC-MS experiments of fraction III-8/5a showed the presence of two peptides with molecular masses of 240 and 226 Da, respectively. Fragmentation of the pseudomolecular ion m/z 241 in the ESI-positive mode once more revealed the fragment ions m/z 72 and m/z 44, indicating an alanyl residue as well as the fragments m/z 170 and m/z 124 showing evidence for an

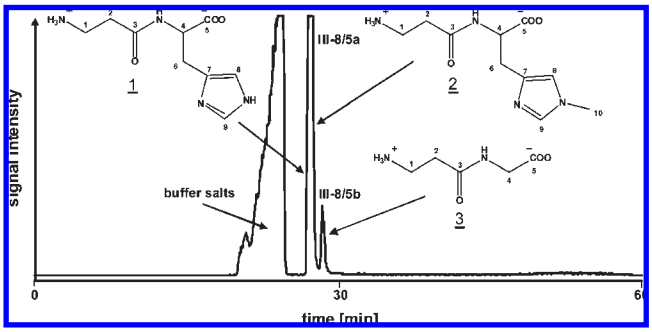

Figure 4. Rechromatography of fraction III-8/5, isolated from chicken broth, by means of HILIC-ELSD, and chemical structures of β-alanyl-L-histidine (**1**), β-alanyl-*N*-methyl-L-histidine (**2**), and β-alanylglycine (**3**).

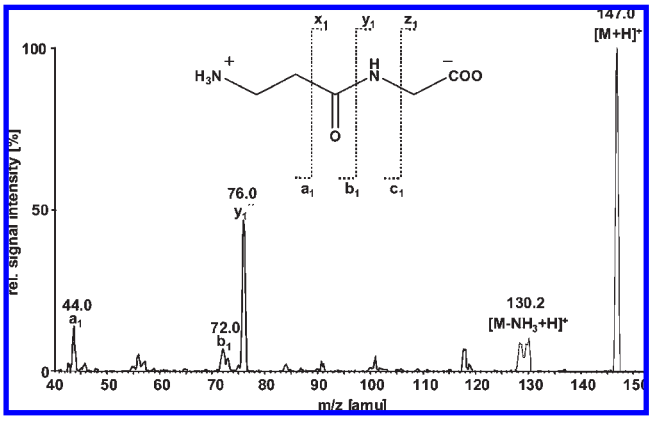

Figure 5. LC-MS/MS spectrum (ESI^+) of β-alanylglycine isolated from fraction III-8/5b.

N-methylhistidine moiety. Comparison of chromatographic (retention time), spectroscopic data (MS, NMR), and sensory data with those obtained for the synthetic reference peptides led to the identification of the taste modulator as β-alanyl-*N*-methyl-L-histidine, which is well-known in the literature as anserine (*35*, *36*). In addition, β-alanyl-L-histidine (carnosine) was identified as the taste-modulating peptide exhibiting a molecular mass of 226 Da. Although the occurrence of anserine and carnosine in chicken was reported already about 80 years ago (*35*), the taste modulating β-alanylglycine was, to our best knowledge, not previously identified in chicken meat or in any other food product.

Sensory Activity of β-Alanyl Dipeptides. Prior to sensory analysis, the purity and identity of the β-alanyl peptides were checked by HPLC-MS, ion chromatography, and ^{1}H NMR spectroscopy. To gain insight into the intrinsic taste of these peptides, first, human threshold concentrations were determined in water (pH 6.5) by means of an ascending three-alternative forced-choice test (*30*). Anserine, carnosine, and β-alanylglycine were found to exhibit a slightly sour and nonspecific astringent taste sensation in concentrations exceeding their threshold concentrations of 45, 57, and 67 mmol/L. The value of 45 mmol/L found for anserine is somewhat higher when compared to the threshold concentration of 10 mmol/L reported for a burning and bitter sensation (*36*) and was about 100 times above the threshold range of 0.047−0.094 mmol/L reported for a slightly sweet and bitter taste of carnosine and anserine (*37*), but it has to be considered that all previously reported sensory studies were performed using these peptides in a nonspecified purity or using the nitrate salt of anserine rather than using the purified peptide.

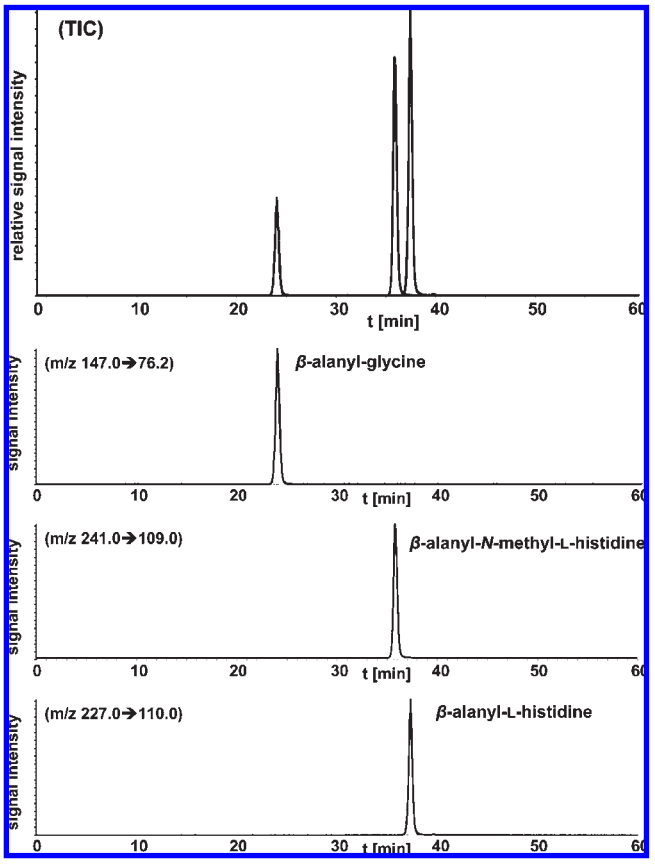

Figure 6. Mass chromatography of β-alanyl peptides in DBC broth.

Quantitative Analysis of β-Alanyl Dipeptides in DBC Broth and Taste Recombination/Omission Experiments. To confirm the sensory impact of the identified peptides on the taste of the DBC broth, anserine, carnosine, and β-alanylglycine were quantitatively determined by means of HILIC-MS/MS running in the MRM mode (**Figure 6**). Analysis of these β-alanyl dipeptides in the chicken broth revealed high amounts of 1340 mg/L of anserine, 660 mg/L of carnosine, and 302 mg/L of the previously not reported β-alanylglycine.

To confirm the impact of the β-alanyl dipeptides on the sour-thick and characteristic white-meaty mouthfeel of the DBC broth, a total taste recombinant (tRec) was prepared by adding carnosine, anserine, and β-alanylglycine in their "natural" concentrations to the solution of the basic taste recombinant bRec. The sensory panel was then asked to evaluate the taste profile of the tRec solution and to compare it to the bRec solution as well as the authentic DBC by scoring the given taste descriptors on a linear scale from 0 (not detectable) to 5 (strong taste impression). The presence of the β-alanyl dipeptides in tRec was detected by all members of the sensory panel. Compared to bRec, in particular, the intensity of the thick-sour and white-meaty mouthfeel was found to be strongly increased (2.6→3.8, **Table 5**) and reached almost the intensity of 4.0 observed for the DBC broth. As the intensities of the basic taste qualities of the tRec solution were very close to those of the authentic DBC broth, it was concluded that the typical taste of the chicken broth could be mimicked by a cocktail of the 57 components summarized in **Table 2**.

To analyze the relevance of the individual taste compounds and to study interactions between different tastant groups, systematic taste omission experiments were performed as successfully applied in previous taste investigations (*19*, *23*, *38*, *39*). To achieve this, multiple taste recombinants lacking in any one of the tastant groups I−VI (**Table 2**), or in one or more individual taste compounds, were evaluated by means of triangle tests using two samples of the total taste recombinant (tRec) as the control. Those panelists who detected any difference in the taste profile were asked to rate the intensity of the taste descriptors sour, sweet, bitter, salty, umami, and thick-sour and white-meaty mouthfeel on a 5-point scale (**Table 6**).

Table 5. Taste Profile Analysis of Double-Boiled Chicken (DBC) Broth, Basic Taste Recombinant (bRec), Total Taste Recombinant (tRec), and Reduced Taste Recombinant (rRec)

	intensities for individual taste qualities[a]						
fraction	sweet	bitter	salty	umami	sour	thick-sour mouthfeel	viscosity
DBC broth	0	0	2.0	4.1	3.5	4.0	3.5
bRec[b]	0	0	2.0	4.3	3.4	2.6	3.5
tRec[c]	0	0	2.0	4.0	3.5	3.8	3.5
rRec[d]	0	0	2.0	3.8	3.4	3.6	3.5

[a] The intensity of the individual taste qualities was evaluated on a linear scale from 0 to 5. The data are given as the mean of triplicates (RSD for each data point < ±0.3 scale point). [b] The basic taste recombinant solution (bRec) contained the tastant groups I−V in the concentrations given in **Table 2** as well as gelatin (8900 mg/L). [c] The total taste recombinant solution (tRec) contained the tastant groups I−VI in the concentrations given in **Table 2** as well as gelatin (8900 mg/L). [d] The reduced taste recombinant solution (rRec) contained L-glutamic acid (1830 μmol/L), 5′-AMP (120 μmol/L), and 5′-IMP (350 μmol/L), the sour-tasting lactic acid (22600 μmol/L), chlorides and phosphates of sodium (8700 μmol/L), potassium (29470 μmol/L), and ammonium (8500 μmol/L), and gelatin (8900 mg/L), as well as the thick-sour mouthfeel enhancing peptides L-anserine (5583 μmol/L), L-carnosine (2920 μmol/L), and β-alanylglycine (2968 μmol/L).

In an initial set of experiments, entire tastant groups, containing all of the compounds provoking the same taste quality, were omitted. A partial recombinant lacking the bitter amino acids and nucleosides (group I) was prepared, but none of the panelists could detect any difference when compared to the total recombinant tRec, thus demonstrating that the bitter-tasting compounds did not contribute to the taste of the DBC broth.

The omission of group II, containing the umami-tasting amino acids and 5′-nucleotides as well as the umami-like succinic acid, was detected by all sensory panelists due to a complete loss of the umami taste (4.0→0) as well as a strong decrease of the thick-sour mouthfeel (3.8→0.7) (**Table 6**). Besides a slight effect on saltiness (2.0→1.5) and bitterness (0.0→0.3), the perceived sour taste was found to be strongly influenced (3.5→0.4). Additional partial taste recombinants were prepared, lacking either the amino acids L-glutamic acid and L-aspartic acid, the 5′-nucleotides, or succinic acid. Omission of L-glutamic acid and L-aspartic acid induced a stronger decrease of umami taste (4.0→1.0) than the omission of the 5′-nucleotides (4.0→2.8), thus demonstrating these amino acids as key drivers for the perceived umami taste. Similarly, the thick-sour and white-meaty mouthfeel (3.8→0.8) was found to be reduced in the absence of both amino acids, whereas the lack of the nucleotides induced only a minor decrease (3.8→3.3). Interestingly, the sour taste (3.5→0.5) was strongly affected by the omission of L-glutamic acid and L-aspartic acid, suggesting a contribution of L-glutamic acid to the sour taste of the chicken broth. In addition, an increase in bitterness (0.0→0.3) was found in the absence of the amino acids, thus confirming earlier reports on the bitter masking activity of glutamic acid (*40*, *41*). Compared to the amino acids and 5′-nucleotides, the omission of succinic acid was not detectable by a single panelist, thus excluding this compound as a sensory-active key component even though a DoT value of 1.4 was calculated (**Table 2**).

In a next experiment, the salty group III, containing inorganic cations and anions, was omitted from the total recombinant. All panelists determined this partial recombinant to be less salty (2.0→0.0), less umami (4.0→1.0), and less sour (3.5→0.7) when compared to the total recombinant (**Table 6**). In addition, a

Table 6. Influence of the Omission of Tastant Groups or Individual Taste Compounds on the Taste Profile of the Total Taste Recombinant tRec

omission of	no.[a]	description of taste difference[b] (change in intensity)
group I (bitter)	0	no differences observed
group II (umami)	8	loss of umami taste (4.0→0.0); decrease of sourness (3.5→0.4), thick-sour mouthfeel (3.8→0.7), and salty taste (2.0→1.5); increase in bitterness (0.0→0.4)
L-glutamic acid, L-aspartic acid	8	decrease of umami taste (4.0→1.0), sourness (3.5→0.5), thick-sour mouthfeel (3.8→0.8), and salty taste (2.0→1.5); slight increase in bitterness (0.0→0.3)
succinic acid	0	no differences observed
5′-nucleotides	7	less umami (4.0→2.8); slight decrease of thick-sour mouthfeel (3.8→3.3)
group III (salty)	8	loss of saltiness (2.0→0.0); decrease in thick-sour mouthfeel (3.8→0.3), umami (4.0→1.0), and sourness (3.5→0.7); slight increase in sweetness (0.0→0.3) and bitterness (0.0→0.2)
Na^+	8	decrease of umami taste (4.0→1.3), thick-sour mouthfeel (3.8→1.3), sourness (3.5→1.4), and saltiness (2.0→0.6)
K^+	7	decrease of umami (4.0→2.0), thick-sour mouthfeel (3.8→1.5), sourness (3.5→1.5), and saltiness (2.0→0.4), slightly bitter (0.0→0.2)
group IV (sweet)	0	no difference observed
group V (sour)	7	decrease in sourness (3.5→1.8), umami (4.0→3.2), saltiness (2.0→1.8), and thick-sour mouthfeel (3.8→3.2)
group VI (β-Ala peptides)	8	decrease of thick-sour mouthfeel (3.8→2.6) and sourness (3.5→3.3); slight increase of umami taste (4.0→4.3)
L-anserine	8	decrease of thick-sour mouthfeel (3.8→3.4) and sourness (3.5→3.3); slightly increased umami taste (4.0→4.2)
L-carnosine	8	decrease of thick-sour mouthfeel (3.8→2.9) and sourness (3.5→3.3); slight increase of umami taste (4.0→4.2)
β-Ala-Gly	8	decrease of thick-sour mouthfeel (3.8→3.1) and sourness (3.5→3.3); slight increase of umami taste (4.0→4.2)

[a] Number of panelists (in total, eight) 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 should be evaluated on a scale from 0 (not detectable) to 5 (strongly detectable). The data are given as the mean of triplicates (RSD for each data point < ±0.3 scale point).

strong decrease of the thick-sour and white-meaty mouthfeel sensation (3.8→0.3) was observed. To further analyze the role of the predominant cations sodium and potassium for the taste of the DBC broth, partial taste recombinants were prepared lacking in one or the other cation. Both partial recombinants were identified by eight or seven of the eight panelists as being different from tRec; the omission of sodium ions induced a strong decrease of umami (4.0→1.3), sourness (3.5→1.4), saltiness (2.0→0.6), and, interestingly, the thick-sour, white-meaty mouthfeel (3.8→1.3). Although the potassium ion was found in the DBC broth as the predominant cation in a concentration of almost 30 mmol/L, the omission of potassium had a lower effect on the umami taste (4.0→2.0) as well as the thick-sour and white-meaty mouthfeel (3.8→1.5) when compared to the omission of sodium (**Table 6**).

A partial recombinant prepared by omission of the sweet-tasting group IV containing sugars, amino acids, and alditols was not perceived by any of the panelists (**Table 6**), thus excluding these sweet compounds as key contributors to the taste of the chicken broth.

Omission of taste group V, containing the organic anions, resulted in a lower intensity of sourness (3.5→1.8), besides a minor decrease in the umami intensity (4.0→3.2) and thick-sour mouthfeel (3.8→3.2). Lactic acid is the only compound in taste group V exceeding its taste detection thresholds in the DBC broth, thus suggesting this acid as the key tastant in group V (**Table 6**).

In the last set of experiments, partial recombinants were prepared by omitting the β-alanyl dipeptides (group VI). All panelists were able to pick up a difference between the partial recombinant lacking the three β-alanyl dipeptides and the tRec solution. Besides the strong decrease of the typical thick-sour and white-meaty mouthfeel (3.8→2.6), a slight increase of umami (4.0→4.3) and a slight decrease of sour taste (3.5→3.3) were perceived (**Table 6**). The sensory panel described this partial recombinant as showing a strong reduction in the white-meaty character when compared to the tRec solution.

To investigate the contribution of the individual peptides to the taste profile of chicken broth, three partial recombinants lacking one or the other peptide were prepared. Eight of eight panelists detected a sensory difference between the tRec and the three partial recombinants (**Table 6**). The omission of L-carnosine induced the most pronounced reduction of the thick-sour, white-meaty orosensation (3.8→2.9), followed by β-alanylglycine (3.8→3.1) and L-anserine (3.8→3.4) with somewhat lower effects. These findings clearly demonstrate that subthreshold concentrations of the β-alanyl dipeptides contribute to the characteristic white-meaty, thick-sour orosensation of chicken broth. Although the addition of L-carnosine and L-anserine to beef broth (*36*) as well as a commercial soup stock (*37*) has already been reported to induce a "thicker, heavier, and more meaty" taste, this is the first time that subthreshold concentrations of the previously unidentified β-alanylglycine contributes to the characteristic white-meaty, thick-sour orosensation of chicken broth.

On the basis of the findings of the reconstitution and omission experiments, a reduced taste recombinant (rRec) was prepared containing only the 13 compounds that were evaluated with a higher taste impact. This tastant cocktail contained the umami compounds L-glutamic acid, 5′-AMP, and 5′-IMP, the sour-tasting lactic acid, chlorides, and phosphates of sodium, potassium, and ammonium, and gelatin as well as the thick-sour mouthfeel-enhancing peptides L-anserine, L-carnosine, and β-alanylglycine. Comparison of the taste profiles of the tRec and the rRec revealed that the mixture of the 13 key components created a taste profile which was rather close to that of the DBC broth (**Table 5**).

On the basis of the data obtained, it can be concluded that the β-alanyl dipeptides, including the previously not reported

β-alanylglycine, are important contributors to the thick-sour mouthfeel and white-meaty character of chicken broth. Although these peptides were found to exhibit only a faint sour and slightly astringent intrinsic taste, subthreshold concentrations of these peptides, when present together with L-glutamic acid and specific cations, do induce the typical thick-sour mouthfeel and white-meaty character of poultry meat. Investigations on the mechanism of this interactive effects between the individual components as well as structure−activity studies on the peptides are currently in progress and will be published elsewhere.

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