Sensory assessment of fish and chicken protein hydrolysates – evaluation of NMR metabolomics profiling as a new prediction tool

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Abstract

Nuclear magnetic resonance (NMR) metabolomics profiling was evaluated as a new tool in sensory assessment of protein hydrolysates. Hydrolysates were produced based on different raw materials (cod, salmon and chicken), enzymes (Food Pro PNL and Bromelain) and hydrolysis time (10 and 50 min). Influence of raw material and hydrolysis parameters on sensory attributes were determined by traditional descriptive sensory analysis and ^{1}H NMR spectroscopy. Raw material had major influence on attribute intensity and metabolite variation, followed by enzyme and hydrolysis time. However, the formation of bitter taste was not affected by raw material. Partial least squares regression (PLSR) on ^{1}H NMR- and sensory data provided good models (Q^2 = 0.55 - 0.89) for 11 of the 17 evaluated attributes, including bitterness. Significant metabolite-attribute associations were identified. The study confirms the potential prediction of the sensory properties of protein hydrolysates from cod, salmon and chicken based on ^{1}H NMR metabolomics profiling.

Keywords

NMR metabolomics, multivariate analysis, enzymatic protein hydrolysis, sensory evaluation, bitter taste

Introduction

A major challenge in the production of protein hydrolysates for human consumption is the formation of bitter and unpalatable tastes [1]. Bitter taste development is related to the formation of small hydrophobic peptides generated in the hydrolysis process [1-5], but also substrate-specific metabolites will contribute to the taste sensation [6-8]. An objective evaluation of sensory properties is imperative for quality assessment of food grade hydrolysates, and is preferably performed by a trained taste panel [9]. However, in case of assessment of numerous attributes in flavor intensive samples, a descriptive sensory analysis is often limited to a small number of samples and does not provide information on the chemical composition and its influence on the assessed attributes. Nuclear magnetic resonance (NMR) spectroscopy has been applied as a "magnetic tongue" in sensory studies of canned tomatoes, olive oil and coffee beans [10-12], and may be a promising alternative or supplemental tool for sensory evaluation of protein hydrolysates. The "magnetic tongue" method uses NMR spectroscopy and multivariate calibration to relate metabolite composition to sensory profiles. The purpose is to obtain a correlation between sample chemical composition and the presence and intensity of sensory attributes. The acquisition of metabolomic data separates NMR from other instrumental sensory analyses, such as the electronic tongue, which generates a signal that may be attributed to a certain property[13], but does not provide information on the chemical composition.

Food grade residues after meat deboning and fish filleting operations contain a significant amount of muscle proteins and connective tissue. Enzymatic protein hydrolysis of such materials is a mild processing technology that decreases the molecular weight, and increase the water-solubility of the peptides. This facilitates their recovery as a protein hydrolysate and is considered a promising industrial approach for improved valorization of such materials [1]. Besides peptides and free amino acids, protein hydrolysates contain numerous of other water-soluble metabolites, salts,

vitamins etc., [8, 14, 15] that may influence the sensory profile. For instance, trimethylamine oxide (TMAO) is a common metabolite in fish, and its degradation product trimethylamine (TMA) has an unpalatable fishy flavor. Moreover, lipid oxidation products may cause rancid flavor [6], while sodium chloride and acids contribute to salty and acidic flavor, respectively [16]. Understanding of how such compounds affect sensory properties is important in the production of flavor-neutral protein hydrolysates.

Several scientific studies have addressed flavor development in protein hydrolysates based on marine and poultry substrates [1, 3, 5, 17, 18]. However, to our knowledge, no studies have compared the sensory attributes of hydrolysates based on these raw materials at otherwise similar hydrolysis conditions. Such studies may improve the understanding of sensory attribute development depending on raw material and hydrolysis parameters (i.e. enzyme specificity, efficiency, enzyme to substrate ratio, time and temperature). Unsorted residual meat and fish products consist of a mixture of heads, backbones, cuttings and, in the case of fish, viscera. This gives significant variation of raw material composition and possible endogenous enzyme activity. To avoid such complex variability, pure muscle fillets were chosen as a model substrate in this study.

The objectives of this study were to: 1) Assess the formation of bitter taste, and other sensory attributes of protein hydrolysates based on salmon, cod and chicken muscle protein at identical hydrolysis conditions, 2) Evaluate the use of ^{1}H NMR spectroscopy to assess the hydrolysate metabolite composition and 3) Evaluate the potential use of ^{1}H NMR in sensory profiling of protein hydrolysates through metabolite-attribute associations.

Materials and Methods

Materials

Filets of Atlantic salmon (*Salmo salar)* and cod (*Gadus morhua*) were purchased fresh from a local fish distributor. Fresh chicken filets (*Gallus gallus domesticus*, Ross 308) were purchased from a local supermarket. All raw materials were vacuum packed and stored at - 20°C until use. The proteases used were Bromelain BR1200 (EC 3.4.22.32, Enzybel, Waterloo, Belgium) and FoodPro PNL (EC 3.4.24.28, DuPont, Wilmington, DE). Peptide standards were purchased from Sigma-Aldrich (Oslo, Norway) except lysozyme (Fluka biochemicals, Buchs, Switzerland) and Alberta standards (Alberta Peptide Institute, Department of Biochemistry, University of Alberta, Edmonton, Canada). All chemicals for analyses were of analytical grade.

Enzyme activity assay

The enzyme activity of Bromelain and FoodPro PNL was determined by non-specific protease activity assay with casein as described by Cupp-Enyard [19], with some modifications. Solutions of 0.65% (w/v) casein in 50 mM potassium phosphate buffer (pH 6.5) was subjected to hydrolysis by different enzyme concentrations for 10 min at 50°C. The reaction was terminated by adding 1:1 of 110 mM trichloroacetic acid solution and filtered through a 0.45 µm polyether sulfone syringe filter and added Folin & Ciolcaltea's (0.5 mM) reagent. Absorbance was measured at 660 nm (Evolution 220, Thermo scientific, Waltham, MA) and the results compared with an L-tyrosine standard curve. The protease activity was given as units (U) defined as micromoles of tyrosine equivalents released from casein per minute.

Chemical analysis

Proximate composition of the raw materials was determined. Analysis of nitrogen (N) was performed by the Kjeldahl method (ISO 5983-2) [20] and crude protein was estimated based on

107 substrate-specific N-to-protein-conversion-factor [21, 22]. Fat content was analyzed by the Bligh &
108 Dyer method [23]. Ash was determined by combustion of raw material at 550°C (ISO 5984-2) [24].
109 Dry matter was determined by drying at 103°C (ISO 6496-2) [25]. Molecular weight distribution
110 analysis was done by size exclusion chromatography (SEC) (1260 series HPLC Agilent
111 Technologies) with a Superdex Peptide 10/300GL column (GE Healthcare, Uppsala, Sweden),
112 acetonitrile with TFA as eluent and UV detection at 190-600 nm. The following components
113 were used to calibrate molecular weights (MW): carbonic anhydrase (MW 29000), lysozyme
114 (MW 14300 Da), Cyt C (MW 12400), aprotinin (MW 6500), alberta 4 (MW 3249.38), insulin A
115 (MW 2531.64), alberta 3 (MW 2441.54), gastrin I (MW 2126.28), alberta 2 (MW 1633.7),
116 polymyxin (MW 1470), substrate P (MW 1347.63), [Val 4]-Ang III (MW 917.06), alberta 1
117 (MW 825.86), (Leu)3 (MW 357.49) and Gly (MW 75.07). Amino acid composition was
118 quantified by fluorescence detection with excitation/emission at 250/395 nm. Proteins were
119 hydrolyzed to free amino acids with 6N HCl and amino acids derivatized with 6-aminoquinolyl-
120 *N*-hydroxysuccinimidyl carbamate before passing through the HPLC column (Waters Accq Tag
121 3.9 x 150 mm) and detector [26]. Cystein (and cystine) was determined after performic acid
122 oxidation. Asparagine and glutamine were estimated based in the release of ammonia in the HCl
123 digest compared to a neutral control sample [22]. Released ammonia was quantified by the method
124 of Conway and Byrne [27]. Tryptophan was chemically determined by the method of Miller [28]. All
125 chemical analyses were performed in duplicate.

126 *Enzymatic protein hydrolysis*

127 Raw material was partly thawed at 4 °C overnight and minced in a kitchen grinder (aperture 4
128 mm, Electrolux AKM 3110 W, Stockholm, Sweden). The mince was mixed with water (1:1
129 (w/w)) and heated to 50 °C in a modified R10Bear Varimixer (A/S Wodschow &Co. Brøndby,

Denmark) while stirred (20 rpm). At 50 °C, 10 U of enzyme were added per g of protein in the raw material. Proteolytic activity was terminated after 10 or 50 min by heating to > 90 °C in a microwave oven (Menumaster commercial, Cedar Rapids, IA), and kept at this temperature for minimum 10 min. After cooling to ~40 °C, the slurry was separated by centrifuged at 15000 × *g* for 10 min (Sorvall LYNX 6000, Thermo scientific, Waltham, MA). The water phase was filtered through a Seitz-T2600 filter (Mall Corporation, East Hills, NY). Particles and lipids were removed by utrafiltration through a Vivaflow 200 cross flow cassette (Sartorius, Goettingen, Germany) with nominal molecular weight cut-off of 100 kDa. The hydrolysates were stored at -30 °C. An overview of all hydrolysates is shown in Table 1.

Sensory analysis

Generic descriptive analysis [9] was conducted by a highly trained panel of eight assessors at Nofima, Ås, Norway. The assessors are regularly tested and trained in accordance to ISO 8586 [29], and have extensive experience in sensory assessment of protein hydrolysates. Hydrolysate samples diluted to 1% protein concentration were served in duplicated balanced, randomized order at room temperature. Sensory attributes were evaluated using an unstructured line scale ranging from no intensity (1) to high intensity (9). Consensus between panelists was verified before assessment of experimental samples by a calibration/pre-test with two samples deemed high and low in generic flavor intensity and bitterness. This, along with previous experience, gave basis for the evaluated attributes that are listed in Table 2 with their respective descriptions.

NMR spectroscopy and data processing

The hydrolysates were diluted to 2% protein concentration using distilled water, and further to 1% with 200 mM sodium phosphate buffer (pH 6.8) containing 20% D_2O with 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). A volume of 550 µl was added to 5 mm NMR tubes. ^1H and ^1H-

^{13}C spectra were acquired at 300K using a Bruker AVANCE NOE ultrashielded 600 MHz spectrometer with cryoprobe (Karlsruhe, Germany). The ^{1}H NOESY (Bruker, noesygppr1d pulse program) data were acquired with 4 dummy scans, 32 real scans, 4 seconds relaxation delay, 96k time-domain points, and spectral width of 29.8 ppm. ^{1}H-^{13}C HSQC spectra (Bruker, hsqcetgpsisp2 pulse program) were attained for peak identification purposes with 16 dummy scans, 8 scans, 2048 data points, 256 increment in F1, and spectral width of 165 and 16 ppm for F1 and F2, respectively. The NMR spectra were processed using TopSpin (v. 4.0.4, Bruker BioSpin, Karlsruhe, Germany). Before Fourier transformation the free induction decay (FID) was zero filled to 128K points and an exponential line broadening of 0.3 Hz was applied. All spectra were phased and referenced relative to DDS. Prior to multivariate analysis, the data was reduced by a factor of ten through averaging, and spectral regions containing DSS, water and trimethylamine-oxide (TMAO) were removed.

Statistical analysis

Analysis of variance (ANOVA) of the sensory profiling data was performed using Minitab (v18.1, Pennsylvania State University, PA). First, a two-way mixed effects ANOVA model was conducted to assess differences between products for all sensory attributes. Product was set as a fixed variable and assessor and interaction effects were set as random variables [30]. Two-factor interactions were tested but removed from the model as they were non-significant. Tukey's pairwise comparison was applied where significant ($p<0.05$) differences were found. Then, another mixed effects ANOVA was used to evaluate the individual fixed effects of raw material, enzyme and hydrolysis time on sensory attributes, still treating assessor as a random variable. Tukey's pairwise comparison was applied where significant ($p<0.05$) differences were found.

Fifty-fifty Multivariate ANOVA [31] performed in MATLAB (R2018b, The Mathworks, Inc Natick, MA), was used to evaluate the effects of raw material, enzyme and hydrolysis time on the pareto-scaled ^{1}H NMR spectra. This method is based on Principal Component Analysis (PCA) and handles multiple collinear responses. The method calculates overall sums-of-squares and p-values for each experimental factor. Rotation testing [32, 33] was used to compute familywise adjusted single response p-values. The model contained main effects only. Two-factor interactions were tested but removed from the model as they were non-significant.

Principal component analysis (PCA) and partial least squares regression (PLSR) was performed using Unscrambler v.10.4.1 (Camo, Oslo, Norway). Sensory data was unit variance-scaled, while ^{1}H NMR data was pareto-scaled [34]. All variables were centered. PCA models were computed for sensory data and ^{1}H NMR data separately. Predictability of sensory attribute intensity from metabolite composition was evaluated by PLSR with full cross-validation. Each attribute response (y-variable) was modelled with the reduced ^{1}H NMR dataset (x-variables). Models of sufficient predictability were evaluated for sensory description markers by identifying ^{1}H NMR signals correlating (R^2>0.5) with changes in predicted sensory attribute intensity (MATLAB R2018b, The Mathworks, Inc Natick, MA) [10, 11].

Results and Discussion

Composition of raw material and protein hydrolysates

The residuals after deboning and filleting operations contains variable ratio of muscle connective- and bone tissue. To avoid this type of variability in the raw material, this study was conducted based on pure muscle tissue as a model substrate. The amino acid composition of cod, salmon and chicken substrates revealed comparable levels for all raw materials (Table 3). The most prominent difference in amino acids was the relatively high level of glutamate in chicken

198 compared to the other raw materials. The calculated substrate specific nitrogen-to-protein
199 conversion factors (Table 3) deviated from the commonly used factor of 6.25 for all raw
200 materials. The observed discrepancy can be ascribed to variations in non-protein nitrogen
201 compounds, such as non-protein amino acids and nucleotides [22, 35].

202 The molecular weight distribution (MWD) of the hydrolysates (Table 4) showed low levels of
203 peptides above 6 kDa. The products showed some variations in peptide levels below 6 kDa
204 depending on raw material and hydrolysis parameters. As the hydrolysis reaction continues, the
205 enzymes will increase the amount of water-soluble peptides and continue the digestion of already
206 released peptides. The reduced level of peptides < 0.2 kDa with prolonged hydrolysis time is
207 caused by the general increased in released water-soluble peptides. The observed difference was
208 lower than expected based on a pure endopeptidase activity, indicating some exopeptidase
209 activity in agreement with previous studies [3]. Bromelain gave a higher release of peptides,
210 evident by the higher nitrogen levels in the hydrolysates (Table 4). Bromelain has a broad
211 selectivity for protein cleavage sites, while Food Pro PNL preferably hydrolyzes peptide-bonds
212 containing hydrophobic amino acids [36].

213 *Sensory evaluation*

214 The perception of substrate-specific and unpalatable tastes affects the acceptability of enzymatic
215 protein hydrolysates. Depending on the intended application of a protein hydrolysate, some
216 flavors may be desired, such as umami or a fresh fish flavor, whereas bitter taste is undesirable.
217 Proper choice of enzyme and processing conditions may improve some sensory properties [1, 3, 18,
218 37], however; the effects of raw material variation is less studied. All hydrolysates had a high
219 overall flavor intensity (Table 5) while the attributes sweet taste, acidic, sea and rancid flavor,
220 and fatty mouthfeel were low. Several hydrolysates had higher intensity scores for bitter taste,

fish flavor and astringency. Significant differences were found for all sensory attributes, except metallic, sea, cloying and fatness (Table 5). However, sweet taste, pork flavor and rancid flavor were found non-significantly different based on Tukey's pairwise comparison. This, along with slightly elevated p-values indicates that a type I error should not be disregarded for the latter attributes.

Raw material was found to be the most important factor influencing sensory attributes (Table 6). However, raw material did not influence the bitter taste intensity and bitterness was solely dependent on choice of enzyme and hydrolysis time, as previously observed [3, 18, 37]. This indicates that studies addressing effects of processing conditions on bitter taste may be transferrable to other substrates, although, additional raw materials, including plant and dairy based substrates, should be included in new studies to verify said transmissibility, or potential limitations thereof.

In general, Food Pro PNL products had lower bitterness scores compared with Bromelain products, and the former has also been found to give less bitter taste in hydrolysates based on salmon head and backbones compared with Alcalase and Promod 671 L [3]. Bromelain has been reported to result in both bitter and umami taste in chicken hydrolysates [5]. In this study, umami taste, along with, sweet and salt taste, and sour, acidic, swine and chicken flavors, were found to be solely dependent on raw material, and not influenced by choice of enzyme. The formation of fish flavor was, as expected, mostly related to raw material variation, but also influenced by hydrolysis time. The overall flavor intensity and astringency was influenced by hydrolysis time, likely due to an increased release of small peptides of 0.2-1 kDa (Table 4). Rancid flavor intensity was low for all products (Table 5). However, Bromelain gave slightly higher rancid flavor (Table 5), also reflected in Table 6. This may be an effect of increased oxidation due to possible lipolytic activity in the applied enzyme product [38]. The lipid contents of the hydrolysates

were < 0.1 % (data not shown) independent of lipid contents in raw materials and confirms the efficiency of membrane filtration for defatting of hydrolysates.

Principal Component Analysis (PCA, Figure 1) was used to evaluate the association between hydrolysates, sensory attributes and MWD. Two principal components (PCs) were found to be relevant for the interpretation of the results. The first and second PCs explained 40 and 31 %, respectively, whereas the third and fourth PCs explained 12 and 7 %, respectively (not shown).

In the score plot (Figure 1a), PC1 explains the combined effect of enzyme and hydrolysis time. PC2 explains raw material associated variation, with some overlap between salmon and chicken. The correlation loading plot (Figure 1b) displays two clear groups based on proximity, where group 1 indicates positive associations between bitter taste, flavor intensity and astringent, cloying and metallic flavors with peptides of 0.2-1 kDa. This confirms the link between these sensory properties and the formation of small peptides containing hydrophobic amino acids, in agreement with previous studies [2-4, 37]. The second group consists of the attributes fullness, fatness, umami, salt, sweet,, acidic and chicken; positively associated to the dipeptides and free amino acids < 0.2 kDa. Umami is known to be positively associated with glutamic acid [4]. Fish, sea, rancid and pork flavors demonstrated < 50% explained variance and were regarded as less relevant for the interpretation of the sensory attribute variance between samples. The hydrolysates showed small variation in peptides > 4 kDa (Table 4), and molecules in this range displayed a negative association to group 1, indication that an increased ratio of > 4 kDa peptides would result in lower intensity of the given attributes.

Effect of hydrolysis parameters on metabolite composition determined by ^{1}H NMR

The NMR spectroscopy was conducted on 1% dilutions on protein basis to enable direct comparison with the sensory evaluation. ^{1}H NMR analysis of the various protein hydrolysates

revealed similar spectra, but with varying peak intensities, depending on raw material (Figure 2), and hydrolysis parameters (not shown). The most obvious difference in metabolite composition, as an effect of raw material, was the intense TMAO peak in cod hydrolysates compared to the others (Figure 2). Additional prominent signals affected by raw material variation were lactate, alanine, dimethylamine (DMA), anserine and creatine. Of these signals, DMA was the only metabolite of which the highest intensity was found in cod hydrolysates. There was considerably more anserine in hydrolysates based on chicken and salmon, compared with cod. Anserine is a known metabolite in vertebrate organisms, and its absence in the cod hydrolysates may be partly explained by drip-loss during thawing [39] and/or endogenic enzyme activity [40].

The resulting PCA score plots (Figure 3a and b) was similar to the one based on sensory data (Figure 1a), indicating that ^{1}H NMR data could be useful to assess sample differences affecting sensory properties. The hydrolysates based on cod formed a separate cluster, whereas there was more overlap between the salmon and chicken samples (Figure 3a and b), indicating similarities in metabolite composition of the two latter hydrolysates. PC1-3 explained 39, 23, and 20% of sample variation, respectively. PC1 (Figure 3a, b and c) mainly describes the differences in raw material which had a strong positive association with lactate and anserine (Figure 3c). PC2 was associated with choice of enzyme, and indicates that FoodPro PNL liberated more free valine and less isoleucine, leucine and small peptides containing these amino acids, than Bromelain in the hydrolysis process (Figure 3d). This was evident by the negative correlation of the PC2 loading with the spectral area around 0.9 ppm. The higher levels of the hydrophobic amino acids isoleucine and leucine in hydrolysates based on Bromelain may explain the higher bitterness found in these products [41]. The variation described by PC3 is less definite, but within each group of raw material and enzyme, there is an association with hydrolysis time. Prolonged hydrolysis

time also increased peak intensities of the identified amino acids (Figure 2), and reduced the concentration of the non-protein dipeptide anserine (not shown). The latter may be attributed to increased nitrogen yield (Table 4) and dilution to the 1% test protein concentration.

The multivariate ANOVA (Table 7) revealed that most of the variation in metabolite composition could be ascribed to the differences between raw materials (51 %), followed by choice of enzyme (17%) and hydrolysis time (13%). This was also reflected in the number of ^{1}H NMR signals that were significantly affected according to rotation tests and correlates with the observed major importance of raw material given by the sensory analysis (Table 6).

Association between hydrolysate metabolites and sensory attributes

To assess the relationship between metabolites and sensory attributes, PLSR models were established based on the ^{1}H NMR data and sensory attribute scores. Models showing acceptable prediction ability based on cross-validation ($Q^2 > 0.5$) were established for the following attributes: Bitter, sweet, salt, umami, sour, flavor intensity, acidic, chicken, pork, fullness and fatness (Table 8).

Fish taste could not be modelled ($Q^2 = 0.19$), despite highly significant variation in the two-way ANOVA analysis (Table 6). The peak area for TMAO was removed prior to multivariate analysis, as the high TMAO content in cod hydrolysates had too high leverage on the models, despite pareto-scaling. TMAO is not known to be related with any strong smell or flavor [42], and its exclusion should not have an effect on the data interpretation. On the other hand, the breakdown products of TMAO, DMA and TMA [43], will contribute to fish flavor [6]. The ^{1}H NMR spectra of hydrolysates based on cod protein, showed the highest content of these metabolites (Figure 2), and displayed a higher positive association with fish flavor compared with salmon in the sensory analysis (Figure 1). However, the variations in TMA and DMA did not explain the

313 variation in fish flavor in this study. This may be due to possible contributing effect of volatile
314 alcohols and carbonyls [44] not identified in the ^{1}H NMR spectra due to low concentrations and or
315 peak overlap.

316 All attributes modelled by PLSR were found to associate with metabolites identified by ^{1}H NMR
317 spectroscopy (Table 9). Bitterness showed a positive association to the presence of isoleucine and
318 leucine, which is well documented in several studies [1, 41, 45], and a negative association with
319 glycerol, inosine 5'-monophosphate (IMP) and lactate. Flavor intensity was mostly dependent on
320 the same metabolites as bitterness. Reduction of lactic acid has been found to increase bitterness
321 and reduce intensity of umami and salt [46]; in agreement with associations observed in this study
322 (Table 9).

323 The attributes sweet, acidic, fullness, chicken, umami, fatness and salt (group 2, Figure 1b) had
324 high correlation in the sensory analysis, making it difficult to separate them in the PLSR models
325 (Table 9). IMP and glutamate compounds are known to contribute to umami taste. In addition,
326 IMP will enhance the umami properties of glutamate and glutamate peptides [47, 48]. In this study,
327 glutamate showed a negligible positive association to umami taste, whereas a strong association
328 was observed for IMP (Table 9). This indicates that the presence of compounds enhancing umami
329 taste may be more important for sensory score of this attribute than the glutamate concentration
330 itself.

331 The dipeptide anserine was found to associate with several sensory attributes, most of which can
332 be considered palatable (Table 9). The compound has been described as a contributor in making
333 the pleasant flavors of broth linger in the mouth [49], and may explain the association of the
334 chicken hydrolysates with fullness and other palatable attributes (Figure 1a and b). The products
335 based on chicken and salmon had high anserine peak intensity (Figure 2). However, only one

salmon product (Sa-P-10) correlated with the palatable attributes in group 2 in Figure 1b, despite the comparable content of anserine in all salmon hydrolysates. Other studies have described anserine as bitter [7] and sour [46]. Although creatine was a prominent metabolite in all samples, its variation proved only significant in the development of pork flavor (negative association). A previous study found that creatine did not affect basic tastes [50], which is in agreement with this study. However, the study did find creatine to improve upon certain mouthfeel attributes not included in this study, thus there might be a creatine-attribute association not detected in the hydrolysates.

This study confirms the potential prediction of the sensory properties of protein hydrolysates from cod, salmon and chicken based on ^{1}H NMR metabolomic profiling; a new and promising tool in the analysis of food products. The data sets from ^{1}H NMR- and sensory analysis displayed similar hydrolysate groupings, and the obtained models found associations between metabolites and sensory attributes that have previously been demonstrated by traditional methods. For most of the sensory attributes, development during processing was solely dependent on raw material and not influenced by choice of enzyme. The formation of bitter taste was not affected by raw material, indicating a comparable release of bitter peptides independent of substrate.

More studies are needed to make statistically more robust prediction models. In addition, raw material-specific studies will most likely generate improved metabolite-attribute associations.

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References

1. Fu, Y.; Chen, J.; Bak, K. H.; Lametsch, R., Valorisation of protein hydrolysates from animal by-products: perspectives on bitter taste and debittering methods: a review. *International Journal of Food Science & Technology* **2019,** *54* (4), 978-986.

2. Kim, H. O.; Li-Chan, E. C. Y., Quantitative structure-activity relationship study of bitter peptides. *J Agr Food Chem* **2006,** *54* (26), 10102-10111.

3. Aspevik, T.; Totland, C.; Lea, P.; Oterhals, A., Sensory and surface-active properties of protein hydrolysates based on Atlantic salmon (Salmo salar) by-products. *Process Biochem* **2016,** *51* (8), 1006-1014.

4. Fu, Y.; Liu, J.; Hansen, E. T.; Bredie, W. L. P.; Lametsch, R., Structural characteristics of low bitter and high umami protein hydrolysates prepared from bovine muscle and porcine plasma. *Food Chem* **2018,** *257*, 163-171.

5. Maehashi, K.; Matsuzaki, M.; Yamamoto, Y.; Udaka, S., Isolation of peptides from an enzymatic hydrolysate of food proteins and characterization of their taste properties. *Biosci Biotech Bioch* **1999,** *63* (3), 555-559.

6. Lindsay, R. C., Fish flavors. *Food Reviews International* **1990,** *6* (4), 437-455.

7. Ramalingam, V.; Song, Z.; Hwang, I., The potential role of secondary metabolites in modulating the flavor and taste of the meat. *Food Res Int* **2019,** *122*, 174-182.

8. Shumilina, E.; Ciampa, A.; Capozzi, F.; Rustad, T.; Dikiy, A., NMR approach for monitoring post-mortem changes in Atlantic salmon fillets stored at 0 and 4 degrees C. *Food Chem* **2015,** *184*, 12-22.

9. Lawless, H. T.; Heymann, H., Descriptive analysis. In *Sensory evaluation of food. Principles and practices*, 2nd ed.; Heldman, D. R., Ed. Springer: New York, 2010; pp 227-253.

10. Lauri, I.; Pagano, B.; Malmendal, A.; Sacchi, R.; Novellino, E.; Randazzo, A., Application of "magnetic tongue" to the sensory evaluation of extra virgin olive oil. *Food Chem* **2013,** *140* (4), 692-699.

11. Malmendal, A.; Amoresano, C.; Trotta, R.; Lauri, I.; De Tito, S.; Novellino, E.; Randazzo, A., NMR Spectrometers as "Magnetic Tongues": Prediction of Sensory Descriptors in Canned Tomatoes. *J Agr Food Chem* **2011,** *59* (20), 10831-10838.

12. Wei, F. F.; Furihata, K.; Miyakawa, T.; Tanokura, M., A pilot study of NMR-based sensory prediction of roasted coffee bean extracts. *Food Chem* **2014,** *152*, 363-369.

13. Podrazka, M.; Baczynska, E.; Kundys, M.; Jelen, P. S.; Witkowska Nery, E., Electronic Tongue-A Tool for All Tastes? *Biosensors (Basel)* **2017,** *8* (1).

14. Sundekilde, U. K.; Jarno, L.; Eggers, N.; Bertram, H. C., Real-time monitoring of enzyme-assisted animal protein hydrolysis by NMR spectroscopy - An NMR reactomics concept. *Lwt-Food Sci Technol* **2018,** *95*, 9-16.

15. Liaset, B.; Espe, M., Nutritional composition of soluble and insoluble fractions obtained by enzymatic hydrolysis of fish-raw materials. *Process Biochem* **2008,** *43* (1), 42-48.

16. Farmer, L. J., The role of nutrients in meat flavour formation. *Proc Nutr Soc* **1994,** *53* (2), 327-33.

17. Slizyte, R.; Opheim, M.; Storro, I.; Sterten, H., Simple Technologies for Converting Rest Raw Materials of Atlantic Salmon (Salmo salar) into High-Quality, Valuable, and Tasty Feed Ingredients. *J Aquat Food Prod T* **2017,** *26* (5), 604-619.

18. Gildberg, A.; Arnesen, J. A.; Carlehög, M., Utilisation of cod backbone by biochemical fractionation. *Process Biochem* **2002,** *38* (4), 475-480.

19. Cupp-Enyard, C., Sigma's Non-specific Protease Activity Assay - Casein as a Substrate. *J Vis Exp* **2008,** (19).

20. ISO Official Method 5983-2. Animal feeding stuffs - Determination of nitrogen content and calculation of crude protein content. Part 2: Block digestion and steam distillation method International Organization for Standardization, Geneva, 2009.

21. Sriperm, N.; Pesti, G. M.; Tillman, P. B., Evaluation of the fixed nitrogen-to-protein (N:P) conversion factor (6.25) versus ingredient specific N:P conversion factors in feedstuffs. *J Sci Food Agr* **2011,** *91* (7), 1182-1186.

22. Aspevik, T.; Egede-Nissen, H.; Oterhals, A., A Systematic Approach to the Comparison of Cost Efficiency of Endopeptidases for the Hydrolysis of Atlantic Salmon (Salmo salar) By-Products. *Food Technol Biotech* **2016,** *54* (4), 421-431.

23. Bligh, E. G.; Dyer, W. J., A Rapid Method of Total Lipid Extraction and Purification. *Can J Biochem Phys* **1959,** *37* (8), 911-917.

24. 5984-2, I., ISO Official Method 5984-2. Animal feeding stuffs - Determination of crude ash. International Organization for Standardization, Geneva, 2002.

25. 6496-2, I., ISO Official Method 6496-2. Animal feeding stuffs - Determination of moisture and other volatile matter content. International Organization for Standardization, Geneva, 1999.

26. Cohen, S. A.; Michaud, D. P., Synthesis of a fluorescent derivatization reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino-acids via high-performance liquid-chromatography. *Analytical Biochemistry* **1993,** *211* (2), 279-287.

27. Conway, E. J.; Byrne, A., An absorption apparatus for the micro-determination of certain volatile substances: The micro-determination of ammonia. *The Biochemical journal* **1933,** *27* (2), 419-29.

28. Miller, E. L., Determination of Tryptophan Content of Feedingstuffs with Particular Reference to Cereals. *J Sci Food Agr* **1967,** *18* (9), 381-&.

29. 8586-1, I., ISO Official Method 8586-1. Sensory analysis – General guidance for the selection, training and monitoring of assessors. International Organisation for Standardization, Geneva, 1993.

30. Naes, T.; Langsrud, O., Fixed or random assessors in sensory profiling? *Food Qual Prefer* **1998,** *9* (3), 145-152.

31. Langsrud, O., 50-50 multivariate analysis of variance for collinear responses. *J Roy Stat Soc D-Sta* **2002,** *51*, 305-317.

32. Langsrud, O., Rotation tests. *Stat Comput* **2005,** *15* (1), 53-60.

33. Moen, B.; Oust, A.; Langsrud, O.; Dorrell, N.; Marsden, G. L.; Hinds, J.; Kohler, A.; Wren, B. W.; Rudi, K., Explorative multifactor approach for investigating global survival mechanisms of Campylobacter jejuni under environmental conditions. *Appl Environ Microb* **2005,** *71* (4), 2086-2094.

34. van den Berg, R. A.; Hoefsloot, H. C. J.; Westerhuis, J. A.; Smilde, A. K.; van der Werf, M. J., Centering, scaling, and transformations: improving the biological information content of metabolomics data. *Bmc Genomics* **2006,** *7*, 142.

35. Mariotti, F.; Tome, D.; Mirand, P. P., Converting nitrogen into Protein - Beyond 6.25 and Jones' factors. *Crit Rev Food Sci* **2008,** *48* (2), 177-184.

36. Brenda The comprehensive enzyme information system. http://www.brenda-enzymes.org/ (accessed 16.09.19).

37. Liu, B. Y.; Zhu, K. X.; Peng, W.; Guo, X. N.; Zhou, H. M., Effect of sequential hydrolysis with endo- and exo-peptidase on bitterness properties of wheat gluten hydrolysates. *Rsc Adv* **2016,** *6* (33), 27659-27668.

38. Caro, Y.; Dhuique-Mayer, C.; Turon, F.; Pina, M.; Reynes, M.; Graille, J., The biocatalytic activity of bromelain in ester synthesis reaction: difference between the intrinsic lipase activity and thermal catalysis. *Biotechnol Lett* **2001,** *23* (24), 2035-2039.

39. Martinez, I.; Bathen, T.; Standal, I. B.; Halvorsen, J.; Aursand, M.; Gribbestad, I. S.; Axelson, D. E., Bioactive compounds in cod (Gadus morhua) products and suitability of H-1 NMR metabolite profiling for classification of the products using multivariate data analyses. *J Agr Food Chem* **2005,** *53* (17), 6889-6895.

40. Yamada, S., The biochemistry of anserine and carnosine degrading enzymes. In *Imidazole dipeptides: chemistry, analysis, function and effects*, Preedy, V. R., Ed. The Royal Society of Chemistry: Cambridge, UK, 2015; pp 118-138.

41. Kato, H.; Rhue, M. R.; Nishimura, T., Role of Free Amino-Acids and Peptides in Food Taste. *Acs Sym Ser* **1989,** *388*, 158-174.

42. Lynum, L., Nitrogenholdige ekstrativstoffer (English: Non protein nitrogen). In *Fisk som råstoff (English: Fish as raw material)*, 2 ed.; Tapir Akademisk Forlag: Trondheim, Norway, 1997; pp 67-70.

43. Nielsen, M. K.; Jorgensen, B. M., Quantitative relationship between trimethylamine oxide aldolase activity and formaldehyde accumulation in white muscle from gadiform fish during frozen storage. *J Agr Food Chem* **2004,** *52* (12), 3814-3822.

44. Josephson, D. B.; Lindsay, R. C., Enzymatic Generation of Volatile Aroma Compounds from Fresh Fish. *Acs Sym Ser* **1986,** *317*, 201-219.

45. Kirimura, J.; Shimizu, A.; Kimizuka, A.; Ninomiya, T.; Katsuya, N., Contribution of Peptides and Amino Acids to Taste of Foodstuffs. *J Agr Food Chem* **1969,** *17* (4), 689-&.

46. Schlichtherle-Cerny, H.; Grosch, W., Evaluation of taste compounds of stewed beef juice. *Z Lebensm Unters F A* **1998,** *207* (5), 369-376.

47. Kurihara, K., Umami the Fifth Basic Taste: History of Studies on Receptor Mechanisms and Role as a Food Flavor. *Biomed Res Int* **2015,** *2015*, 189402.

48. vandenOord, A. H. A.; vanWassenaar, P. D., Umami peptides: assessment of their alleged taste properties. *Z Lebensm Unters F A* **1997,** *205* (2), 125-130.

49. Macleod, G.; Seyyedain-Ardebili, M., Natural and Simulated Meat Flavors (with Particular Reference to Beef). *Crc Cr Rev Food Sci* **1981,** *14* (4), 309-437.

50. Shah, A. K. M. A.; Ogasawara, M.; Egi, M.; Kurihara, H.; Takahashi, K., Identification and sensory evaluation of flavour enhancers in Japanese traditional dried herring (Clupea pallasii) fillet. *Food Chem* **2010,** *122* (1), 249-253.

Table 1: Overview of the hydrolysates produced in the study.

Raw material	Enzyme	Hydrolysis time	Sample name
Cod	FoodPro PNL	10 min	Co-P-10
Cod	FoodPro PNL	50 min	Co-P-50
Cod	Bromelain	10 min	Co-B-10
Cod	Bromelain	50 min	Co-B-50
Chicken	FoodPro PNL	10 min	Ch-P-10
Chicken	FoodPro PNL	50 min	Ch-P-50
Chicken	Bromelain	10 min	Ch-B-10
Chicken	Bromelain	50 min	Ch-B-50
Salmon	FoodPro PNL	10 min	Sa-P-10
Salmon	FoodPro PNL	50 min	Sa-P-50
Salmon	Bromelain	10 min	Sa-B-10
Salmon	Bromelain	50 min	Sa-B-50

Table 2: Sensory attributes and their descriptions used in the descriptive analysis of fish and chicken protein hydrolysates.

Attribute	Description
Flavor intensity	Strength of all flavors in the sample
Sweet taste	Basic sweet taste (sucrose)
Salt taste	Basic salt taste (sodium chloride)
Sour taste	Basic sour taste
Bitter taste	Basic bitter taste
Umami taste	Basic umami taste
Acidic flavor	Related to a fresh, balanced taste from organic acids
Metallic flavor	Related to taste of metal (ferrous sulphate)
Chicken flavor	Related to taste of chicken meat
Swine flavor	Related to taste of swine/pork meat
Sea flavor	Related to taste of fresh, salty sea
Fish flavor	Taste of boiled white fish
Cloying flavor	Non-fresh, nauseating flavor
Rancid flavor	All rancid flavors (grass, hay, stearin, paint)
Fullness (mouthfeel)	Textural properties related to flow resistance
Astringent (mouthfeel)	Related to complex feeling of contractions and dryness of the mouth
Fatness (mouthfeel)	Surface textural property related to perception of fat in a product

Table 3: Amino acid and proximate composition (g kg^{-1}; N=2) of cod (*Gadus morhua*), chicken (*Gallus gallus domesticus*) and salmon (*Salmo salar*) muscle protein. Nitrogen to protein conversion factors were calculated based on Sriperm, et al. [21].

	Cod	**Chicken**	**Salmon**
Alanine	8.0 ± 0.3	12.2 ± 0.2	11.1 ± 0.2
Arginine	9.0 ± 0.4	14.2 ± 0.0	11.4 ± 0.1
Asparagine*	8.3	9.6	8.7
Aspartate	6.7 ± 0.5	10.7 ± 0.1	10.4 ± 0.3
Cysteine	1.9 ± 0.0	2.7 ± 0.0	2.6 ± 0.0
Glutamate	13.4 ± 0.7	21.4 ± 0.1	16.4 ± 0.2
Glutamine*	9.2	10.6	9.6
Glycine	6.1 ± 0.3	9.9 ± 0.7	9.3 ± 0.1
Histidine	3.0 ± 0.1	6.4 ± 0.0	5.4 ± 0.0
Isoleucine	7.0 ± 0.3	11.4 ± 0.1	9.9 ± 0.1
Leucine	12.2 ± 0.5	18.0 ± 0.2	15.7 ± 0.0
Lysine	13.5 ± 0.3	19.2 ± 0.1	17.4 ± 0.1
Methionine	5.1 ± 0.2	6.8 ± 0.1	6.6 ± 0.1
Phenylalanine	5.8 ± 0.2	8.9 ± 0.2	8.2 ± 0.1
Proline	4.6 ± 0.2	7.8 ± 0.2	6.6 ± 0.1
Serine	6.3 ± 0.3	8.6 ± 0.0	7.7 ± 0.0
Threonine	6.4 ± 0.2	10.1 ± 0.1	9.4 ± 0.1
Tryptophan	1.8 ± 0.0	3.1 ± 0.0	2.8 ± 0.0
Tyrosine	5.0 ± 0.3	7.1 ± 0.0	6.5 ± 0.2
Valine	7.3 ± 0.2	11.1 ± 0.1	10.7 ± 0.0
Total nitrogen	27.2 ± 0.2	36.6 ± 0.1	30.6 ± 0.1
NH3 (acid digest)	2.14 ± 0.02	2.47 ± 0.27	2.24 ± 0.03
Nitrogen to protein factor	5.3	5.3	5.2
Lipids	6.5 ± 0.1	30.5 ± 0.7	150 ± 1.7
Ash	11.7 ± 0.1	11.7 ± 0.1	12.0 ± 0.1
Dry matter	191.0 ± 1.3	258.5 ± 0.0	348.9 ± 1.1

*calculated based on released NH_3 and assuming a 1:1 ratio of released NH_3 between Asp:Glu [22].

493 Table 4: Proximate molecular weight distribution (MWD) and nitrogen levels (N) of hydrolysates
494 made from cod (Co), chicken (Ch) and salmon (Sa) muscle protein, with the proteases Bromelain
495 (B) and FoodPro PNL (P) for 10 and 50 minutes.

MW (kDa) (%)*	**Co-P-10**	**Co-P-50**	**Co-B-10**	**Co-B-50**	**Ch-P-10**	**Ch-P-50**	**Ch-B-10**	**Ch-B-50**	**Sa-P-10**	**Sa-P-50**	**Sa-B-10**	**Sa-B-50**
>20	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
15-20	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
10-15	0.6	< 0.1	< 0.1	< 0.1	0.3	0.2	< 0.1	< 0.1	0.3	< 0.1	< 0.1	< 0.1
8-10	1.0	0.3	0.1	< 0.1	0.5	0.2	0.2	< 0.1	0.7	0.2	0.2	< 0.1
6-8	2.8	1.1	1.0	0.2	1.8	0.7	1.0	< 0.1	2.1	0.8	1.0	0.2
4-6	8.3	4.3	5.9	2.2	5.2	2.7	4.9	2.1	5.5	2.9	4.1	1.6
2-4	22.9	16.2	23.1	14.0	12.8	9.8	17.1	10.5	14.7	10.7	15.8	9.1
1-2	18.2	20.9	24.6	24.4	12.7	13.4	19.2	17.4	14.3	16.0	19.8	18.5
0.5-1	12.1	18.7	16.1	23.6	9.9	13.9	14.2	19.2	11.3	17.2	14.6	21.7
0.2-0.5	9.8	16.3	8.3	17.1	13.4	17.3	12.0	19.2	14.0	19.1	14.1	22.7
<0.2	24.2	22.2	20.8	18.5	43.3	41.8	31.4	31.3	37.0	33.0	30.4	26.3
N (g $100g^{-1}$)**	0.6	0.8	0.6	0.9	0.6	0.9	0.9	1.2	0.6	0.9	0.7	1.0

496 *Repeatability (r = sr*2.8) limits for duplicate acceptance: >20 kDa = 0.5, 10-20 kDa = 0.1, 8-10 kDa = 0.0, 0.2-8 kDa = 0.1,
497 < 0.2 kDa = 0.2
498 ** Duplicate sample variation ≤ 0.01 g $100g^{-1}$

499 Table 5: Mean sensory intensity values* in hydrolysates based on chicken (Ch), cod (Co) and salmon (Sa) muscle protein with

500 Bromelain (B) or FoodPro PNL (P) for 10 and 50 min**.

Product	Flavor Intensity	Sweet taste	Salt taste	Sour taste	Bitter taste	Umami taste	Acidic flavor	Metallic flavor	Chicken flavor	Pork flavor	Sea flavor	Fish flavor	Cloying flavor	Rancid flavor	Fullness	Astringent	Fatness
Co-P-10	5.2 ^{b}	2.0 ^{a}	2.1 ^{ab}	2.0 ^{b}	5.0 ^{bcd}	3.0 ^{cd}	1.6 ^{b}	4.2 ^{a}	1.8 ^{c}	1.4 ^{a}	1.1 ^{a}	4.2 ^{ab}	3.4 ^{a}	1.4 ^{a}	2.8 ^{ab}	3.3 ^{b}	1.9 ^{a}
Co-P-50	5.9 ^{ab}	2.0 ^{a}	2.3 ^{ab}	2.1 ^{b}	6.1 ^{abc}	3.5 ^{bcd}	1.5 ^{b}	4.1 ^{a}	2.8 ^{abc}	1.7 ^{a}	1.1 ^{a}	3.5 ^{abc}	3.4 ^{a}	1.2 ^{a}	2.8 ^{ab}	4.1 ^{ab}	1.9 ^{a}
Co-B-10	5.6 ^{ab}	1.9 ^{a}	2.2 ^{ab}	2.1 ^{b}	5.7 ^{abcd}	2.9 ^{d}	1.5 ^{b}	4.1 ^{a}	2.0 ^{c}	1.5 ^{a}	1.0 ^{a}	3.2 ^{abc}	3.7 ^{a}	1.7 ^{a}	2.6 ^{ab}	3.6 ^{ab}	1.8 ^{a}
Co-B-50	6.4 ^{ab}	1.8 ^{a}	1.9 ^{b}	2.6 ^{ab}	6.9 ^{a}	3.0 ^{d}	1.8 ^{ab}	4.7 ^{a}	2.2 ^{bc}	1.5 ^{a}	1.2 ^{a}	5.1 ^{a}	3.9 ^{a}	1.1 ^{a}	2.5 ^{b}	4.2 ^{ab}	1.9 ^{a}
Ch-P-10	6.0 ^{ab}	2.4 ^{a}	3.1 ^{a}	3.0 ^{ab}	4.6 ^{cd}	5.3 ^{a}	2.9 ^{a}	4.1 ^{a}	4.4 ^{a}	2.0 ^{a}	1.0 ^{a}	2.0 ^{bc}	2.9 ^{a}	1.0 ^{a}	3.2 ^{ab}	3.6 ^{ab}	2.3 ^{a}
Ch-P-50	5.7 ^{ab}	2.1 ^{a}	2.9 ^{ab}	3.1 ^{ab}	4.9 ^{cd}	4.3 ^{abcd}	2.7 ^{ab}	4.5 ^{a}	3.3 ^{abc}	1.9 ^{a}	1.0 ^{a}	2.9 ^{abc}	2.8 ^{a}	1.1 ^{a}	3.3 ^{ab}	3.7 ^{ab}	2.0 ^{a}
Ch-B-10	6.1 ^{ab}	2.3 ^{a}	2.9 ^{ab}	3.2 ^{ab}	5.5 ^{abcd}	4.4 ^{ab}	2.7 ^{ab}	4.1 ^{a}	4.0 ^{ab}	2.0 ^{a}	1.0 ^{a}	1.8 ^{c}	2.9 ^{a}	1.2 ^{a}	3.6 ^{a}	3.8 ^{ab}	2.1 ^{a}
Ch-B-50	6.5 ^{ab}	2.4 ^{a}	2.9 ^{ab}	3.3 ^{a}	6.1 ^{abc}	4.4 ^{abc}	2.3 ^{ab}	4.4 ^{a}	3.5 ^{abc}	2.2 ^{a}	1.0 ^{a}	1.7 ^{c}	3.2 ^{a}	1.3 ^{a}	3.3 ^{ab}	3.9 ^{ab}	2.2 ^{a}
Sa-P-10	5.9 ^{ab}	2.5 ^{a}	3.0 ^{ab}	2.8 ^{ab}	4.2 ^{d}	4.7 ^{ab}	2.7 ^{ab}	4.3 ^{a}	3.3 ^{abc}	1.2 ^{a}	1.1 ^{a}	3.5 ^{abc}	2.7 ^{a}	1.2 ^{a}	3.4 ^{ab}	3.3 ^{ab}	2.2 ^{a}
Sa-P-50	6.7 ^{ab}	2.4 ^{a}	3.0 ^{ab}	2.8 ^{ab}	5.8 ^{abcd}	4.1 ^{abcd}	2.1 ^{ab}	4.6 ^{a}	2.4 ^{bc}	1.3 ^{a}	1.2 ^{a}	5.0 ^{a}	3.7 ^{a}	1.4 ^{a}	3.5 ^{ab}	4.0 ^{ab}	2.4 ^{a}
Sa-B-10	6.4 ^{ab}	2.3 ^{a}	3.2 ^{a}	3.1 ^{ab}	6.0 ^{abc}	4.0 ^{abcd}	2.0 ^{ab}	4.6 ^{a}	2.7 ^{abc}	1.5 ^{a}	1.1 ^{a}	4.0 ^{abc}	3.9 ^{a}	1.7 ^{a}	3.5 ^{a}	4.2 ^{ab}	2.3 ^{a}
Sa-B-50	6.9 ^{a}	2.1 ^{a}	2.7 ^{ab}	3.4 ^{a}	6.9 ^{ab}	3.7 ^{bcd}	1.8 ^{ab}	4.8 ^{a}	2.5 ^{bc}	1.3 ^{a}	1.0 ^{a}	5.0 ^{a}	4.3 ^{a}	2.0 ^{a}	3.1 ^{ab}	4.7 ^{a}	2.1 ^{a}
p (product)	0.010	0.047	0.001	<0.001	<0.001	<0.001	<0.001	0.084	<0.001	0.020	0.440	<0.001	0.125	0.035	0.002	0.049	0.201

501 *Mean sensory attribute intensity provided by duplicate evaluations by eight panelists for each product.

502 **Different letters indicate statistical difference ($p<0.05$) between the products by two-way mixed effects model ANOVA and Tukey's

503 comparison test.

Table 6: Significance of hydrolysis parameters (Enzyme: Bromelain and Food Pro PNL, Raw material: Chicken, salmon and cod, Time: 10 and 50 min) on attribute intensity.

Attribute	Enzyme	Raw material	Time
Flavor intensity			*
Acidic		**	
Sweet		*	
Salt		**	
Sour		**	
Bitter	**		***
Umami		***	
Chicken		**	
Swine		*	
Fish		***	*
Rancid	**		
Fullness		**	
Astringent			*

*p ≤ 0.05, ** p ≤ 0.01, ***p ≤ 0.001

Table 7: Fifty-fifty MANOVA of ^{1}H NMR spectra acquired from protein hydrolysates based on different raw materials (cod, salmon and chicken), two enzymes (Bromelain and Food Pro PNL) and 10 and 50 min of hydrolysis time. Rotation testing was used to compute familywise adjusted single response p-values.

Source	Degrees of freedom (df)	Explained variance (%)	p-value[a]	# NMR shifts significantly affected[b]
Raw material	2	51	<0.001	240
Enzyme	1	17	<0.001	44
Hydrolysis time	1	13	<0.001	6
Residuals	7	19		

[a] p-values estimated by 50-50 F-test.
[b] familywise adjusted p-values. limit 0.05

511 Table 8: Summary of the partial least squares (PLS) models based on NMR data and attribute

512 intensity

	Calibration observations			Calibration fit	Predictive ability
Attribute model	F[a]	R^2	Q^2	RMSEC (Y)[b]	RMSECV (Y)[c]
Flavor intensity	7	0.99	0.68	0.01	0.28
Bitter taste	2	0.86	0.71	0.29	0.47
Sweet taste	1	0.80	0.68	0.10	0.14
Salt taste	1	0.90	0.93	0.13	0.18
Sour taste	4	0.98	0.89	0.06	0.16
Umami taste	2	0.93	0.80	0.19	0.36
Acidic flavor	3	0.93	0.75	0.13	0.27
Chicken flavor	3	0.89	0.55	0.26	0.57
Pork flavor	4	0.93	0.66	0.08	0.21
Fullness (mouthfeel)	1	0.88	0.80	0.12	0.17
Fatness (mouthfeel)	1	0.91	0.82	0.05	0.08

[a] Number of factors included in model, [b] Root mean squared error of calibration, [c] Root mean squared error of cross validation

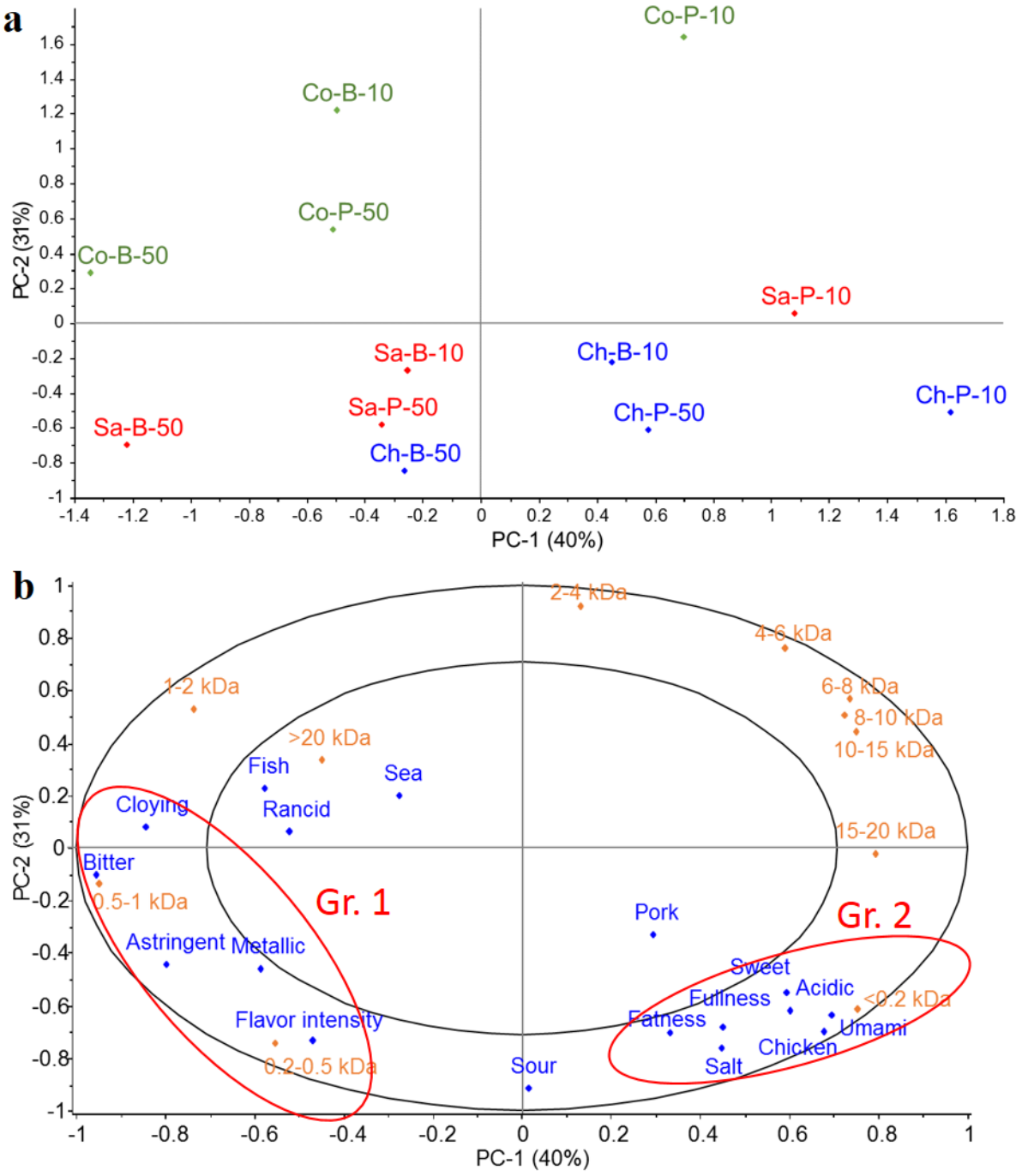
513 Table 9: Compounds found to be negatively (-) or positively (+) associated with sensory

514 attributes ($p < 0.05$) based on partial least squares (PLS) modelling of sensory attribute intensity

515 and NMR spectroscopy data.

Compund	ppm	Bitter	Chicken	Fullness	Acidic	Sweet	Salt	Umami	Sour	Fatness	Pork	Flavor int.
Isoleucine	0.93	+										+
Leucine	0.95	+										+
Valine	0.98/1.03							+				
Lactate	1.32/4.11	-	+	+	+	+	+	+	+	+		
Alanine	1.47		+	+	+	+	+	+	+	+		
Glutamate	2.34/3.76		+								+	
Anserine	2.69/3.78/7.12			+		+	+	+	+	+		
TMA	2.87				-							
Creatine	3.02/3.92										-	
Glycerol	3.55/3.65	-										
IMP	6.08/8.22/8.34	-			+			+				-
Tyrosine	6.89/7.18		+		+			+			+	

516



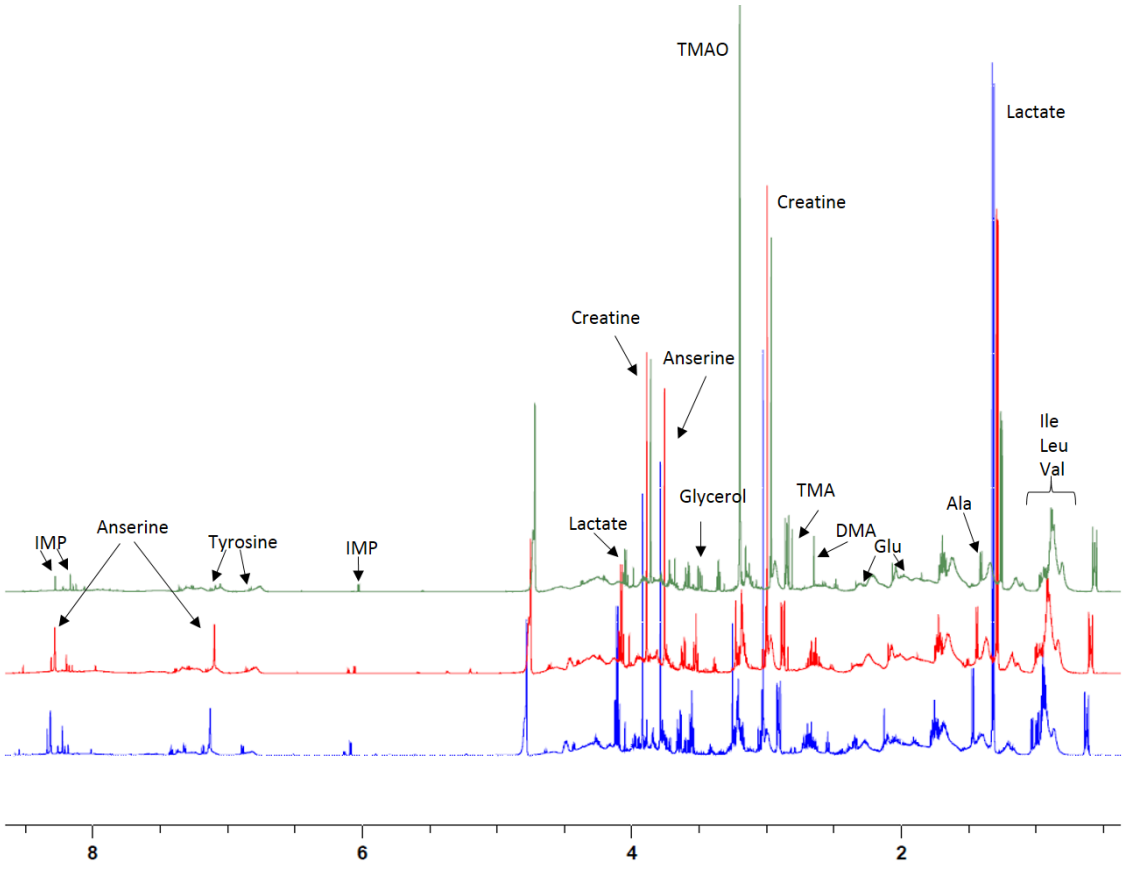
517

518 Figure 1: Principal component analysis score plot (a) shows similarities and differences between

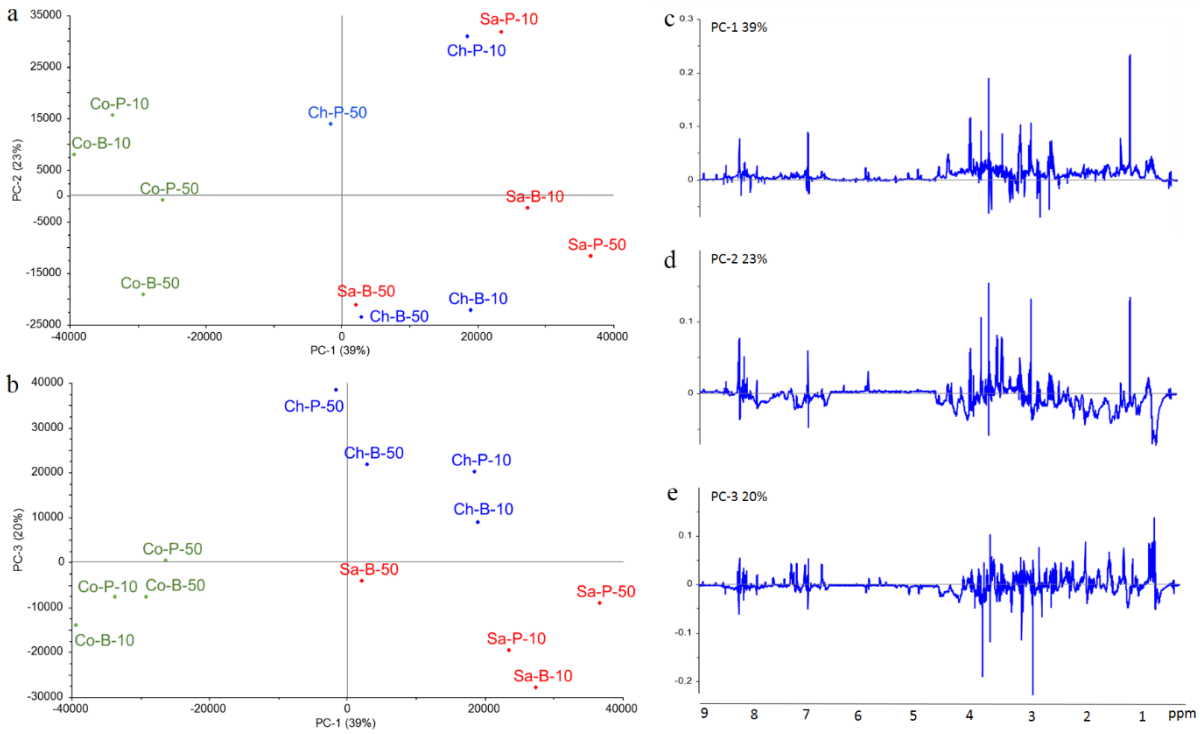
519 hydrolysate products based on cod (Co), salmon (Sa) and chicken (Ch) muscle protein with

520 Bromelain (B) or FoodPro PNL (P) for 10 or 50 min. The correlation loading plot (b) illustrates

associations between molecular weight distribution and sensory data. Group 1 contains unpalatable tastes and flavors associated to 0.2-1 kDa molecules. Group 2 contains tastes and flavors considered palatable and associated to molecules less than 0.2 kDa.



524 Figure 2: Proton NMR spectra acquired from protein hydrolysates based on cod (green), salmon
525 (red) and chicken (blue) hydrolyzed with Food Pro PNL for 50 minutes (horizontal offset: 0.04
526 ppm). Unambiguously identified peaks are marked.



527

528 Figure 3: Principal component analysis (PCA) score plot (a: PC1 and PC2, b: PC1 and PC3), and
529 loading plot (c: PC1, d: PC2, e: PC3) of NMR-data. The score plot illustrates the association
530 between hydrolysates from cod (Co), salmon (Sa) and chicken (Ch) muscle protein with
531 Bromelain (B) or FoodPro PNL (P) for 10 or 50 min, based on peak intensities in the NMR
532 spectra. The loading plots show how the various peaks influence the three principal components
533 included in the figure (negatively or positively, and to what degree). Peak assignment
534 corresponding to the ppm scale in the loading plots are given in Figure 2.

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