Meat Science 150 (2019) 65–76

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Consumer perception of snack sausages enriched with umami-tasting meat protein hydrolysates

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ARTICLE INFO

Keywords:
Functional ingredients
Meat protein hydrolysates
Umami
Sausages
Consumer acceptance

ABSTRACT

Adequate protein intake is necessary to maintain muscle mass and function. Nutritionally dense(r) products are increasingly sought after by consumers that need a heightened protein intake, such as active young and elderly individuals. This paper focuses on functional snack sausages enriched with umami-tasting meat protein hydrolysates (MPH), developed by systematically varying in recipe, meat type and MPH content. A consumer study (N = 100) was conducted where young and elderly consumers evaluated perceived acceptability and sensory quality of samples. Additionally, textural (Warner–Braztler shear test) and chemical (amino acids) analyses on the same samples were conducted to give a complete characterization of the functionality of the hydrolysates. Both the consumer test and the instrumental analyses consistently indicated that the enrichment with MPH had minor or no influence on the perceptual quality of the sausages, suggesting that this ingredient can be added to increase the nutritional density of a reference meat product without negatively affecting consumer acceptability.

1. Introduction

Proper nutrition is essential for physical health and consumption of dietary protein is pivotal for the maintenance of muscle mass as they provide essential amino acids. Increasing awareness of the importance of an adequate protein intake in humans (Morais, Chevalier, & Gougeon, 2006) has moved the food industry towards the development of new protein-enriched functional foods, designed to cater to specific consumer segments interested in improving and maintaining muscle mass, such as athletes and elderly (Vegari, Tibuzzi, & Basile, 2010).

Accordingly, foods with increased protein content are currently one of the fastest-growing product categories targeting particularly image- and health-focused consumers (Banovic et al., 2018). Previous research has shown that younger (18–35) consumer who regularly exercise tend to be especially interested and knowledgeable regarding dietary protein, their sources, as well as their role in muscle building (Childs, Thompson, Lillard, Berry, & Drake, 2008). Young consumers also tend to be the most positively predisposed towards high protein functional foods, which is way a substantial part of these products is currently targeted at this segment (Childs et al., 2008; Kreger, Lee, & Lee, 2012; Oltman, Lopetcharat, Bastian, & Drake, 2015).

Aging and elderly consumers are another strategic market segment for protein containing and protein-enriched functional foods, both from a commercial and public health perspective. Compared to young people, the need for dietary protein in elderly people is increased because of the age-related loss of muscle mass, known as *sarcopenia* (Giacalone et al., 2016; Meinert, Broge, de, Bejerholm, & Jensen, 2015). Essential amino acids are primarily accountable for the amino acid-induced stimulation of muscle protein anabolism in elderly (Volpi, Kobayashi, Sheffield-Moore, Mittendorfer, & Wolfe, 2003). The European Union Geriatric Medicine Society (EUGMS) in cooperation with other scientific organizations conducted an international study to review the dietary protein needs in elderly (PROT-AGE Study Group), concluding that elderly citizens should consume an average intake between 1.0 and 1.2 g/protein/kg/day (Bauer et al., 2013). Meeting this recommendation is both a public health challenge and a potentially huge opportunity for the food industry (Giacalone et al., 2016): according to a recently released study by Eurostat (2017) in the European Union, already 19.2% of the population is over 65 years old, and this percentage is expected to rise sharply in the near future.

Proteins can be consumed from natural food products such as dairy, eggs or meat. A common approach in food product development lies in the enrichment of conventional food products with functional products such as, protein isolates, concentrates and hydrolysates (Baugreet,

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https://doi.org/10.1016/j.meatsci.2018.12.009
Received 25 January 2018; Received in revised form 10 November 2018; Accepted 17 December 2018
Available online 19 December 2018

Kerry, Botinestean, Allen, & Hamill, 2016; Meinert et al., 2015). This approach is very prominent in the dairy industry, and common functional ingredients well described in the literature include for example whey protein supplements in the context of sports nutrition.

Meat proteins, on the other hand, have received less attention in the literature, despite having similar nutritional value and application potential to their dairy counterparts. Meat protein hydrolysates (MPH) are produced from slaughterhouse side streams (Martinez-Alvarez, Chamorro, & Brenes, 2015), where some of the remaining parts of the animals are used to extract valuable proteins through enzymatic hydrolysis to create a pure protein supplement. In addition to being a convenient way to increase daily protein intake, recent research has linked MPH to potential health benefits for the consumers, e.g. ACE-inhibitors[1] were recently found in MPH, which could help reducing hypertension (Meinert et al., 2015).

Not much is known regarding how MPH behaves in food applications. In general, MPH are characterized by umami taste, associated with meaty and savory sensations (Zhang, Klebansky, Fine, et al., 2008). Through the hydrolysis process, some MPH possess a certain bitterness compared to other hydrolysates (Aehle, 2007; Fitzgerald & O'Cuinn, 2006; Saha & Hayashi, 2001), although the specific properties of the MPH depend on the used raw materials and the chosen manufacturing process, as well as on the amino acids composition. This may often pose some challenge in a product development context, as one might expect that the bitter taste might reduce the consumer acceptability of MPH-enriched products. Conducting an enzymatic hydrolysis is often favored because a more precise control of the degree of hydrolysis[2] (DH) can be made as well as a resulting peptide and amino acid profile (Lahl & Braun, 1994). Through higher and more extensive hydrolysis functional properties such as stability, viscosity and gel-forming ability decrease (Mahmoud, 1994). Nevertheless, flavor contribution is increased due to release of amino acids and some peptides. This means that enzymatic hydrolysates are well suited for flavor and flavor enhancer as well as for protein supplement (Synowiecki, Jagielka, & Shahidi, 1996) and can have minimal influence of the texture of a product for possible food application. Further, it has been shown that umami taste increases the appetite in elderly people (Mouritsen, 2012).

The application of MPH in human food is not common yet, and there is currently an interest from both industry and academia to explore the added value from this ingredient in food applications (Meinert, Tøstesen, Bejerholm, Jensen, & Støier, 2013, 2015). As already mentioned, while there is an extensive literature on dairy protein hydrolysates, there is currently a dearth of research on MPH; in particular, there is little to no published research documenting the behavior of these ingredients in selected food applications.

To address this gap in research, the aim of this work was to explore the potential of umami tasting meat protein hydrolysates as a functional food ingredient. The focal meat product was snack sausages (70 g) from beef and pork. Sausages are a familiar meat item within the Danish diet, with familiarity being strongly related to product acceptance of functional foods (Ares, Giménez, & Gámbaro, 2009). The choice of focusing on snack sausages was motivated by existing data on Danish dietary patterns showing an adequate total daily intake of dietary protein, but also that this is primarily concentrated within meals, especially dinner (DTU National Food Institute, 2015). Recent research, however, suggests that more frequent and more evenly distributed protein consumption throughout the day is associated with better muscle mass and functionality (Loenneke, Loprinzi, Murphy, & Phillips, 2016; Tessier & Chevalier, 2018). Hence, functional snack sausages could be a good option for consumers who are looking for protein-rich snack options that are also quick and convenient.

Situated within this context, this paper reports on 1) sensory, 2) textural and 3) compositional changes associated with MPH enrichment in beef and pork snack sausages, as well as 4) the effect of MPH enrichment on consumer acceptability.

2. Materials and methods

2.1. Samples

2.1.1. Meat protein hydrolysates

Danish Crown Ingredients, which develops novel feed and food ingredients based on animal side streams from the various Danish Crown slaughterhouse operations, developed umami tasting MPH used as functional ingredient in this study. Two MPH were produced from lean beef and pork meat respectively. The hydrolysis was performed by heating the slurried minced raw material in water to 55 °C – 60 °C. An enzyme (confidential) was added and incubated at 53 °C – 56 °C. The hydrolysis process was stopped by heating the mixture to 90 °C, followed by fat separation. The resulting MPH were concentrated and spray dried. Proximate analysis showed that the MPH had the following composition: ≥82% crude protein, < 10% fat, < 2% carbohydrates, < 10% ash and ≥95% dry matter. Identical values were obtained for both beef and pork MPH.

2.1.2. Sausage recipe and preparation

The snack sausage samples were processed as a low fat industrial recipe. The ingredients were: minced meat (Beef or Pork), iced water, MPH, spice blends (Normal (Classic frankfurter blend), Merguez or Curry), vacuum salt, nitrite salt, phosphate and ascorbic acid. Three design factors (Table 1) recipe (“Normal”, “Curry”, “Merguez”), meat type (Beef or Pork) and MPH concentration (0%, 7.5%, 12.5%) were systematically varied during the product development. The beef sausages were enriched with beef protein hydrolysates, whereas pork sausages were enriched with pork protein hydrolysates. The choice of using three difference recipes allowed us to span a sensory range that is representative of the current market, as well as to evaluate whether product familiarity could have an impact on the acceptability of the sausages by comparing a familiar recipe (Normal) to a moderately novel (Curry) and a very novel one (Merguez). Furthermore, the interest in testing the Curry and Merguez recipes was that they both contain trigeminal stimulants (e.g., capsaicin) which have the potential to suppress potential bitterness associated with the hydrolysis process (Ley, 2008; Simons, Boucher, & Carstens, 2003). All ingredients were mixed using an industrial blending machine. MPH was slurried in iced water before the addition. The mix were filled into sheep casings (DAT-Schaub) dried, smoked, boiled and chilled to 10° before packing. Only one batch per type of sausage was produced, from which all samples used in the instrumental and sensory tests came from. The overall experimental design used for developing the test sausages is shown in Table 1, which reports the combinations of the design factors developed for this study, as well as the abbreviations for the samples used in the remainder of the paper.

Table 2 reports a nutritional analysis of each sausage type where all data is referred to 100 g of the sample (data refers to the “Normal” recipe from beef and pork). As shown in Table 2, pork sausages contained more fat than beef sausages. Their fat content was slightly above 10% fat, which is usually the maximum threshold used for describing sausages as “low fat” (for reference, pork sausages in Denmark typically contain up to 20–25 g fat per 100 g). The beef sausages generally contained more water and protein than the pork sausages, except for the samples with 0% MPH.

The sausage samples used for texture analysis and the consumer test were pre-cooked and frozen. Prior to testing, the sausages were thawed

[1] A substance that inhibits the activity of angiotensin converting enzyme and which is used in the treatment of hypertension and heart failure (Lant, 1987).

[2] Degree of hydrolysis can be expressed as the ratio of amino nitrogen to total nitrogen (AN/TN), or percent of peptide bonds cleaved, which is a measure of the extent of hydrolytic degradation of protein (Mahmoud, 1994).

Table 1
Design used to formulate the samples varying in meat type (beef, pork), MPH content (0%, 7.5%, 12.5%) and recipes (Normal, Merguez, Curry). Row values indicate sample names used in the reminder of the paper.

		Meat type					
		Pork			Beef		
		Normal	Merguez	Curry	Normal	Merguez	Curry
Protein Content	Ref(0%)	No_0_P			No_0_B		
	7.5%	No_7.5_P	Mg_7.5_P	Cu_7.5_P	No_7.5_B	Mg_7.5_B	Cu_7.5_B
	12.5%	No_12.5_P	Mg_12.5_P	Cu_12.5_P	No_12.5_B	Mg_12.5_B	Cu_12.5_B

Table 2
Nutritional information on the test sausages (Normal recipe).

Meat type		Fat (g/100 g)	Protein (g/100 g)	Carbohydrates (g/100 g)	Water (g/100 g)
Beef	0.0%	7.8	16.7	0.4	72.24
	7.5%	5.1	22.1	< 0.3	69.09
	12.5%	5.5	25.2	< 0.3	66.42
Pork	0.0%	11.6	18.1	< 0.3	67.69
	7.5%	12.0	20.1	< 0.3	64.63
	12.5%	11.2	23.6	< 0.3	61.24

at 5 °C over night, then placed on trays and prepared for about 7 min in a steam cooking oven, preheated to a temperature of 130 °C.

2.2. Instrumental analyses

2.2.1. Texture analysis (Warner-Bratzler shear test)

To give a quantitative measure of the firmness of the re-heated sausages, the force necessary to shear the sausages was measured using a Texture Analyzer (Stable Micro Systems, Godalming, UK) equipped with a 3 mm thick blunt steel blade with a 73° V cut into the lower edge (Warner-Bratzler blade). The blade was lowered into a 4 mm wide slit in a small table making sure that it was not touching the sides of the slit. The sausages were placed on the table below the blade, which was lowered at a constant speed of 2 mm/s and using a load cell of 5 kg (max. force 50 N). Once a trigger force of 0.2 N was reached, data collection started and the force (resistance to shearing) vs. the (deformation) distance was plotted until a max. distance of 30 mm. The blade then retracted to its initial position. We evaluated the maximum shear force, as well as the total shear work required to cut through the sausage.

Each sausage was cut three times – once in the middle, and then in the middle of the resulting halves. Three sausages ($n = 3$) of each type were cut, thus giving a total of nine cuts pr. type of sausage.

2.2.2. Amino acidic (glu and asp) analysis (HPLC)

Cooked frozen sausages were thawed, had the skin removed and were finely minced adding 30% water to ease the process. 6 g of the sausage meat mixture was transferred to a 50 mL centrifuge tube and 20 mL 0.1 M HCl (Sigma Aldrich, Brøndby, Denmark) was added. The tubes were shaken on an orbital shaker at 5 °C over night. Afterwards, they were centrifuged for 10 min at 5000 rpm (Sorval ST8, Thermo Fisher, Slangerup Denmark). The supernatant was then transferred to clean centrifuge tubes and prior to analysis, protein was precipitated from the samples by mixing 600 µL of the samples with 600 µL of 0.4 mM perchloric acid (Sigma Aldrich). The samples where then stored for 10 min at 5 °C before they were centrifuged for 10 min at 14000 rpm. After this they were filtered through a 0.2 µm RC filter (Phenex, Phenomenex, Værløse, Denmark) into a Sarsted 96 well plate (In-Vitro, Fredensborg Denmark). Extractions were performed in duplicate ($n = 2$).

Detection and quantification of the free amino acids (glutamate and aspartate) were done by HPLC-MS using a Shimadzu LCMS 2020 MS equipped with an electrospray interface (ESI) (Mouritsen, Calleja, Duelund, & Frøst, 2017). The HPLC consisted of a DGU20-A5 in-line degasser, two LC-20 CE pumps, a high-pressure mixer, an SIL20A-HT autosampler, a CTO 10 Column oven, and an SPD-20A UV detector, all from Shimadzu (Holm & Halby Brøndby, Denmark). Amino acid separation was performed on a 75x3mm Imtak Intrada amino acid column (Imtakt Corporation, Kyoto, Japan) by a gradient of acetonitrile with 0.1% formic acid and 100 mM NH_4HCO_2 over a time course of 30 min. The gradient consisted of first 4 min with 14% 100 mM NH_4HCO_2 followed by an increase of the 100 mM NH_4HCO_2 to 100% over the next 16 min. This level was then maintained for 2 min, and subsequently returned to 14% over 3 min, and allowed to re-equilibrate for the rest of the time.

Individual amino acids were detected by selected ion monitoring (SIM) at appropriate m/Z values and were quantify by comparison with a standard curve prepare from commercial available amino acid standard from Sigma-Aldrich. All samples were diluted 10-fold with 0.1 M HCl. Each extract was measured twice.

2.3. Consumer test

2.3.1. Participants

One-hundred consumers (61 men and 39 women) participated in the consumer test on a voluntary basis. All were regular consumers of sausages and did not have any food allergies (self-reportedly). The majority ($N = 79$, 37% women) were young (Age 21–39, Mean = 26.8 ± 3.6), physically active consumers, recruited through an internal database at the University of Southern Denmark. The remaining consumers ($N = 21$, 61% women) were elderly (Age 68–93, Mean = 77.1 ± 6.7), independently living and physically active, and were contacted with assistance from a local rehabilitation center located in central Odense.

2.3.2. Experimental procedures

The consumer test was conducted in a central location test (CLT) facility at the University of Southern Denmark. The lighting and temperature of the room was always held constantly to have identical conditions in each session (Lawless & Heymann, 2010). A maximum of 12 participants per session were included. Upon arrival, participants were invited to seat and received a short introduction by the experimenters.

After re-heating sausages, samples were cut and distributed on an individual serving tray for each participant. To avoid fatigue, a reduced experimental design, where each participant tested seven (out of 14) samples, was used for the consumer test. One sample cup consisted of three to four small sausage pieces (approximately 15 g) in a 4 oz. aluminum foil cup. The seven samples were placed on individual trays so the consumers could taste them in the order specified on the tray. The serving order was obtained using a repeated William's Square design with 14 treatments split into 2, resulting into a design where each sample was evaluated an equal amount of times in total, as well as an equal amount of time in each serving position. Each sample was blind labeled with three-digit-number printed on each cup.

Crispbread and water were provided to all participants to cleanse their palate in between samples.

The participants tasted each sample monadically and filled out a questionnaire. The first part of the questionnaire consisted of seven sheets (one for each sample), where consumers were asked to first evaluate the overall acceptability on a 9-point hedonic scale, and then to complete a check-all-that-apply (CATA) questionnaire with the following 20 attributes: *Artificial*, *Bitter*, *Cozy*, *Dry*, *Exceptional*, *Familiar taste*, *Fat*, *Hard texture*, *High quality*, *Hot*, *Intense*, *Mild*, *Pleasant*, *Salty*, *Soft texture*, *Sour*, *Spicy*, *Sweet*, *Umami (meaty)*, *Well balanced*. Attributes were developed with point of departure on existing sensory literature on processed meat (e.g. Meinert et al., 2015), and later refined and modified based on qualitative tastings among the authors and collaborators. A pilot test ($N = 10$) was conducted to confirm the applicability of the attributes to the test samples and that they were easily understood by the consumers.

The order in which the attributes appeared on the ballot was randomized between each sample and between each consumer to avoid order biases (Ares & Jaeger, 2013). Consumers further rated how close each sample was to a sausage they would normally eat, on a 5-point scale (1 = Not close, 5 = Very close) and completed another CATA questionnaire which asked them to check all situations in which they would eat each individual sample of a list with eight options: *Before/after training*, *Breakfast*, *Brunch*, *Between meals*, *Dinner*, *Lunch*, *Snack*, *Special events*.

The second part of the questionnaire consisted of questions on the participants' background such basic demographics, questions about their level of exercise as well as their frequency consumption of sausages and in what situations participants consume sausages. Finally, participants were asked some questions about their consumption and attitudes towards protein-enriched food (these data are not included in the paper and are only reported for completeness).

2.4. Data analysis

All statistical analyses were conducted in the `R` (R Core Team, 2017), using either native functions or functions from the `FactoMineR` package (Lê, Josse, & Husson, 2008). For analyses of inferential nature, a significance level of $p < 0.05$ was considered.

2.4.1. Instrumental data (Texture analyzer and HPLC)

Regarding the data from the Warner-Bratzler shear test, mean Peak Positive Force (hardness) and total positive area (consistency) between the test sausages were compared by means of Analysis of Variance (ANOVA). For each response variable, separate 2-way ANOVA models were run using samples and the three design factors as main effects, whereas replicate and location of the cut were used as random effects. Where significant main effects were found, pairwise comparisons were carried out by Tukey's Honestly Significant Difference (HSD) test to uncover significant differences between individual means.

Glutamate and aspartate concentrations were determined from the MS chromatograms, and corrected for various dilutions, reporting the extracted amino acid content in mg per 100 g of sausage. The mean values were calculated from the two different extractions each measured in duplicate.

2.4.2. Consumer test data (CATA and liking)

CATA data were coded as binary (1/0) depending on whether an attribute was checked or not. A contingency table crossing attributes and samples was then obtained, containing the frequency of each mentioned attribute for each test sausage. To uncover which of the CATA attributes significantly discriminated between the samples, a Chi-squared test (with Yates continuity correction) was conducted. Furthermore, multivariate relations between attributes and samples were visually explored by means of correspondence analysis (Greenacre, 2017).

To investigate differences in acceptability, means of overall liking were analyzed by analysis of variance (ANOVA) using samples as main effect. To provide a more conservative estimation of main effects, consumers were included in the model as random effect. An additional 3-way ANOVA was carried out to investigate the main effect and the three design factors (meat type, protein content, recipe) as main effects, again including consumers as random effect.[3] To consider a possible effect of the consumer background on acceptability, an additional ANOVA model using gender, age, and their interactions as main effect, with individual consumers as random. Where significant main effects were found, pairwise comparisons were carried out by Tukey's Honestly Significant Difference (HSD) test to uncover significant differences between individual means.

Finally, in order to uncover driver of liking/disliking, a penalty analysis was conducted to evaluate the drop (or increase) in mean liking associated to the presence (or absence) of each individual CATA attributes (Ares, Dauber, Fernández, Giménez, & Varela, 2014).

3. Results

3.1. Texture analyses

Fig. 1 (a–b) shows the total work (force x distance) associated with shearing the different sausages (grouped according to hydrolysate content), and the maximum shear force resistance (normally this is the force just before cutting through the skin of the sausage) of the sausages. Significant differences between the samples for both variables were observed from their respective ANOVA models (Total work: $F_{(13,111)} = 36.27$, $p < 0.001$); Resistance ($F_{(13,111)} = 47.02$, $p < 0.001$). It can be seen from Fig. 1 that the two types of force measurements show the same trend, and are therefore described collectively.

Consistently, the beef sausages required a statistically significant higher work to shear as compared to the pork sausages of similar type and hydrolysate concentration. Accordingly, a significant main effect of meat type was found for both total work ($F_{(1,123)} = 36.35$, $p < 0.001$) and resistance ($F_{(1,123)} = 122.8$, $p < 0.001$).

For the pork and beef Normal sausages, the firmness is unaffected when varying the hydrolysate content between 0%, 7.5% and 12.5% (Fig. 1). The same is true for pork and beef Curry sausages with 7.5% and 12.5% hydrolysate content, and for the pork Merguez sausages of 7.5% and 12.5% hydrolysate content. The beef Merguez sausages of 12.5% hydrolysate content require significantly more work to shear than the corresponding 7.5%, but the maximum shear force for the same types of sausages is not statistically different from each other. Accordingly, significant differences of protein content was found for the total work ($F_{(2,122)} = 10.41$, $p < 0.001$) but for the resistance ($F_{(1,123)} = 3.988$, $p = 0.21$) no significant differences were found. The general picture is therefore that the hydrolysate concentration does not affect the firmness of the sausages.

The type of recipe (Normal, Curry and Merguez) did affect the firmness of the sausages. For both pork and beef, the Normal sausages are significantly firmer than the corresponding Curry and Merguez sausages. The pork Curry and Merguez sausages showed similar firmness for both 7.5% and 12.5% hydrolysate content. The beef Curry sausage was firmer than the beef Merguez sausage for 7.5% hydrolysate, but for the 12.5% hydrolysate, the opposite was true (and in both cases, the numerical difference is much smaller as compared to the difference to the Normal sausages). Significant differences between the

[3] The choice of not including samples in the 3-way model was motivated by the fact that we used a fractional factorial design in the consumer test, meaning that it was not possible to separate the sample effect from the effect of the design factors.

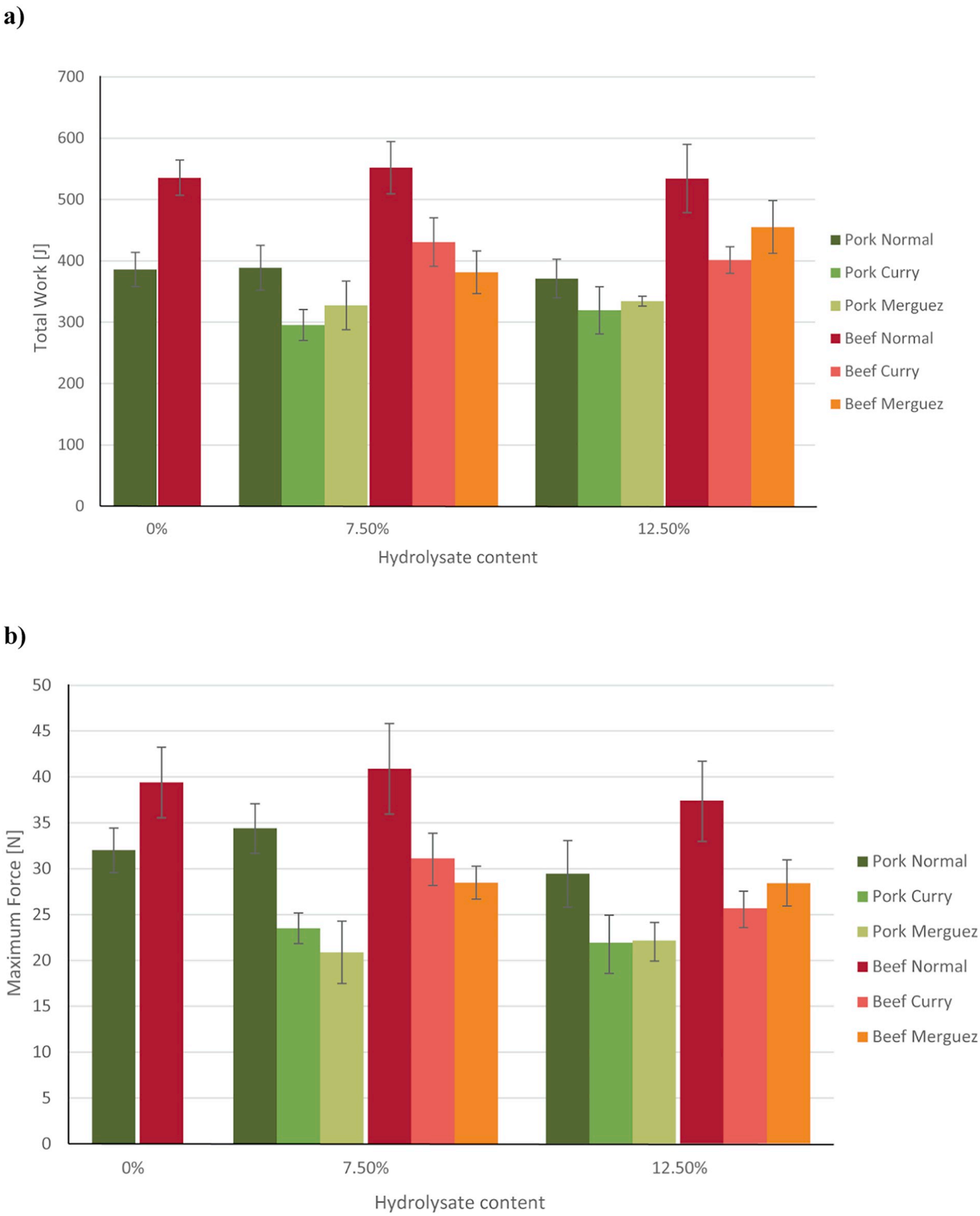

Fig. 1. Total work associated with shearing the different sausages.

recipes were found for the total work ($F_{(2,122)} = 68.33$, $p < 0.001$) as well as for the resistance ($F_{(2,122)} = 23.69$, $p < 0.001$).

3.2. Quantification of umami-tasting amino acids

We quantified free amino acids responsible for the umami taste, glutamic acid and aspartic acid (Lindemann, Ogiwara, & Ninomiya, 2002), for the normal pork and beef sausages with varying content of added meat protein hydrolysate (Fig. 2.).

As seen from the plot, the concentration of free glutamic and aspartic acid increase with increasing concentration of added meat protein hydrolysate in a roughly linear manner. This is not surprising since the enzymatic hydrolysis during the production of the MPH should generate a certain amount of free amino acids, and as the MPH is known to contain both glutamic acid and aspartic acid (data from an amino acid composition analysis of acid hydrolyzed MPH shows roughly 13% glutamic acid and 7% aspartic acid for both pork and beef hydrolysates). Nevertheless, it shows that for both beef and pork normal

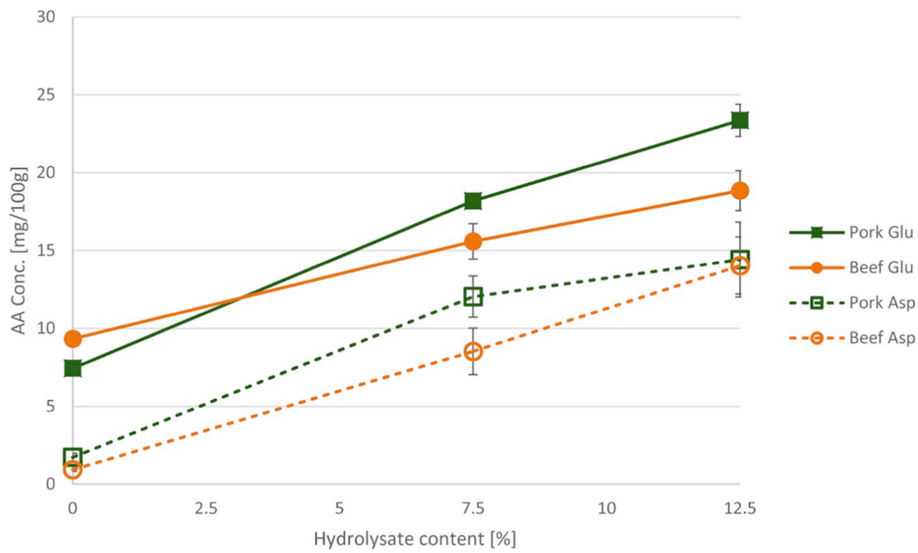

Fig. 2. Glutamic and aspartic acid concentrations for beef and pork normal sausages depending on protein hydrolysate concentration.

sausages, the concentration of umami contributing amino acids increase with increasing levels of hydrolysate.

3.3. Consumer test results

3.3.1. Perceptual characterization (CATA)

Table 3 shows the frequency of occurrence of all CATA for each individual sample. Overall, the attribute *Spicy* was the most often used with checking rate of 12%, followed by *Salty* with 9%. The attributes *Well balanced*, *Pleasant*, *Mild* and *Familiar taste* followed with 6%. The least checked attributes were *Cozy* (1%), *Bitter*, *Sour*, *Exceptional* (2% each), *Sweet* (3%) and *High quality* (3%). Table 3a also shows that half of the perceptual attributes (10 out of 20) significantly discriminated between the sausage samples. Furthermore, no significant differences were observed with regards to appropriateness for use (Table 3b). In this case, the attribute *Dinner* was the most used with an overall checking rate of 39.4%, followed by *Lunch* (33%), *Brunch* (31%), and *Snack* (29%).

Associations between perceptual attributes and samples were evident from the CA model. Fig. 3 shows the bi-plot visualizing the relation between attributes and samples on the first two CA dimensions (explaining 62.29% of the original variance). Examining the bi-plot, it is apparent that the first dimension related to flavor intensity (*Hot* vs. *Mild*), whereas the second dimension was mainly related to texture (*Soft* vs. *Hard Texture*) (Fig. 3).

Dimension 1 mostly discriminated samples by recipes, as we can see the main opposition to be between sample Mg_12.5_P, described by the attributes *Exceptional*, *Spicy* and *Hot*, to sample No_7.5_P, which was characterizes as being *Mild*. The lower left quadrant shows three of the four Merguez samples: Mg_12.5_B was perceived as *Exceptional* and *Intense* whereas Mg_7.5_B can be classified as being *Spicy*. Cu_7.5_B can be described as *Salty*.

Dimension 2 mostly opposed samples perceived as *Bitter*, *Hard texture*, and *Artificial* to samples perceived as *Pleasant*, *Well balanced*, *Fat* and *Sweet*. This difference seems primarily related to meat type, as we can see that all pork samples are positively loaded on this dimension, whereas all beef samples are negatively loaded (Fig. 3).

The lower right corner is dominated by the samples of the type Normal (Fig. 3). No_0_B was mostly perceived as being *Salty*, and also had the most *Umami* taste according to the participants. No_7.5_B was mostly perceived as *Dry* and *Sour*, while No_12.5_B was associated to the attributes *Artificial* and *Bitter*. Cu_12.5_B was mostly described as *Sour* and *Artificial*.

The top right corner was again dominated by the sausage type Normal and pork. No_12.5_P was perceived as *Well balanced*. The samples No_7.5_P were best described as being *Mild*, and No_0_P as having a *Familiar taste*.

The upper left corner described Cu_12.5_P associated to attributes such as *Fat*, *Pleasant* and *Soft texture*. Mg_7.5_P was also described as having a soft texture. The left side of the plot shows the *Spicy, Intense and Exceptional* samples as well as the ones having a soft texture and those high in fat. The right side mostly consists of Normal samples and two Curry samples described by attributes such as *Sour*, *Salty*, *Artificial*, *Umami*, *Familiar taste* and *Mild*.

3.3.2. Acceptability

With respect to acceptability, the means of all samples ranged from a lowest of 4.2 (on a 9-pt scale) to a highest of 6.16. The beef/Merguez sample with 7.5% MPH was the most liked sample, whereas the beef/curry with 12.5% MPH was the least liked. Sample means for each sample are shown in Fig. 4. Clear significant difference between the samples were found ($F_{(13,686)} = 3.553$, $p < 0.001$); the superscript letters in Fig. 4 show differences between the samples means.

In general, the Curry sausages seemed to be on average the least liked samples. Furthermore, when only comparing the two samples that share the same recipe and protein content, all pork samples were liked better compared to the respective beef samples, except for the Merguez recipe, where the beef samples seemed to be liked better than the pork samples (Fig. 4).

Fig. 5 displays mean liking ratings corresponding to each design factor (Recipe, Meat type, and MPH content). No significant 3-way interactions between the design factors were observed ($F_{(2,685)} = 1.02$, $p = 0.35$). A significant interaction effect between meat type and recipe were found ($F_{(2,685)} = 6.25$, $p = 0.002$), whereas the other 2-way interaction terms were not significant (MPH content by meat type: $F_{(2,685)} = 0.43$, $p = 0.43$; MPH content by recipe: $F_{(2,685)} = 0.37$, $p = 0.69$). Regarding main effects, significant differences in mean liking by recipe were found ($F_{(2,685)} = 9.21$, $p < 0.001$), with Merguez being the most liked and Curry the least liked (Fig. 5c). Furthermore, pork as a meat type was liked significantly better than beef ($F_{(1,685)} = 7.41$, $p = 0.007$), as shown in Fig. 5. Finally, no differences in acceptability due to MPH concentration was found, though the effect was close to being significant ($F_{(2,685)} = 2.64$, $p = 0.072$). Visual inspection of Fig. 5 and post-hoc pairwise comparison revealed that the difference pertained to the 7.5% (Mean liking: 5.47) and the 12.5% (5.06) levels ($p = 0.06$), whereas mean liking for the 0% level (5.24) was not

Table 3
(a, b). Frequency of mention of CATA attributes for each sample (N ≈ 50 in each sample). Table 3a shows perceptual attributes and Table 3b shows appropriateness for use. CATA attributes are listed by total frequency of mention, in descending order from left to right. Within columns, samples not sharing superscript letters are significantly different according to a χ^2 test. $^{n.s.}$ not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

a)

Product	Spicy	Salty	Umami	Soft Texture	Mild	Familiar Taste	Pleasant	Well Balanced	Intense	Hard Texture
No_0_B	25^{ab}	20	16	17^{ab}	11^{abc}	8^{ab}	9	10	8^{ab}	16^{def}
No_0_P	15^{ab}	21	16	10^{ab}	18^{c}	22^{b}	14	17	4^{a}	6^{abcd}
No_7.5_B	26^{ab}	23	14	5^{a}	16^{c}	18^{ab}	12	10	8^{ab}	25^{f}
No_7.5_P	12^{a}	9	12	5^{a}	24^{c}	16^{ab}	10	11	3^{a}	12^{bcdef}
No_12.5_B	20^{ab}	22	17	11^{ab}	11^{abc}	10^{ab}	11	12	9^{ab}	20^{ef}
No_12.5_P	21^{ab}	22	11	11^{ab}	14^{bc}	24^{b}	11	14	6^{ab}	11^{cdef}
Mg_7.5_B	34^{b}	19	17	12^{a}	9^{abc}	9^{ab}	14	16	20^{b}	10^{bcde}
Mg_7.5_P	31^{ab}	18	7	22^{b}	4^{ab}	9^{ab}	16	12	9^{ab}	2^{ab}
Mg_12.5_B	35^{b}	20	20	14^{ab}	4^{ab}	4^{a}	12	8	20^{b}	8^{abcde}
Mg_12.5_P	34^{b}	15	14	17^{ab}	3^{a}	7^{ab}	10	9	18^{b}	1^{a}
Cu_7.5_B	19^{ab}	10	12	17^{ab}	10^{abc}	7^{ab}	12	6	12^{ab}	7^{abcd}
Cu_7.5_P	22^{ab}	16	10	13^{ab}	20^{c}	13^{ab}	16	11	8^{ab}	10^{bcde}
Cu_12.5_B	18^{ab}	14	13	8^{ab}	9^{abc}	8^{ab}	4	10	9^{ab}	10^{bcde}
Cu_12.5_P	28^{ab}	17	6	22^{b}	14^{bc}	9^{ab}	12	10	9^{ab}	3^{abc}
Total	340	246	185	184	167	164	163	156	143	141
$\chi^2_{(13)}$	29.0	14.1	15.2	29.0	41.9	40.4	10.4	10.2	37.7	58.4
p	**	n.s.	n.s.	**	***	***	n.s.	n.s.	***	***

Product	Hot	Artificial	Fat	Dry	Sweet	High Quality	Bitter	Sour	Exceptional	Cozy
No_0_B	9^{bc}	13	14	9^{ab}	3^{abc}	5	3	7	2^{ab}	3
No_0_P	3^{ab}	5	8	6^{ab}	5^{abcd}	7	2	1	1^{ab}	4
No_7.5_B	6^{abc}	10	8	11^{ab}	5^{abcd}	4	5	5	1^{ab}	1
No_7.5_P	4^{abc}	8	8	16^{b}	2^{ab}	9	1	2	0^{a}	3
No_12.5_B	7^{abc}	15	7	10^{ab}	1^{a}	3	7	7	0^{a}	1
No_12.5_P	6^{abc}	4	9	8^{ab}	2^{ab}	8	2	3	2^{ab}	3
Mg_7.5_B	14^{cd}	9	7	6^{ab}	3^{abc}	10	4	2	7^{b}	1
Mg_7.5_P	22^{d}	7	14	2^{a}	11^{bcd}	8	2	1	1^{ab}	3
Mg_12.5_B	23^{d}	8	3	2^{a}	8^{abcd}	7	3	2	6^{ab}	1
Mg_12.5_P	21^{d}	9	12	4^{ab}	5^{abcd}	8	3	3	4^{ab}	2
Cu_7.5_B	5^{abc}	10	6	11^{ab}	6^{abcd}	1	9	4	5^{ab}	2
Cu_7.5_P	6^{abc}	8	11	9^{ab}	15^{d}	2	3	5	4^{ab}	8
Cu_12.5_B	1^{a}	14	5	11^{ab}	3^{abc}	5	5	4	3^{ab}	2
Cu_12.5_P	7^{abc}	9	13	3^{ab}	14^{cd}	3	5	6	5^{ab}	2
Total	134	129	125	108	83	80	54	52	41	36
$\chi^2_{(13)}$	73.7	13.7	16.9	28.1	44.0	18.0	16.0	14.8	22.8	16.9
p	***	n.s.	n.s.	**	***	n.s.	n.s.	n.s.	*	n.s.

b)

Product	For dinner	For lunch	For brunch	As a snack	In between meals	For special events	For breakfast	Before/after training
No_0_B	16	20	14	15	7	8	4	1
No_0_P	21	21	19	17	8	8	5	3
No_7.5_B	22	17	22	18	11	6	2	2
No_7.5_P	18	13	18	12	12	7	8	6
No_12.5_B	14	12	16	14	9	8	5	1
No_12.5_P	21	18	17	16	15	10	6	2
Mg_7.5_B	28	20	16	15	11	13	3	1
Mg_7.5_P	22	16	13	19	9	12	4	4
Mg_12.5_B	21	17	20	16	8	11	4	2
Mg_12.5_P	22	18	16	16	15	10	6	3
Cu_7.5_B	11	12	12	9	5	12	5	0
Cu_7.5_P	22	16	14	15	13	8	5	2
Cu_12.5_B	18	12	10	8	2	6	5	3
Cu_12.5_P	20	21	11	14	8	6	3	1
Total	276	233	218	204	133	125	65	31
$\chi^2_{(13)}$	11.3	8.6	10.1	8.6	18.3	8.3	6.3	13.7
p	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

different from the other two ($p \geq 0.65$).

Table 4 and Fig. 6 report results of the penalty analysis showing the effect of each CATA on liking. Except for three attributes (*Hot*, *Salty* and *Fat*), all CATA attributes had a significant effect on mean liking (Table 4).

Fig. 6 shows that the attributes *Sour*, *Bitter* and *Artificial* were associated to lower acceptability (mean drop between −1.12 and −1.41 on a 9-pt hedonic scale). The number of consumers responsible for this result was less than 10% of the total number of participants for both attributes. Considering the positive (uppoer) side of the plot, participants liked the samples more when they checked the attributes *Sweet*, *Cozy* and *Exceptional*. The largest overall difference in mean liking was found for the attribute *High quality*: once participants checked this attribute, samples were perceived with an increase of 2.71 compared to

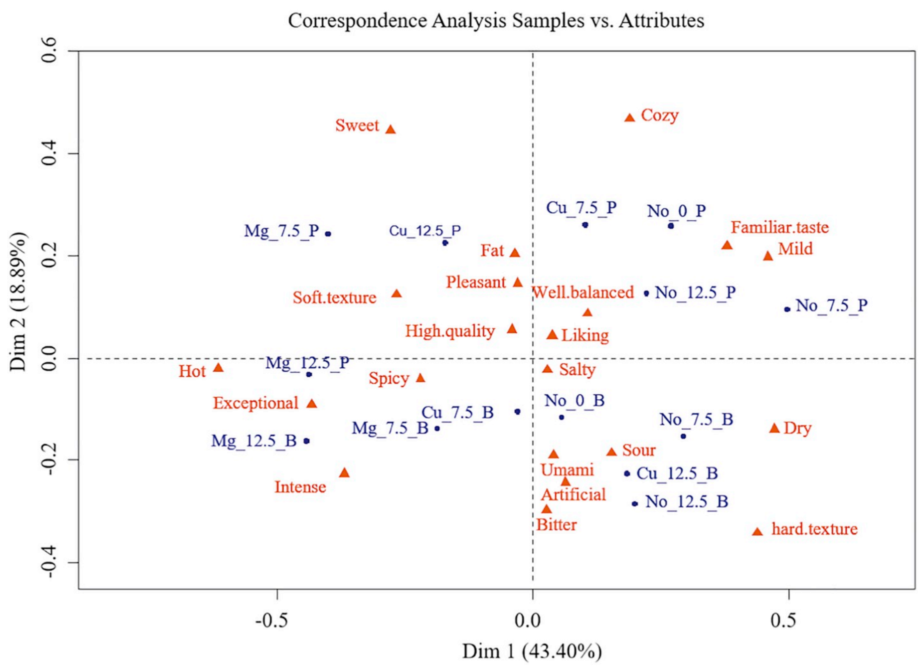

Fig. 3. Bi-plot showing the first and second dimensions of the CA model (62.3% variance explained) visualizing the correspondence of the 14 samples with the 20 CATA attributes.

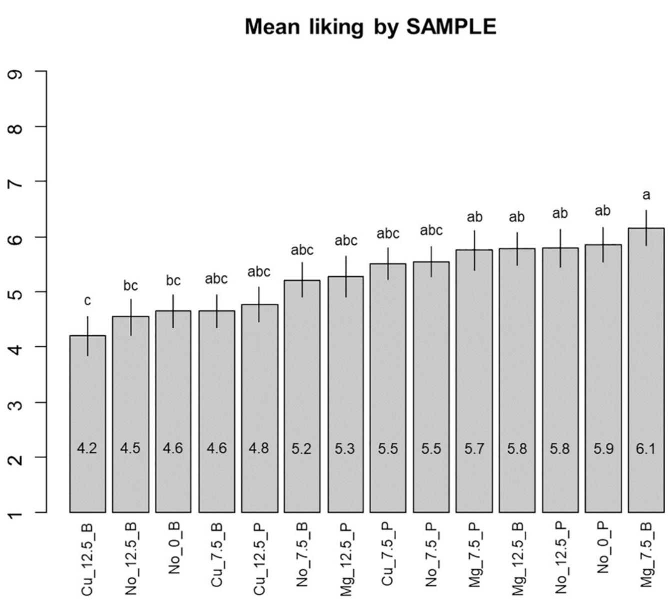

Fig. 4. Mean liking (9-pt scale) and S.E.M. by sample. Means not sharing superscript letters are significantly different (Tukey $p < 0.05$).

samples for which this attribute was not checked. Moving along the x-axis of Fig. 6, it can be seen that attributes such as *Dry*, *Fat* and *Hard texture* were also associated to a drop in mean liking. The mean drop for liking for the attribute *Dry* was nearly 1, and 15% of the consumers used that attribute. Between 18% and 20% did not like the attributes *Fat* or *Hard texture* in the samples, whereas a low positive trend could be seen when the attribute *Hot* was checked. Attributes such as *Intense*, *Mild*, *Soft texture* and *Umami* were perceived positively. These attributes were perceived by 20–26% and their presence was associated to an increase in mean liking of 0.58, 0.83 and 0.75, respectively.

In samples that were perceived as having an *Artificial* taste, the mean dropped by 2.04 points on the 9-pt hedonic scale. Approximately 20% of the participants used that attribute. Proceeding further to the middle of the x-axis, there are three attributes that seem to have a positive effect on the overall liking of the samples: *Familiar taste* adds up to 1.50 points on the liking scale and nearly a fourth of the respondents share this opinion. The attributes *Well balanced* and *Pleasant* were associated to an even larger rise in acceptability (1.96 and 2.25 respectively) and were used by 22% and 23% of the total number of participants.

Many participants (35%) also perceived the sausages to be *Salty*; however, saltiness did not affect liking significantly (Table 4). *Spicy*, the attribute that was checked by most respondents overall, was perceived as positive and its presence marked a 0.58 increase in mean liking.

Finally, with regards to the effect of consumer demographics on acceptability, men liked the sausages significantly more than women (0.8 on a 9-pt hedonic scale), and accordingly the two-way ANOVA revealed a significant main effect of gender ($F_{(1,695)} = 11.5$, $p < 0.001$). Conversely, no main effect of age was found ($F_{(1,695)} = 1.5$, $p = 0.21$), although young people tended to like the sausages more than the elderly did (also 0.8 on a 9-pt hedonic scale). A significant interaction between age and gender was found ($F_{(2,695)} = 10.8$, $p = 0.011$): elderly women liked the sausages significantly less than all other three segments, whereas men of both age groups had a nearly identical mean liking score, thus explaining the lack of a main effect for age.

4. Discussion

In order to enable targeted product development, and to better understand the behavior of MPH in relevant food applications, the main aim of this study was to analyze textural properties, content of umami contributing amino acids associated with MPH-enrichment, and consumers' perception of MPH-enriched snack sausages.

Starting from the latter aspects, the results obtained indicated that consumers could clearly differentiate between the test samples on the basis of both perceptual attributes (CATA) and acceptability (9-pt hedonic scale). The results from the CATA task, in particular, gave a good overview of attributes that discriminated between the samples, as well as indications as to which attributes had a positive or negative effect on consumer liking. Overall, MPH content did not substantially affect consumer perception, and for the two Normal samples the treated

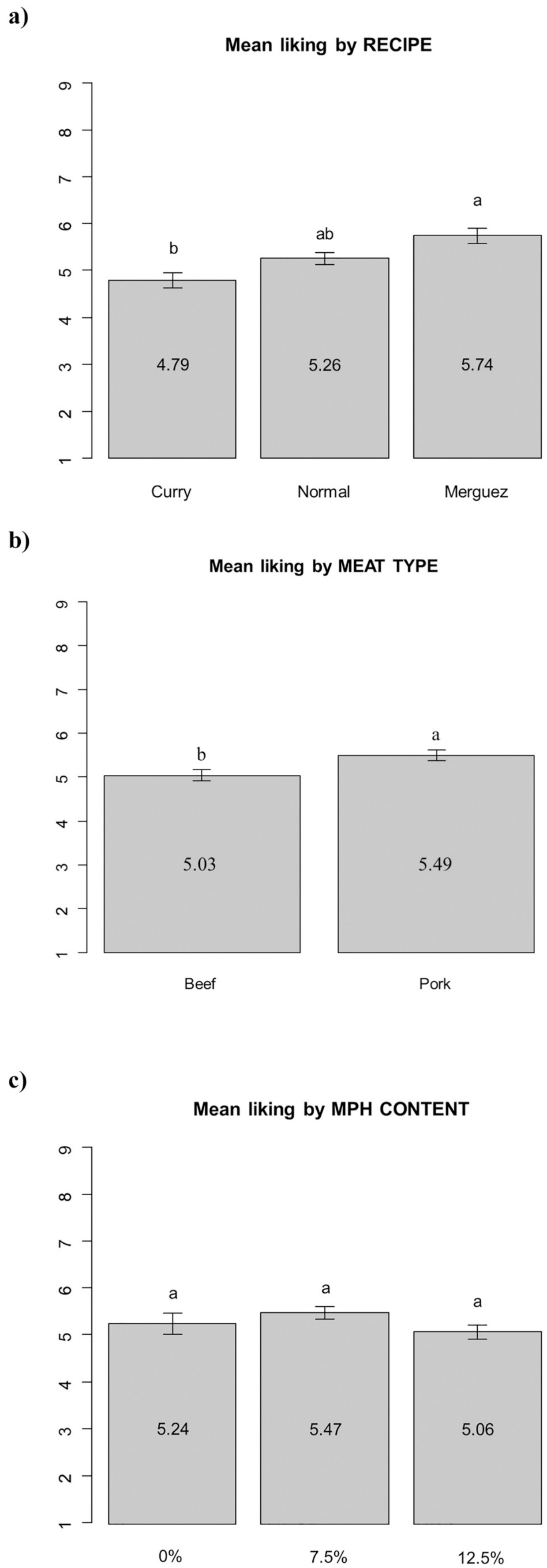

Fig. 5. Bar charts showing mean liking (9-pt hedonic scale) and S.E.M. by (a) recipe, (b) meat type and (c) MPH content. Means not sharing superscript letters are significantly different (Tukey $p < 0.05$).

Table 4
Penalty analysis (N = 100) showing mean drop/gain in mean liking when an attribute is checked or unchecked (0 = unchecked; 1 = checked), as well as the significance of that drop assessed by ANOVA. $^{n.s.}$ not significant; * p < 0.05; ** p < 0.01; *** p < 0.001.

Attribute	Mean Liking		*F*	*p*
Mild	0	5.13	8.40	**
	1	5.71		
Familiar taste	0	4.91	60.25	***
	1	6.41		
Spicy	0	4.98	11.82	***
	1	5.56		
Exceptional	0	5.15	27.52	***
	1	7.02		
Hot	0	5.23	0.85	n.s
	1	5.43		
Soft texture	0	5.05	18.63	***
	1	5.88		
Sweet	0	5.17	8.40	**
	1	5.94		
Dry	0	5.41	17.10	***
	1	4.44		
Artificial	0	5.64	94.19	***
	1	3.60		
Sour	0	5.35	11.82	***
	1	4.23		
Umami	0	5.07	15.22	***
	1	5.82		
Cozy	0	5.19	15.45	***
	1	6.69		
Bitter	0	5.37	19.69	***
	1	3.96		
Salty	0	5.23	0.24	n.s
	1	5.32		
Intense	0	5.15	7.58	**
	1	5.73		
High quality	0	4.95	116.03	***
	1	7.66		
Fat	0	5.34	3.37	n.s
	1	4.93		
Hard texture	0	5.35	3.89	*
	1	4.93		
Well balanced	0	4.83	102.97	***
	1	6.79		
Pleasant	0	4.74	148.34	***
	1	6.99		

samples were remarkably close to the control (0% MPH) sample in all attributes. Rather, the main difference pertained to flavor intensity (hot vs mild), which were related to variation in recipe, particularly between the Merguez and the Normal samples. The second most important difference uncovered was related to textural and taste variation, and was associated to meat type: pork sausages were perceived as being softer and sweeter than the beef ones, which were harder and more bitter. The sensory results on meat type are in good agreement with the textural analysis performed (beef sausages required a higher workload to shear), as well as with previous literature on the sensory differences between these two meat types in processed meat products (Parizek, Ramsey, Galyean, & Tatum, 1981; Rentfrow, Brewer, Weingartner, & McKeith, 2004). Likely, the difference in texture was also associated with the difference in fat content, with the pork sausages having a higher fat content and being softer, in agreement with previous reports (Sofos & Allen, 1977). We should note here that, as the sausages were frozen and thawed, sensory attributes of both texture and flavor could be expected to differ to some extent from those of fresh sausages (DeFreitas, Sebranek, Olson, & Carr, 1997; Dong et al., 2007; Kulkarni, DeSantos, Kattamuri, Rossi, & Brewer, 2011). No differences between the samples regarding appropriateness for use were detected, meaning that all samples were considered equally appropriate for the consumers. The sausages were collectively perceived to be especially appropriate for dinner, lunch, and brunch, reflecting the main occasions in which

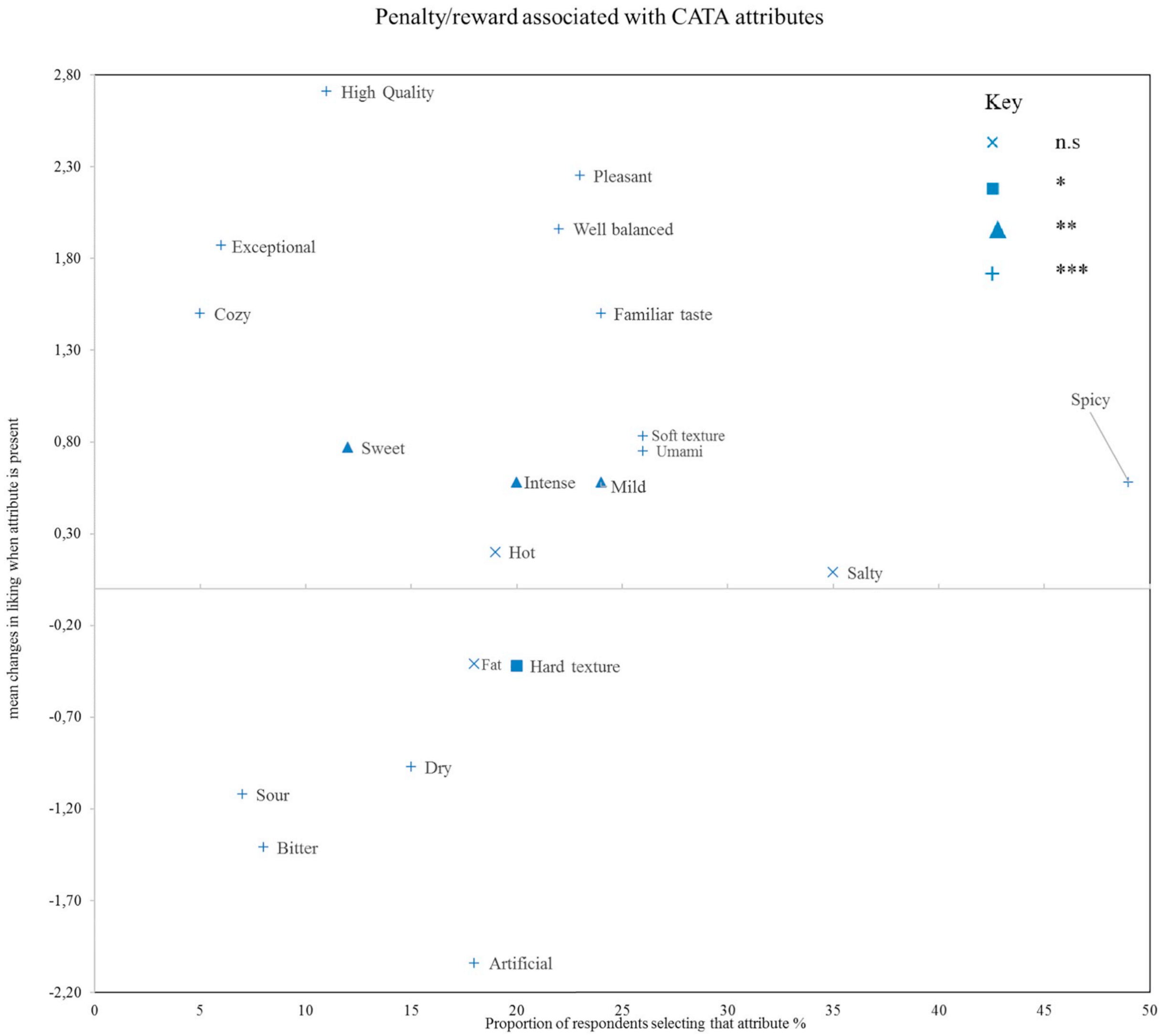

Fig. 6. Penalty analysis showing mean drop/increase associated with the presence of each CATA attributes (ordinata), as well as the percentage of consumers checking that attribute (abscissa).

sausages are currently consumed. Nearly a third of the consumers perceived the sausages to be appropriate as a snack, which was the target context for this specific product application.

The results of the overall hedonic liking score show that the recipe was the most important factor in this case: the Merguez recipe was the one preferred by the consumers, followed by the Normal recipe. Furthermore, consumers overall preferred pork over beef samples. This finding is most likely due to familiarity, since pork sausages are much more common than beef sausages, and Danes on average consume twice as much pork as beef (DAFC, 2015). It should be noted, however, that an interaction effect between meat type and recipe was found, indicating that Beef was liked on par with pork within a specific recipe (Merguez).

Consistently with the indication of the CATA results, the MPH content did not have a significant effect on acceptability, meaning that enrichment with protein hydrolysates did not affect consumer liking; this is of course a positive finding from a product application perspective. Since the protein content does not have any effect on the overall liking of the samples, the protein hydrolysates seemed to be designed in a way that it does not influence the taste or the texture of the product itself. This was further confirmed by the texture analysis, which showed that the protein hydrolysates did not significantly influence the firmness of the sausages, whereas both the recipe and meat type (and corresponding fat content) did affect it. These findings are consistent with the sensory results, where the most important effect was found for the type of recipe, rather than the MPH content. All data consistently show that the hydrolysate content did not have a negative impact on instrumental or sensory attributes.

The instrumental determination of umami tasting amino acids showed a clear increase in umami with increasing MPH, a result confirming previous reports by Lahl and Braun (1994) that enzymatic hydrolysates possess umami flavor as a main characteristic. The higher umami concentration did not result, however, in a corresponding higher umami taste experienced by the consumers. This inconsistency can possibly be explained by masking effects, for example due to an increase in bitterness from bitter tasting amino acids masking the increased umami, or to differences being lower than or close to sensory difference threshold for umami, which would further suggest that the MPH preparation can still be optimized for taste attributes. It is also possible that the lack of differences is related to power, such as that with a higher sample size and/or a method that allows for scaling of sensory intensity (e.g., descriptive analysis or Rate-All-That-Apply (Reinbach, Giacalone, Ribeiro, Bredie, & Frøst, 2014)) differences may have been detected. Finally, it should also be kept in mind that the lack of concept calibration may have cause consumers not to use unfamiliar terms (such as "umami") reliably, and that a trained panel would have been able to detect additional and/or more subtle differences between the samples. On average, consumers liked samples better when attributes such as *High quality*, *Exceptional*, *Pleasant*, *Well balanced* and *Familiar taste* were checked. Attributes such as *Fat*, *Hard texture*, *Dry*, *Artificial*, *Sour* and *Bitter* caused a decrease in overall liking and should be paid attention to in product development applications.

Noting the paucity of literature on MPH in food applications, this study contributes to knowledge in this area by providing a characterization of the flavor and textural properties of MPH as a functional ingredient in food applications. To the best of our knowledge, the only other paper in this area at the time of writing is a study by Meinert and colleagues (Meinert et al., 2015), focusing on sensory properties of MPH enriched saveloy obtained from different types of hydrolysates, and found that the source of hydrolysates the slaughterhouse side stream had an impact on the sensory profile, but not concentration (up to 8%), affected the sensory quality. Here, we extend these previous results with an even larger range of MPH concentration and a wider range of product variation. Additionally, we provide first evidence that from a consumer point of view, enrichment with meat protein hydrolysates does not influence participants perception of the sausage, both with regards to acceptability (no effect of MPH content) and sensory quality (no differences or very minor compared to other factors such as recipe and meat type). The finding that MPH addition was not associated to an increase in bitterness (according to our consumer panel) was important given that, based on extant research, bitter taste might have been expected to be an obstacle to consumer acceptability of MPH-enriched products (Aehle, 2007; Fitzgerald & O'Cuinn, 2006; Saha & Hayashi, 2001). Importantly, this was the case not only for the recipes containing hot spices (Curry and Merguez), where bitterness suppression due to taste-taste interactions may have been expected, but also for the Normal recipe. Taken overall, these results are promising in terms of product development of MPH enriched meat products, as they indicate that this ingredient can be added to increase the nutritional density of a reference meat product without negatively affecting its sensory quality and consumer acceptability.

Of course, this study is not without limitations. There are at least three that are important to point out. Firstly, we focused on MPH-enrichment for a snack sausage application. Future studies are advised to elucidate how these ingredients behave in other relevant products, such as cold cuts, soups and snack bars. Secondly, the consumer test results are immediately applicable to the Danish population but may not directly extend to other cultural and geographic areas that may be characterized by different dietary habits (e.g., regarding pork consumption). Lastly, future research should look at individual differences between consumers, especially between young and older consumers, since aging is associated to many changes or sensory perception as well attitudes towards food (e.g., heightened pickiness) that is important to consider to successfully facilitate the adoption of MPH-enriched products (Song, Giacalone, Bølling-Johansen, Frøst, & Bredie, 2016). For instance, the data collected in this paper showed that the mean liking for the elderly consumers (65+) was 0.8 points (on a 9-pt hedonic scale) lower than that of the young (18–35), which may be due to higher pickiness in the former group, particularly among older women. However, this result is only indicative due to the limited size of the elderly group ($N = 21$) employed in this study. In general, this suggests that the issue of heterogeneity in consumer preferences should be given attention in future research, as it is quite possible that several segments characterized by different drivers of liking exist for this product category. Additionally, this study has only focused on consumer perception in a blind test, whereas in the future it would be interesting to test how the presence of extrinsic factors can modify consumer responses. For example, it would be relevant to study whether nutritional information and health claims can further improve consumer attitudes towards functional snack sausages, in particular among older women that in this study were found to have an overall lower acceptance for these products.

5. Conclusion

This paper focused on consumer responses to sausages from pork and beef meat, enriched with MPH. A consumer study ($N = 100$) was conducted where young (18–35) and elderly (65+) consumers evaluated perceived liking on acceptability, sensory attributes and appropriateness for relevant usage contexts. Additionally, textural (Warner–Braztler shear test) and chemical (umami-tasting amino acids by HPLC) analyses of the same samples were provided to give a more complete characterization of the functionality of the hydrolysates in sausage applications.

The results showed that recipe was the most important factor determining sensory properties and acceptability. Consumers liked the Merguez samples the most, associated to attributes such as *Spicy, Intense, Exceptional and Soft texture*, which were all found to have a positive influence on consumer liking. Meat type was found to have an impact on texture, with beef samples being firmer than pork samples, as well as liking, with pork samples being preferred on average to beef samples in this consumer population. Beef samples were characterized by attributes such as *Fat*, *Hard texture*, *Dry*, *Artificial*, *Sour* and *Bitter*, all of which were found to be associated to reduced acceptability at an overall level.

From an application perspective, the most important (and promising) finding of the study is that the enrichment with meat protein hydrolysates did not influence participants perception of the sausage, suggesting that this ingredient can be added to increase the nutritional density of a reference meat product without negatively affecting its sensory quality and consumer acceptability.

Acknowledgements

We are grateful to our colleagues Erik T. Hansen (Danish Crown Ingredients), Jens Fabricius (Danish Crown Ingredients), Knud Villy Christensen (SDU), and Ulla Lauritsen (SDU) for help with various aspects of the planning and/or data collection.

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