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# Development of Inosine Monophosphate and Its Degradation Products during Aging of Pork of Different Qualities in Relation to Basic Taste and Retronasal Flavor Perception of the Meat

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Inosine monophosphate (IMP) and its degradation products, ribose and hypoxanthine, are all considered to be important constituents in meat flavor formation and development. The present study explored the fate of IMP during the aging of two qualities of pork (pH >5.7 and 5.5 < pH < 5.6) and the potential relationship between IMP, hypoxanthine, and sensory attributes of pork registered both as retronasal and basic taste responses in whole meat, meat juice, and the remaining meat residue. During aging the concentration of IMP decreased with a simultaneous increase in the concentrations of inosine, hypoxanthine, and ribose. The rates at which IMP was degraded to inosine and inosine to hypoxanthine during aging were found to be in agreement with the known rate constants of the dephosphorylation of IMP and the hydrolysis of inosine, respectively. Moreover, high-pH pork resulted in a significantly higher concentration of hypoxanthine throughout storage compared with low-pH pork due to an initially higher concentration of IMP in high-pH meat. The sensory analysis showed increasing intensity in bitterness and saltiness of pork as a function of aging, with the intensity being most pronounced in the meat juice. The increasing bitterness of the pork as a function of aging coincided with the higher content of hypoxanthine in these samples, thereby suggesting that degradation of IMP to hypoxanthine might influence pork flavor. In contrast, IMP was associated with nonaged meat and the sensory attributes meaty and brothy.

**KEYWORDS: Pork; meat quality; flavor; IMP; brothy; hypoxanthine; bitterness**

## INTRODUCTION

The flavor of meat develops largely through the cooking process; however, fresh meat contains nonvolatile constituents that are essential flavor precursors and contribute to the basic taste of cooked meat (*1*). Consequently, many of the constituents in fresh meat undergo substantial changes during the cooking process and subsequently provide roasted, boiled, fatty, and species-related flavors, as well as the characteristic meaty aromas associated with cooked meats. Model experiments have suggested that the Maillard reaction between amino acids and reducing sugars is one of the main pathways in the formation of many of the aroma compounds identified in cooked meat (*2*−*5*). One of the main reducing sugars in meat is the pentose, ribose, originating from the degradation of ribonucleotides (*6*). However, the 5′-ribonucleotides, adenosine monophosphate (AMP), inosine monophosphate (IMP), and guanosine monophosphate (GMP), are also important in meat flavor perception, as they hold umami taste characteristics (*7*, *8*). Umami compounds have a characteristic savory quality, first characterized for monosodium glutamate. Besides the characteristic umami taste, umami compounds have flavor-enhancing properties and are reported to enhance meaty, brothy, mouth-filling, dry, and astringent qualities and to suppress sulfurous notes (*9*). Interestingly, the 5′-ribonucleotide, IMP, may indirectly also contribute to meat flavor through its secondary degradation product, hypoxanthine, which together with several free amino acids and anserine, carnosine, and other dipeptides might contribute with bitter flavor characteristics.

Considering that IMP, ribose, and hypoxanthine in this way all are to be considered important constituents in meat flavor formation and development, an understanding of the postmortem energy metabolism in muscle and the subsequent degradation of the adenosine triphosphate (ATP) metabolite, IMP, during aging and cooking, as schematically outlined in **Scheme 1**, becomes crucial in the further exploitation of flavor development in meat.

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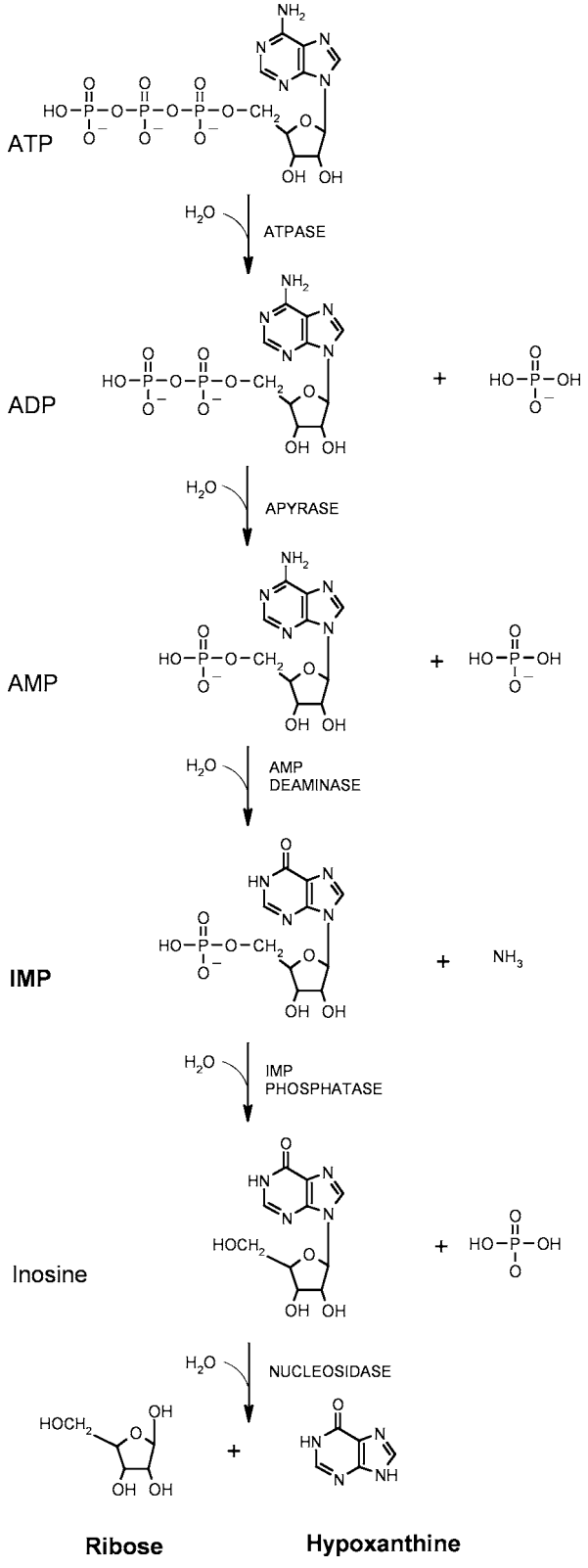
**Scheme 1.** Enzymatic Breakdown of ATP




Several studies have reported a heat-induced increase in ATP metabolites during cooking of different muscle foods, and a significant increase in [inosine] and [hypoxanthine] during cooking has been demonstrated in goat's and sheep's meat (*10*). Moreover, increasing cooking temperatures have been found to result in a significant rise in the concentrations of creatinine, IMP, and AMP in beef broth, with IMP showing the highest correlation to the sensory data of the broth (*11*). The stability of IMP is reported to be both temperature- and pH-dependent due to the residence of weak chemical bonds, for example, glucoside and ester bonds (*12*). Consequently, pH in the fresh meat must be expected to influence IMP degradation through aging and cooking.

Interestingly, the concomitant formation of ribose upon hydrolysis of inosine is not considered in the literature even though ribose, as mentioned above, is known as a major reactant in the Maillard reaction that takes place in meat.

Most flavor studies related to meat have been carried out in model systems or in spiking studies where potential flavor precursors have been added to meat or meat broth with subsequent chemical or sensory analysis.

The present study aims to elucidate for the first time both the chemical and sensory fate of IMP in two different meat qualities (normal versus "high" pH) during storage. Initially, the chemical degradation of inosine monophosphate during aging and cooking of pork is investigated. Subsequently, a sensory study of nonvolatile flavor precursors including IMP and its degradation products of pork of two different qualities in relation to basic taste perception and retronasal flavor perception was carried out.

## MATERIALS AND METHODS

**Experimental Design, First Study.** Four pigs (cross-breeds of Duroc boar and Danish Landrace × Yorkshire dams) reared and slaughtered at The Danish Institute of Agricultural Sciences (DIAS), Foulum, Denmark, were included in the experiment. The pigs were stunned by 85% $CO_2$ for 3 min, exsanguinated, scalded at 62 °C for 3 min, cleaned, and eviscerated within 30 min. M. longissimus dorsi was dissected from the area of the last rib. Rectangular meat samples (3 × 3 × 2 cm), from which all visible fat and connective tissue were removed, were cut, vacuum-packed in pairs, and stored at 4 °C for 1, 3, 5, and 9 days after slaughter. From all meat samples, 400 mg subsamples were taken at different times of aging to determine inosine monophosphate, inosine, and hypoxanthine in the raw meat.

The meat samples were cooked in an oven at 150 °C to an inner temperature of either 70 or 90 °C and subsequently cooled to room temperature. From each cooked sample two subsamples from the outer layer 1 mm and from the center part, respectively, were obtained. Subsequently, extractions were carried out according to the procedure described below for the determination of inosine monophosphate, inosine, and hypoxanthine.

**Experimental Design, Second Study.** Pork carcasses were randomly selected at the slaughter line at Danish Crown, Ringsted, Denmark, according to hot carcass weight (75−79 kg) and meat percent (58.5−63.0%), and 28 carcasses were chosen and grouped according to ultimate pH, with mean values of 5.5 and 5.7 for normal-pH and high-pH groups, respectively (16 carcasses with 5.5 < pH < 5.6, "normal pH", and 12 carcasses with pH >5.7, "high pH"). The pH was measured with a Knick Portamess pH-meter 751 (Berlin, Germany) equipped with an Ingold LOT glass electrode type 3120 (Mettler Toledo, Urdorf, Switzerland). Both loins from the carcasses were excised the day after slaughter, vacuum-packed, and aged at 2 °C for either 2 days (16 loins with normal pH and 8 loins with high pH), 15 days (8 loins from each pH group), or 21 days (8 loins from each pH group), before they were frozen and stored at −20 °C until further analysis.

**Sensory Analysis.** For sensory analysis the loins were thawed at 5 °C over a period of 20 h. Samples for chemical analysis were obtained, and subsequently the loins were roasted in an oven at 100 °C to a core temperature of 75 °C. The roasted loins were allowed to rest for 30 min at room temperature before they were cut into five 1.5 cm thick slices. The meat was served as whole meat, meat juice, and residue. Meat juice and residue were obtained by squeezing two-thirds of the remaining part of the roast in a pneumatic press (≈191 kg of pressure). The meat juice was centrifuged (1000 rpm, 14 °C, 5 min) to remove dissolved fat globules and subsequently brought to 30 °C using a water

bath before serving, whereas whole meat and the residue were served at room temperature.

The sensory attributes included in the analysis were salty, sour, sweet, bitter, umami, meaty, brothy, piggy, fatty, and cooked root vegetables. The intensity was evaluated using a 15 cm nonstructured line scale.

The panel for the sensory analysis received basic training based on ISO, ASTM-MNL 13, DIN 10964, and DIN 10952. The panel consisted of eight assessors—six female and two male—all living in or around Roskilde, Denmark. All assessors were familiar with pork and descriptive analysis. Prior to the analysis, the panelists were given four training sessions on both basic tastes and the samples represented in the experiment. They were also trained in how to distinguish between retronasal flavor perception and basic taste.

The intensity was evaluated using a 15 cm nonstructured line scale, end-anchored with 0 = little and 15 = very much. The assessors were served either two identical meat samples or two identical samples of dry matter residue (1 × 2 cm). The meat juice (15−20 mL) was served in small plastic cups. Panelists used nose clips when evaluating the first sample (basic taste perception) and no nose clip when evaluating the second sample (retronasal flavor perception). During training, the following reference compounds were used: sour (citric acid, 2 g/L), sweet (saccharose, 12 g/L), bitter (quinine hydrochloride, 0.01 g/L), umami (L-glutamic acid monosodium salt, 2 g/L), brothy (Knorr, pork stock cube), and greasy (decoction of oxtails). All three fractions from the same animal were served in the same session in a randomized design.

**Analysis of Inosine 5′-Monophosphate, Inosine, and Hypoxanthine.** Pork samples (50 mg) for chemical analysis were homogenized (Polytron PT-MR 2100) for 10 s in 3 mL of ice-cold 0.6 M perchloric acid (PCA) containing a pH indicator (0.004% bromthymolblue and 0.004% phenolphthalein). The samples were left on an ice bath for 15 min before neutralization with 2.7 mL of ice-cold 0.8 M KOH and the addition of 0.125 mL of ice-cold $KH_2PO_4$ buffer. Subsequently, the mixtures were mixed for 10 s (IKA MS 2 Minishaker), and the pH was adjusted to 7−8 using either KOH or PCA. Finally, the mixtures were centrifuged at 4000 rpm for 10 min at 4 °C (Multifuge 3 S-R, Heraeus, Germany), and 1 mL of supernatant was transferred to an Eppendorf vial and frozen at −80 °C until further analysis.

The samples were thawed and centrifuged at 10000 rpm for 5 min at 4 °C (Eppendorf centrifuge 5417R), and the supernatants were transferred to cold HPLC vials and placed in a thermostated autosampler (1−2 °C) (G.A.F. 4). Analysis of inosine 5′-monophosphate, inosine, and hypoxanthine was carried out by high-performance liquid chromatography (HPLC) (Hewlett-Packard HPLC system series 1100 Germany) using UV detection (210 nm). A 10 μL sample was injected on the column (Lichrospher 250 × 4 mm RP18, Germany) from which the three compounds were separated by isocratic elution using a solvent based on a buffer containing 10 mM tetrabutylammonium hydrogen sulfate and 215 mM $KH_2PO_4$ to which 7.5 mL methanol/L was added. The following flow gradient was used to obtain optimal separation: 0.5 mL/min for 5 min, increasing to 1.5 mL/min during 1 min, and keeping this flow for 9 min before a final decrease to 0.5 mL/min in 0.5 min. Quantification was based on standard curves using external standards and calculations carried out in the included software (HP Chemstation). In the first study, the sample size and extraction volume were increased 8 times.

**Analysis of Ribose.** The concentration of ribose was measured on six samples of high-pH meat. Three milliliters of 70% methanol was added to 2 g of minced meat in a 10 mL centrifuge tube. The mixture was homogenized (Ultra Turrax T 25, Janke & Kunkel, Staufen, Germany) for 1 min at moderate speed, followed by centrifugation at 2000*g* for 3 min. The procedure was repeated three times in all, and the pooled supernatants were evaporated to dryness by air. The centrifugation residues were redissolved in 4 mL of water (Milli-Q, Millipore, Bedford, MA) of which 2 mL was subjected to group separation by ion exchange chromatography as described by Andersen et al. (*13*). Ribose was eluted with 5 × 4 × 4 mL of water, subsequently evaporated to dryness, and finally redissolved in 200 μL of water before reductive amination and high-performance capillary electrophoresis (HPCE) analysis.

The derivatization of carbohydrates by reductive amination was performed as described by Andersen et al. (*13*). with minor modification. Twenty microliters of internal standard D-thyminose (12.5 mM) was added to 10 μL of a pure ribose solution (standard) and to 100 μL of the redissolved purified elute from the anion exchanger. Additionally 12.5 μL of 0.15 M tryptamine (dissolved in 10% propanol) was added to the mixtures and heated for 10 min at 90 °C. Subsequently, 4.5 μL of aqueous sodium cyanoborohydride solution (0.3 g/mL) was added, and the mixtures were heated at 90 °C for 60 min.

Ribose analysis was performed by capillary electrophoresis system ABI 270A-HT (Applied Biosystems, Foster City, CA) using UV detection. For data processing, a Shimadzu (Kyoto, Japan) Chromatopac C-R3A integrator was used. The method is described by Andersen et al. (*13*).

The concentration of ribose in meat was calculated by the equation

$$C_{\text{ribose}}\ (\mu\text{mol/g}) = (\text{RNA}_{\text{ribose}} \times C_{\text{internal standard}}\ (\mu\text{mol}) \times \text{RRF}_{\text{ribose}})/m_{\text{meat}}\ (\text{g})$$

where $\text{RNA}_{\text{ribose}} = \text{NA}_{\text{ribose}}/\text{NA}_{\text{internal standard}}$, $\text{NA}_{\text{ribose}} = (A_{\text{ribose}}/\text{MT}_{\text{ribose}})$, $C$ = concentration, MT = migration time, NA = normalized area, and RNA = relative normalized area.

For the quantification of ribose, relative response factors (RRF) to the internal standard D-thyminose were calculated. RRF was determined as $\alpha_{\text{D-thyminose}}/\alpha_{\text{ribose}}$, where α is the slope of the calibration curve of pure ribose solutions (0.0025−0.1M).

**Data Analysis.** Data analysis was performed using SAS v. 8.02 (SAS Institute Inc., Cary, NC) for analysis of variance with the MIXED procedure and Unscrambler v. 9.1 (Camo Process AS, Oslo, Norway) for principal component analysis (PCA).

The ANOVA model for analyzing chemical data contained animal as random effect in the first experiment and animal nested within pH in the second experiment. Aging time and treatment were set as fixed effect in the first experiment and aging time and pH group as fixed effect in the second experiment, and the interactions were set as fixed effects in both experiments. Interactions were kept in the model for calculating necessary least-square means (LSM). The concentrations of IMP and inosine were used in the model as covariates when inosine and hypoxanthine, respectively, were analyzed.

Data analysis was performed separately on data as to whether a nose clip was used or not and separately for each fraction—meat, meat juice, and residue.

The ANOVA model for analyzing sensory data contained animal nested within pH as random effect, aging time, pH group, and the interaction as fixed effect. The concentrations of IMP and hypoxanthine were used as covariate in the models for analyzing brothy/meaty and bitter/salty/piggy taste, respectively, and removed from the model when nonsignificant. LSM and *p* values in **Table 2** are calculated with a similar model without separating the fractions, excluding chemical data and including assessors as random effect.

Pearson correlation coefficients together with probability values are used for correlation analysis.

PCA was performed on whole meat, meat juice, and the residue fraction, respectively, in combination with the flavor perception method using full cross-validation without standardization of the observations.

## RESULTS

**Experiment 1. Figure 1** shows the concentrations of IMP, inosine, and hypoxanthine in fresh pork during aging. The concentrations of IMP and hypoxanthine were constant throughout the first 72 h, whereas the concentration of inosine increased significantly from 24 to 72 h. From 72 to 216 h the concentration of IMP decreased with a simultaneous increase in the concentrations of inosine and hypoxanthine.

Significant correlations were found between the concentrations of IMP and inosine ($R = -0.68$, $p = 0.0035$), and tendencies to correlation were seen between the concentrations of inosine and hypoxanthine ($R = 0.44$, $p = 0.0910$) during aging, whereas no significant correlation was found between

**Table 2.** Sensory Scores of the Different Meat Fractions Assessed as Retronasal Flavor Perception (WO) and Basic Taste (W)

| | | whole meat | | | | dry matter residue | | | | meat juice | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | aging | normal pH | | high pH | | normal pH | | high pH | | normal pH | | high pH | | aging[a] | | *p* value fraction | pH[a] | |
| | days | WO | W | WO[b] | W | WO | W | WO[b] | W | WO | W[b] | WO | W[b] | WO | W | WO and W | WO | W |
| sour | 2 | 5.64 | 4.78 | 4.5 | 3.34 | 3.77 | 2.58 | 3.55 | 1.91 | 7.32 | 7.35 a | 6.62 | 6.56 b | | | | | |
| | 15 | 5.59 | 4.56 | 4.77 | 3.68 | 3.84 | 2.19 | 3.13 | 2.14 | 7.78 | 7.75 a | 5.56 | 5.53 a | ns | ns | *** | ***/***/* | ***/ns/** |
| | 21 | 5.83 | 4.9 | 4.33 | 3.26 | 3.63 | 2.25 | 2.94 | 2.11 | 7.86 | 7.99a | 6.74 | 6.69 b | | | | | |
| salty | 2 | 1.63 | 1.79 | 1.44 | 1.22 | 1.41 | 1.12 | 1.31 | 0.98 | 4.37 | 7.38 | 3.94 | 6.01 | | | | | |
| | 15 | 1.95 | 2.08 | 1.59 | 1.46 | 1.53 | 1.08 | 1.1 | 0.83 | 4.56 | 7.36 | 6.45 | 5.17 | ns | ns | *** | * | ns/ns/** |
| | 21 | 2.18 | 2.42 | 1.53 | 1.68 | 1.45 | 0.83 | 1.36 | 0.9 | 4.84 | 7.45 | 4.42 | 3.39 | | | | | |
| bitter | 2 | 1.05 | 0.65 | 0.70 a | 0.59 | 1.38 | 0.95 | 1.10 a | 0.82 | 1.99 | 1.61 | 2.04 | 1.37 | | | | | |
| | 15 | 1.42 | 0.99 | 1.09 a | 0.77 | 1.28 | 0.76 | 1.30 a | 0.81 | 2.06 | 1.36 | 2.18 | 1.76 | **/*/ns | ns | *** | ns | ns |
| | 21 | 1.39 | 0.77 | 1.91 b | 1 | 1.84 | 1.02 | 1.92 b | 1.16 | 2.56 | 1.8 | 2.77 | 1.98 | | | | | |
| umami | 2 | 0.75 | 0.1 | 0.58 | 0.4 | 0.58 | 0.08 | 0.93 | 0.12 | 3.24 | 1.52 | 3.22 | 2.01 | | | | | |
| | 15 | 1.03 | 0.61 | 0.81 | 0.15 | 0.57 | 0.15 | 0.38 | 0.03 | 3.36 | 1.73 | 2.53 | 1.71 | ns | ns | *** | ns | ns[d] |
| | 21 | 0.8 | 0.35 | 0.53 | 0.2 | 0.62 | 0.26 | 0.3 | 0.13 | 3.56 | 2.29 | 3.74 | 1.61 | | | | | |
| pork flavor | 2 | 7.89 | 0.66 | 7.5 | 1.13 | 5.62 | 0.25 | 6.68 | 0.47 | 7.08 | 2.17 | 6.82 | 1.87 | | | | | |
| | 15 | 7.46 | 1.19 | 8.09 | 1.08 | 6.46 | 0.37 | 6.36 | 0.23 | 7.03 | 1.9 | 6 | 1.74 | ns | ns | *** | ns | ns |
| | 21 | 8.05 | 1.16 | 7.37 | 0.92 | 6.18 | 0.35 | 5.63 | 0.27 | 7.49 | 1.85 | 6.38 | 2.01 | | | | | |
| brothy | 2 | 2.92 | 0.67 | 0.37 | 0.51 | 1.53 | 0.38 | 2.33 | 0.21 | 6.89 | 2.04 | 6.33 | 1.73 | | | | | |
| | 15 | 3.21 | 0.81 | 2.76 | 0.8 | 1.74 | 0.26 | 2.12 | 0.4 | 7.25 | 1.79 | 5.46 | 1.6 | ns[c] | ns | *** | ns | ns |
| | 21 | 3.44 | 0.73 | 2.58 | 0.57 | 1.67 | 0.34 | 1.11 | 0.29 | 5.96 | 2.04 | 5.94 | 1.9 | | | | | |
| greasy | 2 | 0.25 | 0.04 | 0.23 | 0.07 | 0.16 | 0 | 0.05 | 0.04 | 3.64 | 2.44 | 3.44 | 2.64 | | | | | |
| | 15 | 0.13 | 0 | 0.12 | 0.06 | 0.06 | 0 | 0.1 | 0.03 | 3.4 | 2.79 | 3.46 | 2.78 | ns | ns | *** | ns | */ns/ns |
| | 21 | 0.04 | 0 | 0.36 | 0.2 | 0.05 | 0 | 0.1 | 0 | 3.6 | 3.1 | 3.61 | 3.17 | | | | | |

[a] If the level of significance was different for the three fractions, the levels are stated as whole meat/dry matter residue/meat juice. [b] Different letters in the same column show significant difference for the attribute. [c] A small significant interaction ($p = 0.03$) between pH and aging was seen for the dry matter residue fraction. [d] A small significant interaction ($p = 0.02$) between pH and aging was seen for the meat fraction.

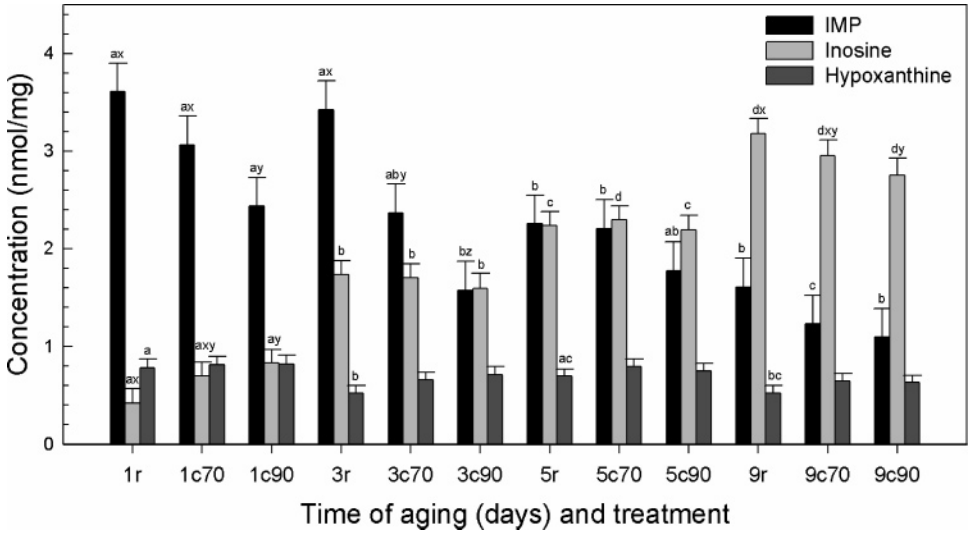



**Figure 1.** Influence of heating on the concentration of IMP, inosine, and hypoxanthine in the center of meat samples as a function of aging: 1c90, 1 day of aging of a center sample heated to 90 °C; r, fresh meat. Letters on bars indicate significant differences between aging time (a–d) within treatment of a metabolite and significant differences between treatments (x–z) within aging time of a metabolite.

the concentrations of IMP and hypoxanthine during aging ($R = -0.31$, $p = 0.2367$).

The sum of the concentration of IMP and its degradation products reached the highest point at 72 h of aging.

**Figure 1** also shows the concentrations of IMP, inosine, and hypoxanthine in fresh meat and in the center of the pork samples aged for different time intervals and heated to a center temperature of 70 and 90 °C, respectively.

Independent of aging time, cooking resulted in a decrease in the concentration of IMP with a concomitant increase in the concentration of hypoxanthine. The concentrations of IMP and inosine in the center of the cooked samples decreased independently of time of aging, whereas the concentrations of IMP and inosine at the surface increased independently of aging compared with the concentrations in the fresh meat (**Figure 2**).

**Experiment 2. Table 1** shows that IMP, independent of pH, in the fresh meat decreased significantly ($p < 0.0001$) both in the samples aged from 2 to 15 days and in those aged from 2 to 21 days. The difference in the concentration of IMP between pork samples from the normal-pH and high-pH groups, with the concentration being highest in the high-pH group, was only statistically significant after 2 days of aging.

The decrease in the concentration of IMP preceded a simultaneous increase in the concentrations of both inosine and hypoxanthine in meat samples of both qualities aged from 2 to 15 days and from 2 to 21 days, respectively.

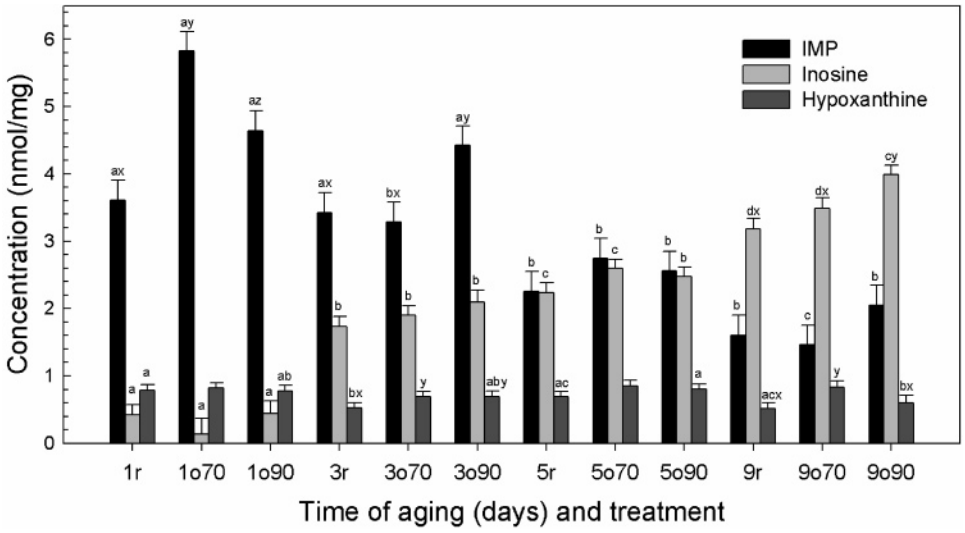



**Figure 2.** Influence of heating on the concentration of IMP, inosine, and hypoxanthine in the outer layer of meat samples as a function of aging: 1o90, 1 day of aging of a surface sample heated to 90 °C; r, fresh meat. Letters on bars indicate significant differences between aging time (a–d) within treatment of a metabolite and significant differences between treatments (x, y) within aging time of a metabolite.

**Table 1.** Change in Concentration of IMP, Inosine, and Hypoxanthine of Meat with High and Normal pH as a Function of Aging Expressed as LSM[a] with Standard Errors

| aging | IMP (nmol/mg) | | inosine (nmol/mg) | | hypoxanthine (nmol/mg) | |
|---|---|---|---|---|---|---|
| days | high pH | normal pH | high pH | normal pH | high pH | normal pH |
| 2 | 4.22 ± 0.16 ax | 3.76 ± 0.11 bx | 1.63 ± 0.23 x | 1.75 ± 0.15 x | 0.74 ± 0.08 ax | 0.52 ± 0.06 bx |
| 15 | 2.52 ± 0.16 y | 2.39 ± 0.15 y | 3.02 ± 0.16 y | 3.34 ± 0.16y y | 1.38 ± 0.07 ay | 1.15 ± 0.07 by |
| 21 | 2.10 ± 0.16 y | 1.86 ± 0.16 z | 3.43 ± 0.19 ay | 4.24 ± 0.21 bz | 1.61 ± 0.07 az | 1.24 ± 0.09 by |

[a] Means with different letters (a, b) within a row of a metabolite indicate significant differences between pH groups (within aging time), $p \leq 0.05$. Means with different letters (x–z) within a column indicate significant differences in concentration of a metabolite between aging times (within pH group), $p \leq 0.05$.

The samples of the high-pH group analyzed for the concentration of ribose had mean values of 0.72 ± 0.38 and 1.33 ± 0.41 nmol/mg of meat at aging times of 2 and 21 days, respectively. The concentration of ribose showed tendencies to significant correlation to the concentration of IMP ($R = -0.74$, $p = 0.0912$) and inosine ($R = 0.75$, $p = 0.0831$).

The scores for the sensory attributes by retronasal flavor perception are shown in **Table 2**. There was a significant difference between the fractions for all attributes, independent of pH and aging, with the highest scores in the meat juice. The differences were most pronounced for the salty, sour, umami, greasy, and especially the brothy attributes. **Table 2** also gives the sensory attributes obtained as basic tastes. Even though the difference between fractions in the brothy attribute was not as pronounced as obtained by retronasal flavor perception, the overall pattern was the same independent of the perception mode. The pork flavor attribute was most intense in the whole meat obtained by retronasal flavor perception; however, both the residue fraction and the meat juice fraction also scored high for this attribute.

Performing sensory analysis with and without nose clips clearly showed that the basic taste attributes, salty, sour, bitter and umami, resulted in almost the same scores independent of mode of perception, whereas the scores of the other sensory attributes, for example, meaty, brothy, and fatty, decreased drastically when the sensory analysis was performed with a nose clip.

Independent of the two sensory analysis approaches, the sensory attribute sour was as expected found to be significantly more pronounced in pork of normal pH (retronasal perception, $p = 0.0014$; and basic taste perception, $p < 0.0001$). Moreover, the sensory attribute salty identified only by basic taste perception was significantly influenced by pH in the meat ($p = 0.006$) and meat juice ($p = 0.0134$) with pork with normal pH being more salty. Finally, the sensory attribute bitter tended to be more pronounced in the pork juice fraction from the high-pH quality ($p = 0.0793$).

The sensory attribute bitter determined by retronasal flavor perception in the pork residue was affected by aging of the meat ($p = 0.0359$), as the residue became more bitter upon prolonged aging. Moreover, the sensory attribute piggy seemed to be more pronounced in whole meat ($p = 0.0791$) and meat juice ($p = 0.0363$) determined by basic taste perception and in whole meat ($p = 0.0513$) determined by retronasal taste perception. Finally, aging of the meat had a positive effect on the sensory attribute salty in whole meat determined by both retronasal flavor perception ($p = 0.039$) and basic taste perception ($p = 0.0297$) and in pork residue ($p = 0.0722$) determined by basic taste perception.

To compare data obtained by sensory analysis with the chemical data on the meat as a function of aging, PCAs were performed on the data. PCA was carried out on data from whole meat, meat juice, and the residue, respectively, and on sensory data obtained both with and without nose clip. **Figures 3** and **4** show score and loading plots of whole pork (**a**) and the corresponding meat juice (**b**) and residue (**c**) on retronasal flavor perception and chemical data. In **Figure 4a**, PC3 expands the aging time and PC2 the meat quality (normal, 5.5 < pH < 5.6 and high, pH >5.7), whereas PC1 expands aging time and PC2 the meat quality in both panels **b** and **c** of **Figure 4**. Panels **a**

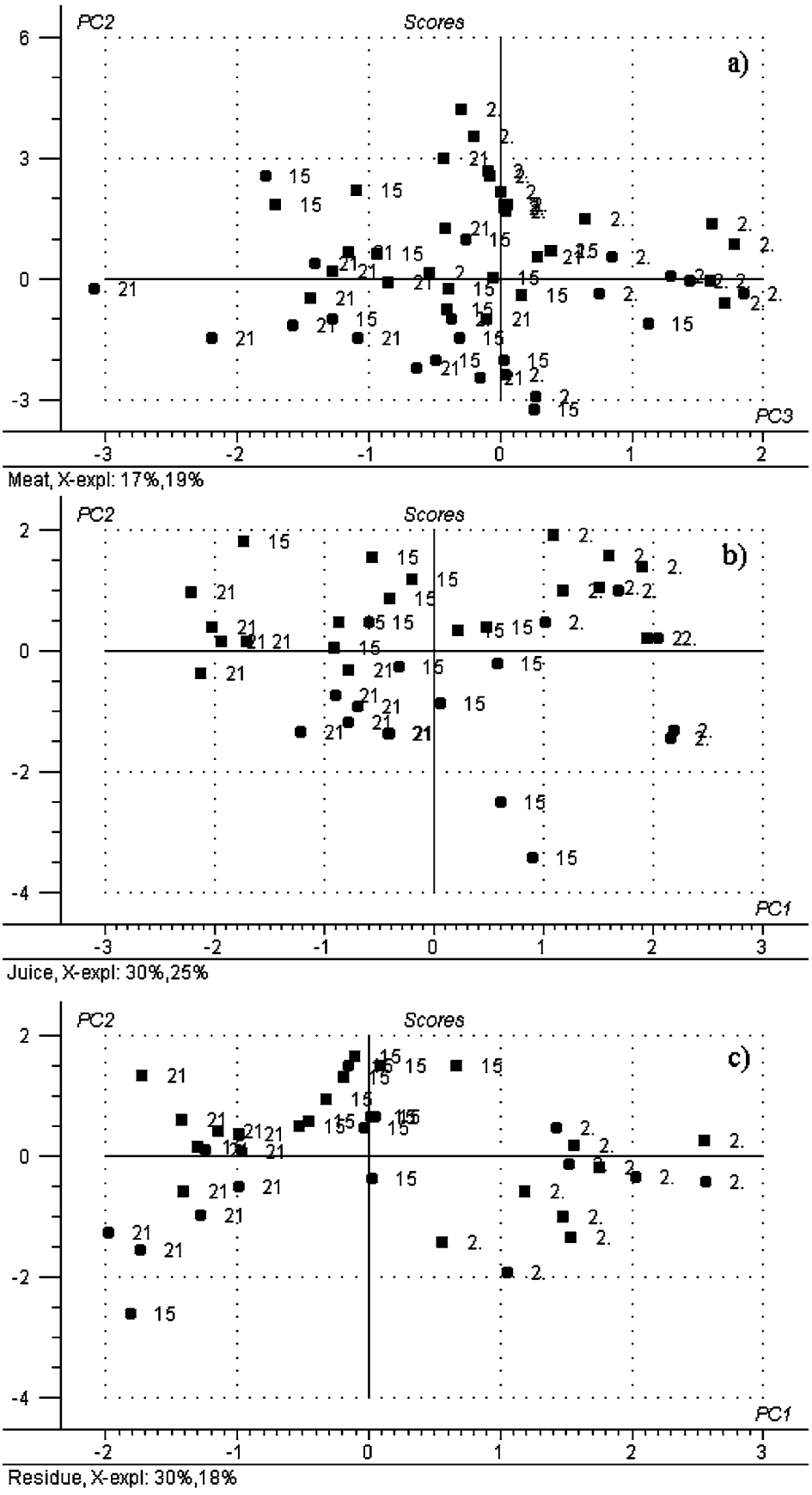



**Figure 3.** Score plots from PCA of meat (**a**), meat juice (**b**), and residue samples (**c**) using retronasal flavor perception. Rectangles represent samples of normal pH, and circles represent samples of high pH.

and **c** of **Figure 4** show that the sensory attribute brothy is associated with meat aged for only 2 days (fresh meat) and IMP. Subsequent correlation analysis showed that brothy determined by retronasal flavor perception was significantly correlated with

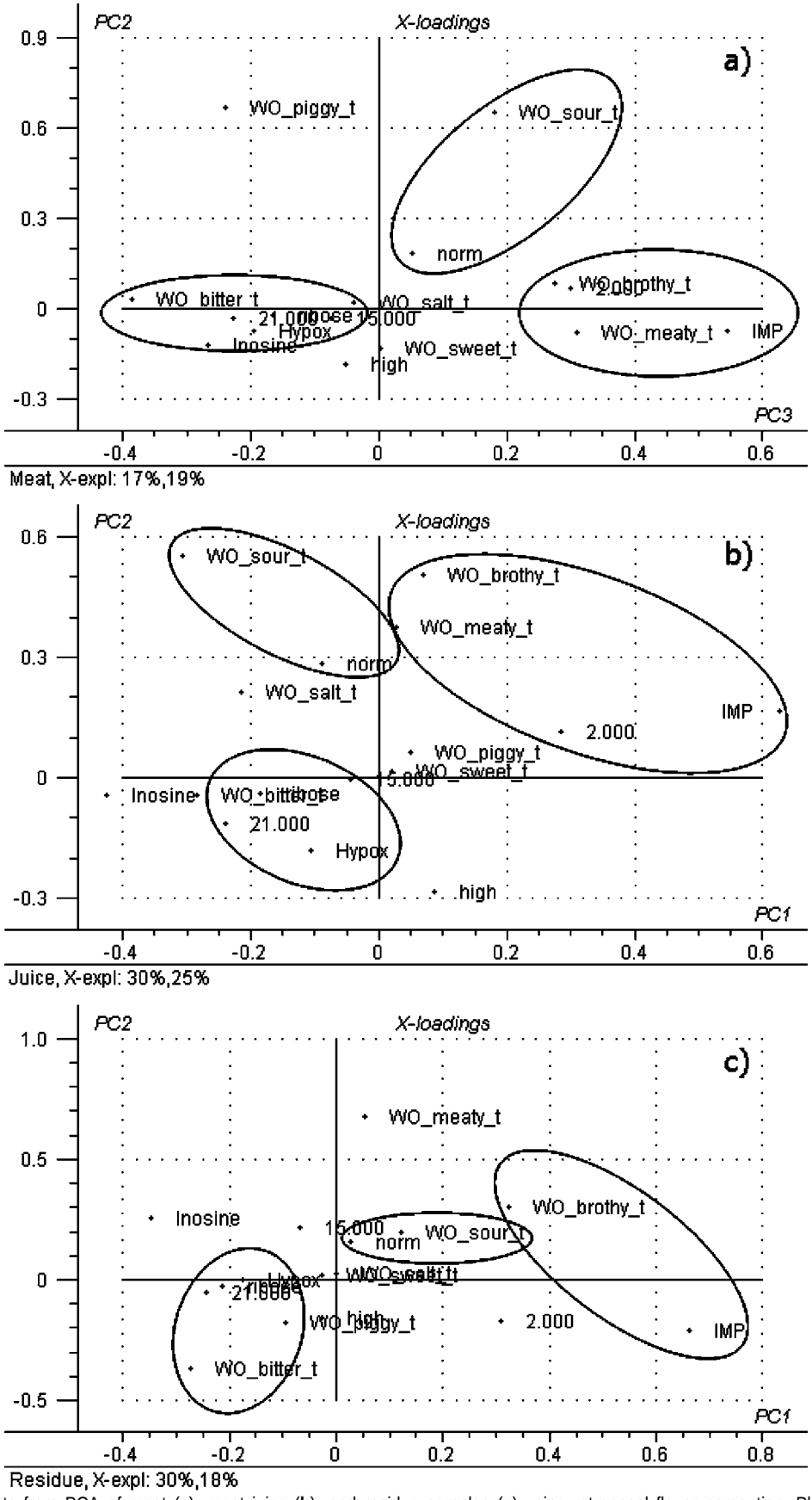



**Figure 4.** Loading plots from PCA of meat (**a**), meat juice (**b**), and residue samples (**c**) using retronasal flavor perception. Plots include pH groups (normal, high), aging times in days (2.000 ≡ 2, 15.000 ≡ 15, and 21.000 ≡ 21 days), chemical data (IMP, inosine, hypoxanthine), and sensory scores (sour, salty, sweet, bitter, meaty, brothy, and piggy). "WO_brothy_t" represents the brothy taste experienced by retronasal flavor perception.

IMP in the pork residue ($R = 0.36$, $p = 0.0265$). Moreover, **Figure 4** clearly shows that the sensory attribute bitter is associated with meat aged for 21 days and the presence of high concentrations of hypoxanthine. Subsequent correlation analysis

showed that hypoxanthine and bitterness determined by retronasal flavor perception tended to correlate positively ($R = 0.31$, $p = 0.0621$).

Finally, **Figure 4** confirms that the normal meat quality is associated with the sensory attribute sour, as also found by analysis of variance.

## DISCUSSION

The fate of the umami compound, inosine monophosphate, and its secondary degradation products, ribose and hypoxanthine, in pork during storage and cooking and their subsequent influence on the flavor development in pork is far from well established. This is so despite the fact that such an understanding could provide valuable information of importance in the marketing of pork products with high eating quality.

The present data showed a decrease in the concentration of IMP and a simultaneous increase in the concentrations of inosine and hypoxanthine in fresh pork during aging and, hereby, resemble data previously reported by Kato and Nishimura (*14*). However, in contrast to the results from Dannert and Pearson (*15*) on beef, we were not able to confirm a temporary accumulation of IMP in the meat during the first day post-mortem.

The maximum concentration of IMP and its degradation products, inosine and hypoxanthine, was reached at 72 h after slaughter (**Figure 1**), which is in agreement with data by Lindahl et al. (*16*), who found that the post-mortem metabolism proceeds up to 2 days postslaughter.

Our data clearly show that inosine accumulates without noticeable increase in the concentration of hypoxanthine during 9 days of aging (**Figure 1**). This can be explained by the fact that the rate at which IMP is dephosphorylated to inosine is much more rapid than the hydrolysis of inosine to hypoxanthine (*7*). Moreover, the degradation of IMP proceeded more rapidly at higher temperature, whereas only small or no differences were found in the concentrations of inosine and hypoxanthine upon increase in heating temperature from 70 to 90 °C (**Figure 1**). This indicates that during heating both inosine and hypoxanthine go into subsequent reactions with other constituents in meat.

In the present study, we found a highly significant difference between the concentrations of IMP, inosine, and hypoxanthine in the center (**Figure 1**) and at the surface (**Figure 2**) of the pork samples, with higher concentration at the surface. This might just be explained by a pronounced dehydration of the outer layer during cooking, even though it cannot be completely ruled out that a thermally induced dephosphorylation of residual ATP/ADP and AMP might also take place. However, additional studies are needed if this aspect has to be elucidated further.

The degradation of IMP was found to be weakly dependent on the meat quality, with pork of normal pH having lower IMP concentration after 2 days of aging (**Table 1**). However, meat quality was not found to influence the degradation of IMP further during aging. This influence of meat quality might be explained by the fact that the stability of IMP, containing weak chemical bonds, for example, glucoside and ester bonds (*12*), is both temperature- and pH-dependent, which is why low pH might accelerate the dephosphorylation of IMP in the period early post-mortem. As expected, the concentration of ribose increased during aging in the samples analyzed. Absolute values of ribose equal to the concentration of hypoxanthine thereby support the stoichiometry of the degradation of IMP. However, no significant relationship was established between ribose and flavor attributes. This might be due to the relatively small number of samples analyzed and the relatively low cooking temperature or the fact that the flavor attributes included in the sensory analysis do not reflect ribose or ribose-related flavor compounds. Further studies are needed to exploit this.

The difference in perception of salty, sour, bitter, and umami attributes (basic tastes) and the other sensory attributes, for example, meaty, brothy, and fatty (flavor), depending on whether sensory analysis was performed with or without nose clip (**Table 2**) suggests that this approach fulfills the required distinction between basic taste perception and retronasal flavor perception.

Brothy flavor in beef and pork broth has been shown to increase with time (*11*, *14*), and it is believed to be influenced by the proteolytic breakdown of proteins to free amino acids and peptides. However, in the present study we were not able to see any development in the brothy flavor attribute as a consequence of aging time.

Compounds contributing to a specific pork flavor, such as 2-methyl-3-furanthiol, and corresponding disulfide from the thermal degradation of thiamin have been reported to have an extremely low odor threshold (*17*), confirmed also by the high scores for pork flavor attribute for all fractions in the present study. The fact that the pork flavor attribute was mainly assessed by retronasal flavor perception indicates that volatile compounds mainly contribute to this flavor attribute. This is in accordance with the general principles of the meat flavor development, as Maillard reaction products and lipid oxidation products, which are mainly volatiles, are thought to be responsible for both the meaty and the species-specific flavor of meats (*6*, *18*).

Unlike the results by Bryhni et al. (*19*) reporting the lower intensities of the pork flavor attribute in meat with a high pH, the pork flavor was not influenced by the pH of the meat in the present study. This might be due to the rather small pH difference and the low cooking temperature used in the present study, which results in only low intensities of the pork flavor attribute.

The difference in pH of the two meat quality groups influenced the sour flavor attribute, resulting in higher scores of sourness of the meat with normal pH, as expected from previous results (*19*). The source of the sour taste might mainly be the lactic acid from a more extensive buildup of lactic acid from post-mortem glycolysis of the meat with low pH (*20*). Schlichtherle-Cerny and Grosch (*21*) showed that lactic acid is the most active ion in beef broth and that 83% of the assessors in a sensory triangle test could recognize the omission of lactic acid in a model system of the broth. Lactic acid is water-soluble, and the low scores for the sour flavor attribute in the residue fraction might just be a result of the combination of the fact that most of the lactic acid is no longer in this fraction, but is in the meat juice, and that it is more easily masked by other flavor compounds present in residue fraction.

The bitter taste can be elicited by hypoxanthine, hydrophobic amino acids, either free or bound in smaller peptides (*22*, *23*), and certain Maillard reaction products, for example, pyrazines (*24*), which might be perceived by basic taste perception as well as by retronasal flavor perception.

The flavor attribute bitter increased as a function of aging in both the meat and the residue fraction as assessed by retronasal flavor perception, with the increase being most pronounced from day 15 to 21.

PCA allows describing data from chemical analysis together with data from sensory evaluation in one analysis. It defines the main effects present in data as principal components (PCs) and display the PC's strength in relation to the whole dataset and each other. Individual samples are placed in the score plot in relation to the loading plot of the variables.

To illustrate the connection between sensory attributes of the two meat qualities during aging and IMP and its degradation products, a PCA of each of the three meat fractions was carried out (**Figure 4**). The obtained PCs clearly distended the time of aging and the quality of the meat in the loading plots. The data showed that the sensory attributes brothy and meaty correlate significantly with nonaged meat and IMP, being most pronounced in whole meat, and thereby support previous data showing that IMP is a desirable flavor enhancer in meat and fish (*25*−*27*). Moreover, the correlation between the sensory attribute bitter, aging for 21 days, and hypoxanthine indicates that the formation of hypoxanthine upon degradation of IMP might contribute to flavor deterioration during storage of pork, as previously suggested to be the case during prolonged storage of fish (*28*). Finally, the PCA data confirm the result of the sensory analysis that the sensory attribute sour is highly correlated to the pH of meat.

In conclusion, the obtained results clearly demonstrate that aging and cooking are important for the development of essential meat flavor precursors. In contrast, the difference in meat quality (pH) within the limits studied in the present study had only negligible effect on the development of the studied flavor precursors. The flavor enhancer inosine monophosphate was found to contribute to the sensory attributes brothy and meaty, whereas its degradation product hypoxanthine was related to the sensory attribute bitter in pork. Finally, the change in sensory attributes from brothy/meaty to bitter taking place during the storage of pork was found to coincide with the continuous degradation of inosine monophosphate to hypoxanthine.

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