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THE DEVELOPMENT IN INOSINE MONOPHOSPHATE AND ITS DEGRADATION PRODUCTS DURING AGING OF PORK OF DIFFERENT QUALITIES IN RELATION TO BASIC TASTE - AND RETRO NASAL FLAVOR PERCEPTION OF THE MEAT

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Introduction

The characteristic flavor of meat mostly develops through the heating of the meat, however, raw meat also inherents several non-volatile constituents that can contribute to the overall flavor of meat (MacLeod, 1986). The 5´-ribonucleotide, inosine monophosphate (IMP) originating from dephosphorylation of the triphosphate 5´-ribonucleotide, adenosine triphosphate (ATP) and its degradation products ribose and hypoxanthine are all considered to be important constituents in meat flavor formation and development (see Scheme 1). IMP contributes with umami taste (Durnford, Shahidi, 1998; Spurvey et al, 1998) and has flavor-enhancing properties, and are reported to enhance meaty, brothy, mouth filling, dry and astringent qualities and suppress sulphurous notes (Kuninaka, 1981) while hypoxanthine displays bitter characteristics, and ribose is considered the most important reducing sugar participating in flavor-producing Maillard reactions in meats upon heating (Mottram, 1998).

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

Scheme 1

$$\text{ATP} \rightarrow \text{ADP} \rightarrow \text{AMP} \rightarrow \text{IMP} \rightarrow \text{Inosine} \rightarrow \text{Hypoxanthine} + \text{Ribose}$$

ATP ≡ adenosine 5´-triphosphate
ADP ≡ adenosine 5´-diphosphate
AMP ≡ adenosine 5´-monophosphate
IMP ≡ inosine 5´-monophosphate

The present study aims to exploit the faith of inherent inosine monophosphate during aging of pork of two qualities, normal pH versus high pH, in relation to basic taste perception and retro nasal flavor perception of the cooked meat.

Objectives

The objective of the study is to identify potential connections between the concentration of flavor precursors and sensory attributes in whole meat, meat juice, and the remaining meat residue in two pork qualities during aging considering both basic taste perception and retro-nasal flavor perception.

Methodology

Pork carcasses were randomly selected at the slaughter-line at Danish Crown, Ringsted, Denmark according to hot carcass weight (75-79 kg), meat percent (58.5-63.0%), and twenty-eight carcasses were chosen and grouped according to ultimate pH (16 carcasses with5.5<pH<5.6, normal pH, and 12 carcasses with pH>5.7, high pH). The pH was measured with a Knick Portamess pH-meter no. 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. The experimental design is schematically outlined in Figure 1.

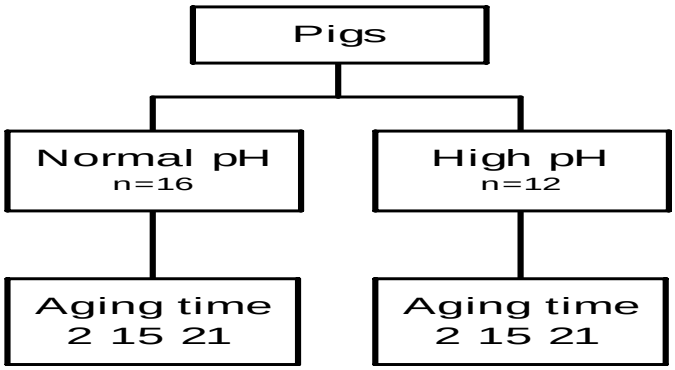

Figure 1. Experimental design (n – number of animals)

For sensory analysis the loins were thawed at 5°C over a period of 20 hours, slice for chemical analysis separated, and subsequently roasted in an oven at 100°C to a core temperature of 75°C. The roasted loins were allowed to rest for 30 minutes at room temperature before they were cut into five 1.5 cm-thick slices. The meat was served both as whole meat, meat juice and residue. Meat juice and residue were obtained by squeezing 2/3 of the remaining part of the roast in a pneumatic press. 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 while whole meat and residue were served at room temperature.

The sensory attributes were as follows: *salty*, *sour*, *sweet*, *bitter*, *umami*, *meaty*, *brothy*, *piggy*, *fatty*, and *cooked root vegetables*. The intensity was evaluated using a 15 cm non-structured line scale. The assessors were either served 2 identical meat samples or 2 identical residue samples (1x2 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 without the nose clip when evaluating the second sample (retro nasal

flavor perception). All three fractions from the same animal were served in the same session in a randomized design.

Pork samples (50 mg) for chemical analysis were homogenized (Polytron PT-MR 2100) for 10 seconds in 3 ml of ice-cold 0.6 M perchloric acid (PCA) containing a pH indicator (bromthymolblue and phenolphthalein 0.004% of each) using Sarstedt 15 ml conical vials. The samples were left on ice-bath for 15 minutes before neutralization with 2.7 ml of ice-cold 0.8 M KOH and addition of 0.125 ml ice-cold KH_2PO_4 buffer. Subsequently the mixtures were mixed for 10 seconds using an IKA MS 2 Minishaker, and the pH was adjusted to 7-8 using either KOH or PCA. Finally, the mixtures were centrifuged using an Eppendorf Centrifuge 5417R (4000 rpm for 10 min at 4°C), and 1 ml supernatant (in duplicate) was transferred to an Eppendorf vial and frozen at -80°C until further analysis.

Analysis of inosine 5´-monophosphate, inosine and hypoxanthine was carried out by high-performance liquid chromatography (HPLC) on a Hewlett-Packard HPLC system series 1100 using UV detection (210 nm). The samples were thawed and centrifuged, and the supernatants were transferred to cold HPLC vials and placed in a thermostatted auto sampler (1-2°C). A 10 µl sample was injected on a Lichrospher 250 x 4 mm RP18 column from which the three compounds were separated by isocratic elution using a solvent based on a buffer containing 10 mM tetrabutylammonium hydrogensulfate 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 in 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).

Data anlysis was performed using Unscrambler v. 9.1 (CAMO PROCESS AS, Oslo, Norway) for Principal Component Analysis (PCA) and SAS v. 8.02 (SAS Institute Inc., Cary, NC, USA) for analysis of variance with the MIXED procedure.

Results & Discussion

Figure 2 shows that IMP independent of pH in the fresh meat decreased significantly ($p<0.0001$) both in the samples aged from 2 to 15 and from 2 to 21 days. The difference in IMP concentration between pork samples from the normal and high pH group with the concentration being highest in the high pH group was only statistical significant after 2 days of aging. This difference in concentration between the normal and high pH group is in agreement with the fact that the stability of IMP is both temperature- and pH-dependent due to the presence of weak chemical bonds, e.g. glucoside and ester bonds (Matoba et al. 1988), with low pH accelerating the dephosphorylation of IMP. The decrease in IMP preceded a simultaneous increase in both inosine and hypoxhantine concentrations in meat samples of both qualities aged from 2 to 15 days and from 2 to 21 days, respectively. The rate by which IMP was degraded to inosine and the rate by which inosine was degraded to hypoxanthine during aging was found to be in agreement with the difference in the rate constants of the dephosphorylation of IMP and the hydrolysis of inosine described by Dunford and Shahidi (1998).

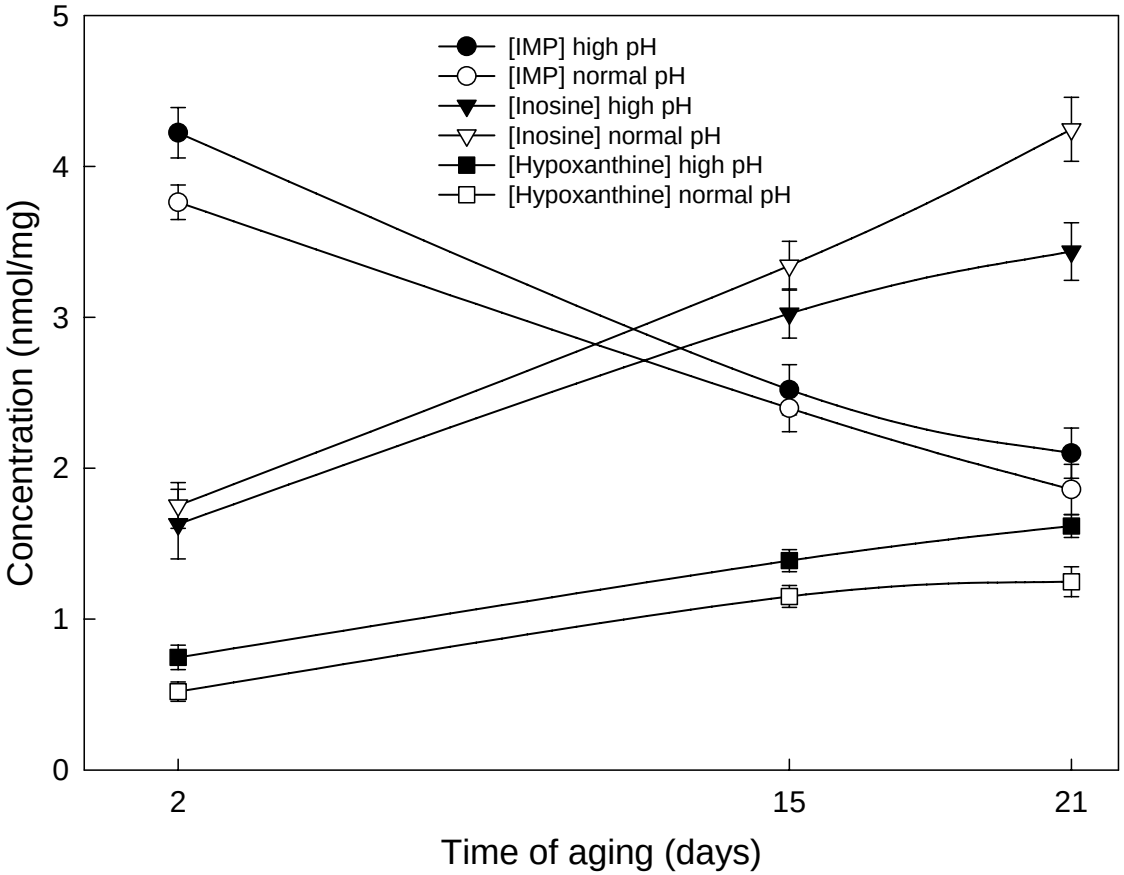

Figure 2. The change in the concentration of IMP, inosine and hypoxanthine of meat with high and normal pH as a function of aging expressed as lsmeans with standard errors

Performing sensory analysis with and without nose clip clearly showed that the basic tastes; *salty*, *sour*, *bitter*, and *umami*, displayed almost the same intensity in the individual samples independent of use of nose clip, while the intensity of the other sensory attributes, e.g. *meaty*, *brothy*, and *fatty*, decreased drastically when the sensory analysis was performed with nose clip. Consequently, the aim of performing the sensory analysis both with and without nose clip to differ between basic taste perception and retro nasal flavor perception of the samples seems to be fulfilled using this approach.

Independent of the two sensory analyses approaches, the sensory attribute *sour* for retro nasal perception ($p=0.0014$) and for basic taste perception ($p<0.0001$) was as expected found to be significantly more pronounced in pork of the normal pH. Moreover, the sensory attribute *salty* identified only by basic taste perception was significantly influenced by pH in meat (0.006) and meat juice (0.0134) with pork with normal pH appearing more salty while the sensory attribute *bitter* tended to be more pronounced in the pork juice fraction of the high pH quality ($p=0.0793$).

The sensory attribute *bitter* determined by retro nasal 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 more pronounced in whole meat ($p=0.0791$) and meat juice ($p=0.0363$) determined by basic taste perception, and in whole meat ($p=0.0513$) by retro nasal taste perception. Finally, aging of the meat had a positive effect on the sensory attribute *salty* in whole meat determined by both retro

nasal 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, principal component analyses (PCA) were performed on the data. PCA analysis was carried out on data from whole meat, meat juice and the residue, respectively, and both on sensory data obtained with and without nose clip. Figure 3 shows loading plots of whole pork (a) and the corresponding meat juice (b) and residue (c) on retro nasal flavor perception and chemical data. In Figure 3a **PC3** expands the aging time and **PC2** the meat quality (*norm* - 5.5<pH<5.6 & *high* – pH>5.7) while **PC1** expands aging time and **PC2** the meat quality in both Figure 3b and 3c. Figure 3a and 3c shows that the sensory attribute *brothy* is associated with meat aged for only 2 days (fresh meat) and presence of the flavor enhancer, IMP. Subsequent correlation analysis showed that *brothy* determined by retro nasal flavor perception was significantly correlated with IMP in the pork residue (R=0.36, p=0.0265). These data confirm previous data, showing that IMP is a desirable flavor enhancer in meat and fish (Maga, 1987; Madruga, 1997; Murata and Sakaguchi, 1989). Moreover, Figure 3 clearly shows that the sensory attribute *bitter* is associated with meat aged for 21 days and presence of high concentrations of hypoxanthine. Subsequent correlation analysis showed that hypoxanthine and bitterness determined by retro nasal flavor perception tended to correlate positively (R=0.31, p=0.0621). Consequently, present data indicate that formation of bitter taste through degradation of IMP to hypoxanthine during aging might be an element in flavor deterioration, as previously suggested to be the case during prolonged storage of fish (Bremner et al., 1988).

Finally, Figure 3 confirms that the normal meat quality is associated with the sensory attribute *sour*, as also found by analysis of variance.

Conclusion

The present study shows that the flavor enhancer inosine monophosphate and its degradation product hypoxanthine contribute to the sensory attributes *brothy* and *bitter* in pork and that the change in sensory attributes from *brothy* to *bitter* taking place upon prolonged storage of pork resembles continuous degradation of inosine monophosphate to hypoxanthine.

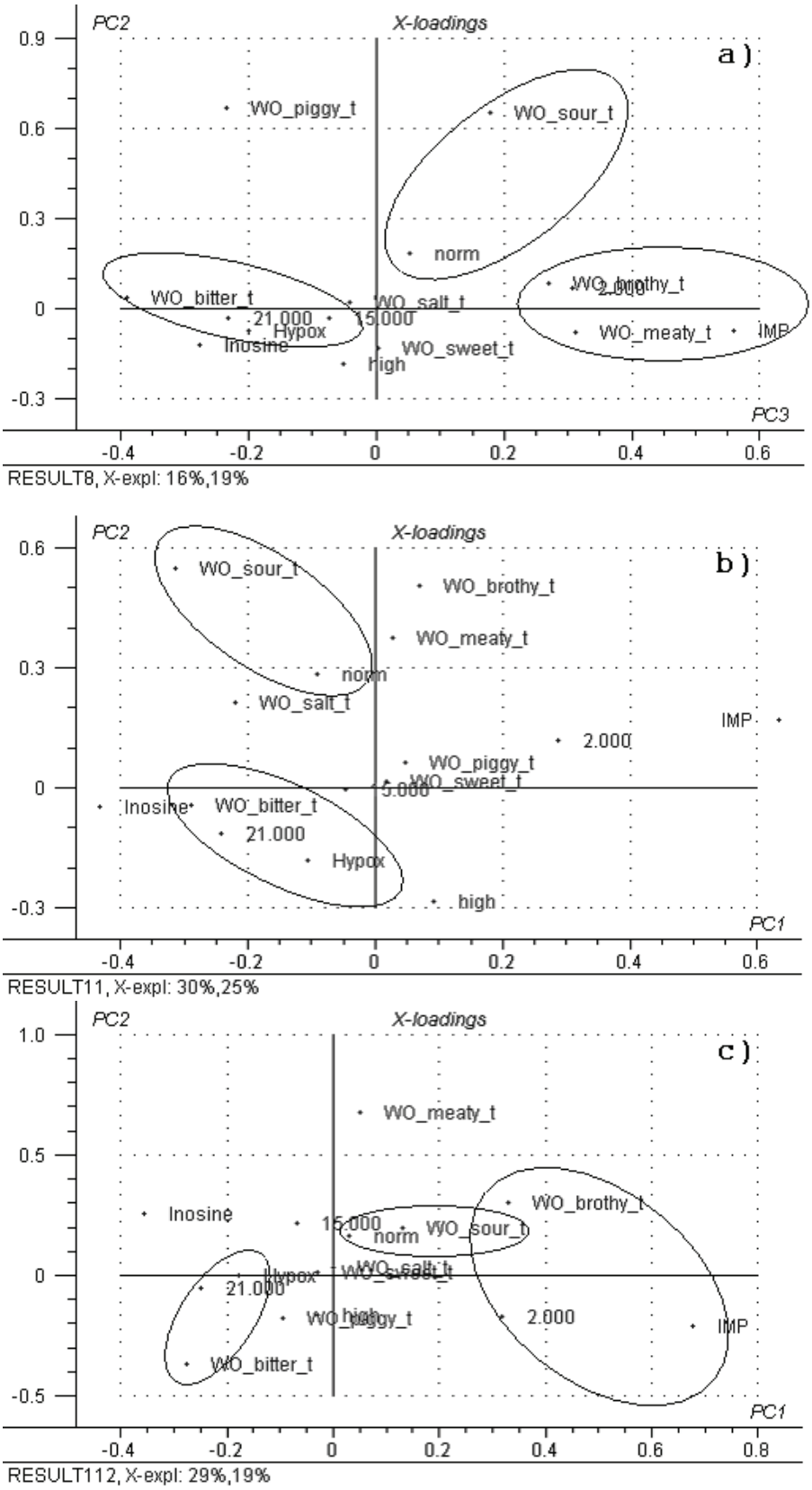

Figure 3. The loading plots of principal component analysis on meat (a), meat juice (b) and residue (c) by retro nasal flavor perception

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