THE FAITH OF THE UMAMI COMPOUND AND FLAVOUR PRECURSOR INOSINE MONOPHOSPHATE DURING AGING AND COOKING OF PORK

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Background

The flavour of meat develops largely through the cooking process, however, raw meat contains several constituents that are non-volatile and contribute to its taste (MacLeod, 1986). Consequently, many of the constituents in raw meat undergo substantial changes during the cooking process and subsequently provide roasted, boiled, fatty and species-related flavours, as well as the characteristic meaty aromas associated with all cooked meats. Model experiments have suggested that the Maillard reaction between amino acids and reducing sugars is one of the main pathways for formation of many of the aroma compounds, which have been identified in cooked meat. The dominating reducing sugar in meat is the pentose, ribose, which originates from the degradation of ribonucleotides (Mottram, 1998). Moreover, nucleotides and related compounds, e.g. the 5´-ribonucleotides, adenosine monophosphate (AMP), inosine monophosphate (IMP) and guanosine monophosphate (GMP), are also important in relation to meat flavour, as they contribute with umami taste (Durnford, Shahidi, 1998; Spurvey et al, 1998). Umami taste has a characteristic savoury quality, first characterized for glutamate. Beside the characteristic umami taste, umami compounds have flavour enhancing properties, and are reported to enhance meaty, brothy, mouth filling, dry and astringent qualities and suppress sulphurous notes (Kuninaka, 1981). Finally, bitterness in meat may be derived from hypoxanthine, which is a degradation product of IMP, together with anserine, carnosine, other dipeptides and several free amino acids.

Considering that IMP, ribose and hypoxanthine all are considered important constituents in meat flavour 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 flavour development in meat.

Scheme 1

ATP ⇒ ADP ⇒ AMP ⇒ IMP ⇒ Inosine ⇒ Hypoxanthine + Ribose

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

IMP has been found to be a desirable flavour enhancer and umami compound in meat and fish (Maga, 1987; Madruga M.S., 1997; Murata, Sakaguchi, 1989). As the dephosphorylation of IMP to inosine is a slow process, IMP has been reported to accumulate in beef and fresh fish muscle (Dannert and Pearson, 1967; Konosu, Yamaguchi, 1989). The IMP concentration has in fish been found to be highest within one to two days post mortem with a subsequent decrease resulting in a less acceptable flavour development (Fletcher and Statham, 1988). Formation of bitter taste through degradation of IMP to hypoxanthine might be an element in flavour deterioration of fish during prolonged storage, as nucleotide degradation is found to contribute to bitter taste in muscle foods (Bremner et al., 1988). Likewise, Cambero et al. (2000a) reported sour and astringent flavour of beef broth at higher cooking temperatures, which also may reflect severe nucleotide degradation.

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 and sheep meat (Arya and Parihar, 1979). Moreover, increasing cooking temperatures have been found to

result in a significant rise in the concentration of creatinine, IMP, AMP in beef broth, with IMP showing the highest correlation to the sensory data of the broth (Cambero et al., 2000b). The stability of IMP is reported to be both temperature- and pH-dependent due to the presence of weak chemical bonds, e.g. glucoside and ester bonds (Matoba et al. 1988). Consequently, pH in the fresh meat must be expected to influence IMP degradation through aging and cooking.
Interestingly, the contaminant 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 reactions, which take place in meat.
Most flavour studies related to meat have been carried out in model systems or in spiking studies where potential flavour pre-cursors have been added to meat or meat broth with subsequent chemical or sensory analysis. The present study is a preliminary study to exploit the faith of inherent inosine monophosphate during aging and cooking of pork, which subsequently will be investigated in relation to the potential contribution of IMP, hypoxanthine and ribose to the formation of volatile flavour compounds and sensory characteristics, as a consequence of aging and cooking temperature.

Objectives

The objective of this study was to investigate the faith of IMP during aging and cooking of pork, by measuring IMP and its degradation products inosine and hypoxanthine.

Materials and methods

The *M. longissimus dorsi* from four pigs (cross-breeds of Duroc boar and Danish Landrace x Yorkshire dams) reared and slaughtered at The Danish Institute of Agricultural Sciences (DIAS), Foulum, Denmark was used in the experiment. Rectangular meat samples (3x3x2 cm), from which all visible fat and connective tissue were removed, were cut, vacuum-packed in pairs and stored at 4°C for 24, 72, 120 and 216 h after slaughter. From all meat samples, 400 mg sub samples were taken at different time of aging.
The meat with different time of aging was cooked in an oven at 150°C to an inner temperature of 70 and 90°C and then cooled to room temperature. From each cooked piece two samples from 1 mm outer layer and two samples from the inner part of the meat were cut, and extractions according to the procedure described below were carried out to determine inosine monophosphate, inosine and hypoxanthine.
Pork samples of 400 mg were mixed for 5 seconds in 24 ml of ice-cold 0.6 M perchloric acid (PCA) containing a pH indicator (bromthymolblue and phenolphthalein 0.004% of each) using Sarstedt 50 ml conical vials. The samples were left on ice-bath for 15 minutes before neutralization with 21.6 ml of ice-cold 0.8 M KOH and addition of 1 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 triplicate) 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).

Results and discussion

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, while the concentration of inosine increased significantly from 24 to 72 h. From 72 h to 216 h the concentration of IMP decreased with a simultaneous increase in the concentrations of inosine and hypoxanthine hereby resembling the data reported previously by Kato and Nishimura (1987).

Significant correlations were found between the concentrations of IMP and inosine (R=-0.55, P=0.0012) and between [inosine] and [hypoxanthine] (R=0.46, P=0.0077) during aging, while no significant correlation was found between [IMP] and [hypoxanthine] during aging (R=-0.26, P=0.1522). This is in agreement with the expected difference in the rate constants of the dephosphorylation of IMP and the hydrolysis of inosine, as described by Dunford and Shahidi (1998).
The observed maximum in the concentration of IMP and its degradation products inosine and hypoxanthine at 72 h after slaughter is in agreement with the data by Lindahl et al. (2003), who found that the post mortem metabolism proceeded after 2 days post slaughter.
Figure 2 shows the concentrations of IMP, inosine and hypoxanthine at the surface (o) and the centre (i) of pork samples aged for different time intervals and heated to a centre temperature of 90°C. In general, cooking resulted in a decrease in [IMP] with a concomitant increase in [inosine] and [hypoxantine] independent of time of aging. The concentrations of IMP and inosine in the centre of the cooked samples decreased independently of time of aging while [IMP] and [inosine] at the surface increased independently of aging compared with the concentrations in the fresh meat. Even though not as drastically, the same pattern was seen when samples were heated to a centre temperature of 70°C (data not shown). The highly significant difference between concentrations in the centre and at the surface of the pork samples is most probably due to dehydration of the outer layer during cooking even though a thermally induced dephosphorylation of residual ATP/ADP and AMP might also be a possibility. This aspect needs to be investigated further.

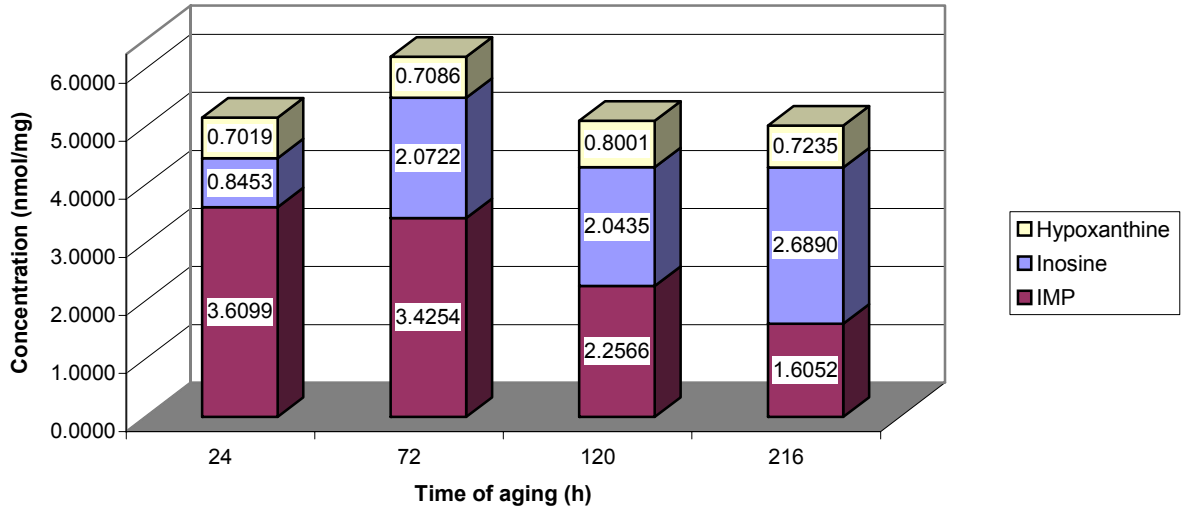

Figure 1. The concentration of IMP, inosine and hypoxanthine during aging of raw pork

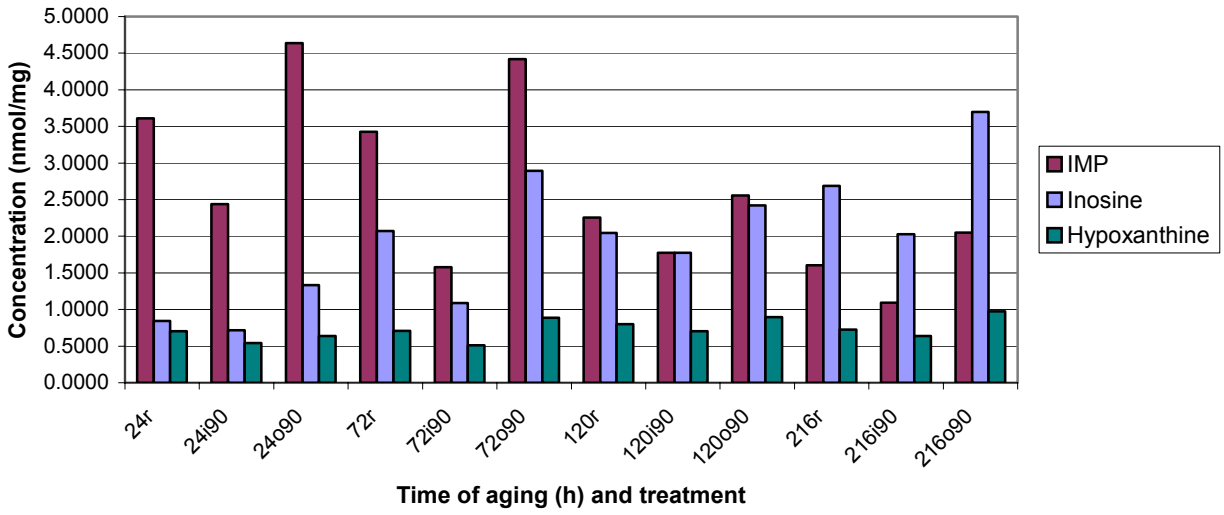

Figure 2. Influence of aging and heat treatment on the concentration of IMP, inosine and hypoxanthine. 24i90 means aging 24h, inside the meat at 90 ºC; r= fresh meat; o= outside layer; I= inside layer

Conclusions

This paper clearly demonstrates that aging and cooking are important for the development of essential meat aroma precursors. However, further studies including sensory analysis are needed to exploit the importance in relation to influence on sensory characteristics.

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