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Biogenic amine concentrations and evolution in "chilled" Canadian pork for the Japanese market

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ABSTRACT

The aim of this study was to evaluate concentrations and evolution of biogenic amines in Canadian pork destined for the Japanese market. At 48 h post-mortem, export quality loins were aged at −1.7 °C for 13, 28, 43 or 58 d (chilled) or 4.0 °C for 5 d (fresh). Increasing concentrations of putrescine, spermine and spermidine were observed with chilled ageing period and were greater in chilled export (43 d at −1.7 °C) than domestic market (5 d at 4.0 °C) pork equivalents. Cadaverine was detected, but was not influenced by ageing conditions, and tyramine was only detected in some samples after 43 days at −1.7 °C. Individual biogenic amines were not correlated with their precursor amino acids. Biogenic amines in Canadian pork for the chilled export Japanese market were not in sufficiently high concentrations to pose a risk of intoxication.

1. Introduction

Amines can be classified as natural polyamines, produced naturally by animal, plant or microorganism metabolism, or biogenic amines, produced by decarboxylation of free amino acids (Bardócz, 1995). In meat, natural polyamines consist chiefly of spermidine, spermine, and putrescine, while biogenic amines include almost all dietary amines. Biogenic amines are needed for many critical functions in mammals. However, consumption of foods containing high concentrations of these amines can present a health hazard through the direct toxic effect of these compounds and medical conditions attributed to biogenic amines include headaches, migraines, gastric and intestinal problems, pseudo-allergic responses, changes in blood pressure and food allergies (Bardócz, 1995; Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994; Shalaby, 1996). Furthermore, serious toxicological problems can result from interactions of biogenic and monoamine oxidase inhibitor (MAOI) antidepressant medicines (Sattler, Häfner, Klotter, Lorenz, & Wagner, 1988). These medicines are available worldwide and estimates suggest that approximately 20% of the European population take such medication regularly (Sattler et al., 1988). As a consequence of the prevalence of biogenic amines in foods and the implications of consumption in human health, a wealth of articles have been published covering a range of aspects related to biogenic amines, including numerous reviews on meat and meat products (Halász et al., 1994; Masson & Montel, 1995; Ruiz-Capillas & Jiménez-Colmenero, 2004; Shalaby, 1996; Stadnik & Dolatowski, 2010; Suzzi & Gardini, 2003).

In meat products, tyramine, phenethylamine, histamine, tryptamine, cadaverine, putrescine, spermine and spermidine have all been detected and occur naturally as a result of metabolic processes in the raw material or because of bacterial contamination or starter cultures (Eerola, Maijala, Roig-Saguès, Salminen, & Hirvi, 1996). Studies on whole fresh meat generally show relatively low levels of biogenic amines compared to fermented and cured products, but some amines, such as histamine, tyramine, putrescine and cadaverine, have been observed in significant concentrations during storage (Bauer et al., 1994; Dadáková, Pelikánová, & Kalač, 2011; Galgano, Favati, Bonadio, Lorusso, & Romano, 2009; Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, & Vidal-Carou, 1996; Kaniou, Samouris, Mouratidou, Eleftheriadou, & Zantopoulos, 2001; Krausová, Kalač, Křížek, & Pelikánová, 2006; Maijala, Nurmi, & Fischer, 1995; Min et al., 2007; Paulsen & Bauer, 2007; Sayem-El-Daher, Simard, & Fillion, 1984; Vinci & Antonelli, 2002; Zee, Simard, & L'Heureux, 1983). In research projects, determining limits and understanding trends means that experimental conditions often include extremes exceeding those of real life situations. This is reflected in the long storage times employed to achieve significant biogenic amine concentrations in many of the aforementioned studies which are not representative of commercial practice. One of the few commercial situations

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requiring long term storage of fresh meat is "chilled" exportation and two studies in the scientific literature show the presence of significant levels of biogenic amines after this type of storage (Krizek, Smith, & Phebus, 1995; Nadon, Ismond, & Holley, 2001).

Chilled exportation of fresh meat comprises chilling meat within 48 h post-mortem to strictly controlled temperatures below 0 °C, without freezing, and holding the meat under these conditions for several weeks. This type of exportation achieves meat that competes in the fresh market at the end of transportation and is of high value. However, despite the economic importance of the chilled market, few studies have investigated the impact of the long storage period during chilled transportation on the ageing processes in meat, and only two studies report on the presence of biogenic amines (Krizek et al., 1995; Nadon et al., 2001). In beef subject to chilled export conditions (100 d at −2 °C), it was concluded that after just 40 d of storage, tyramine intoxication was possible (Krizek et al., 1995). And in pork, it was observed that after 13 weeks under CO_2 at −1.5 °C, concentrations of 60 mg/kg tyramine and 68 mg/kg cadaverine were equivalent to those found in fermented meat products (Nadon et al., 2001). Furthermore, extremely high levels of spermine were found, which at 600 mg/kg were 10–60 times greater than that reported in other studies of fresh or processed meats. To give some indication of the significance of these levels, it is considered that for a "normal" person, 125 mg/kg would be required for tyramine to be toxic, but 6 mg/kg would be toxic if ingested with MAOIs (McCabe, 1986). Cadaverine is not considered toxic individually, but rather enhances the effect of histamine and tyramine by interacting with aminooxidases (Bjeldanes, Schutz, & Morris, 1978; Sattler et al., 1988). And spermine, like a number of biogenic amines, when subjected to heat can give rise to the formation of secondary amines, which in the presence of nitrites can generate nitrosamines considered to possess major carcinogenic properties (Patterson & Mottram, 1974). These results are alarming, particularly considering the value of chilled export meat markets.

Maturation at temperatures below 0 °C was the object of a recent study on chilled export pork for the Japanese market (Ngapo & Vachon, 2016). Pork loins for the local Canadian market and for export to Japan were sorted on-line in a commercial abattoir and, at 48 h post mortem, were stored under conditions simulating those of the domestic market (about 3 °C for 5 days) or chilled export to Japan (−1.7 ± 0.1 °C for 43 days). Intermediary storage periods (13, 28 and 58 d) at the latter temperature were also studied to follow the evolution of maturation. Significant increases in most concentrations of amino acids and oligopeptides were observed with ageing period and, generally, chilled export conditions induced greater protein degradation than conditions for the local market. Given that some of these breakdown products are precursors to amines, the potential exists that pork, after chilled export to Japan, has higher concentrations of amines, particularly biogenic amines, than pork in the local Canadian market.

The aim of this study was to determine the concentrations and evolution of biogenic amines and associated precursors in Canadian pork destined for the Japanese market with particular reference to the long, slow ageing process in chilled transport. The pork loins used in this study are the same loins that were used to study umami in chilled pork for the Japanese market (Ngapo & Vachon, 2016). Methods describing pork collection, portioning, ageing and chilling, physico-chemical measures, sex determination and free amino acids and oligopeptides measurements are reported in this earlier publication. Effects of ageing conditions and meat type on physico-chemical measures and free amino acids and oligopeptide concentrations are also presented in this earlier paper. For completeness, selected methods are briefly presented in the current paper.

2. Materials and methods

2.1. Chemicals, reagents and water

Chemicals and reagents were analytical grade or higher. Water was deionised.

2.2. Pork and collection

At 24 h post-mortem, one boneless, short cut loin from each of 80 pigs (about 110 kg live weight) was collected from a commercial slaughter-line. Export (n = 40; NPPC marbling score of 2–4 (NPPC, 1999), Japanese Pork Colour Standards of 3–4 (Nakai, Saito, Ikeda, Ando, & Komatsu, 1975) and firm to the touch) and domestic (n = 40; loins not meeting export selection criteria) loins were sorted on-line by abattoir staff. Loins were vacuum-packaged, placed in boxes of four, and held at 0.5 °C until refrigerated transport to the research laboratory at 48 h post mortem.

2.3. Portioning and ageing/chilling

Working at 5 °C, a slice (2.5 cm) was removed from the centre of each loin for determination of the drip loss, colour and pH of the meat and the sex of the pig from which the pork was obtained. Three sections (7.5 cm) were taken from either side of the centre slice and vacuum-packaged. One section was held at 4.0 °C (±0.3 °C; HOBO RH/temp dataloggers, H08-003-02, Onset Computer Corporation, Bourne, MA, USA). Five sections were stacked in boxes used for export to Japan and held at −1.7 °C ± 0.1 °C mean core temperature (two Class 'A' PT100 RTDs, with hermetically sealed sensor tips connected to OM-CP-QUADRTD 4-channel temperature data-loggers, Omega Engineering, Inc., Stamford, CT, USA and inserted into loins used only for this purpose). Mean temperatures of the air circulating (−1.84 °C ± 0.17 °C s.d.) and inside the boxes (−1.75 °C ± 0.13 °C s.d.) were measured by, respectively, four and two PT100 RTDs mounted in open-end stainless steel housing. Readings were taken every 5 min during the 58 day period. Sections from each loin were removed at 15, 30, 45 and 60 d post mortem.

Working at 5 °C, the loin sections were portioned upon completion of the 0, 5, 13, 28, 43 and 58 d ageing periods (that is, at 2, 7, 15, 30, 45 or 60 days post mortem). The *longissimus thoracis and lumborum* was isolated from each section and cut into six portions of about equal size which were individually vacuum-packaged and stored at –40 °C until required.

Upon determination of the sex of the animals, a subset of 40 loins was selected that was balanced for domestic and export quality and for male and female animals. Data for the 40 loin subset is reported hereafter.

2.4. Free amino acids

Extraction of free amino acids was undertaken according to Hughes et al. (2002). Duplicate samples (5 g) of partially thawed loin were homogenised (13,500 rpm, 2 × 30 with a 30 s break; IKA T25 DS1 Digital Ultra-turrax with S25N-18G dispersing tool, IKA®-Werke GmbH & Co. KG, Staufen, Germany) in 2% TCA (20 ml) on ice. The homogenate was centrifuged (10,000*g*, 20 min, 4 °C) and the supernatant filtered (syringe driven Millex-AP 25 mm filter units, glass fibre filter; Millipore, Japan). Free amino acids were determined by the Waters AccQ·Tag RP-HPLC method (Waters Corporation, Milford, MA, USA) using pre-column derivatization and reaction of the amino acids with AccQ·Fluor reagent (6-aminoquinolyl-N-hydrosuccinimidyl carbamate). The HPLC system (Waters Corporation, Massachussetts,