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Post-slaughter changes in ATP metabolites, reducing and phosphorylated sugars in chicken meat

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ABSTRACT

The formation of ATP breakdown products in chicken *M. pectoralis major* post-slaughter is reported. The concentrations of metabolites were followed in chicken breast throughout the carcass processing post-slaughter and during chilled storage. The concentration of glucose remains similar throughout the period whilst that of glucose-6-phosphate decreases linearly. Glucose and glucose-6-phosphate concentrations were inversely related to the pHu of the breast meat throughout chilled storage. Rapid post-mortem glycolysis and high pHu values suggest the occurrence of stress at and pre-slaughter. Whilst ATP, ADP and AMP were rapidly broken down, the concentration of IMP rose rapidly and remained high. Concentrations of inosine, ribose and hypoxanthine increased gradually post-slaughter but an initial increase in ribose phosphate was not sustained. Most of the potential ribose present in chicken meat, believed to be important for flavor formation, remains bound in the form of inosine and IMP. There is evidence that additional breakdown pathways for ribose and ribose-5-phosphate may deplete the concentrations of these precursors.

1. Introduction

Post-mortem changes that occur in the conversion of muscle to meat not only alter some of its biochemical and physical properties but also play an important role in improving its acceptability as food (Pearson, 1987). The nature of these changes and their consequences for meat has been reviewed (Greaser, 1986; Pearson, 1987). Many of the biochemical pathways involved during slaughter and the post-mortem ageing period in skeletal muscles were elucidated some years ago and result in the formation of sugars (Lilyblade & Peterson, 1962), organic acids (Bodwell, Pearson, & Spooner, 1965), peptides and free amino acids (Parrish et al., 1969), and metabolites of adenosine nucleotides (Dannert & Pearson, 1967; Davidek & Khan, 1967). These chemical modifications in the ageing meat result in a pool of taste compounds and flavor precursors; these latter react during cooking to form the volatile components of flavour. Recently, a new hypothesis has been proposed by Ouali et al. (2006) suggesting the existence of an early phase, named "apoptosis", prior to traditional steps involved in the conversion of muscle to meat.

The role of IMP for the generation of meat odor and flavor has been demonstrated both in model systems and sensory studies (Farmer, Hagan, & Paraskevas, 1996; Mottram, 1994). Ribose-5-phosphate also causes important changes in beef odor, whilst glucose and glucose-6-phosphate cause much smaller effects (Farmer, Hagan, & Paraskevas, 1998; Mottram, 1998). The addition of small quantities of ribose to raw beef and chicken has been shown to increase the quantities of key odor compounds, as well as meaty and roasted notes, especially in chicken (Aliani & Farmer, 2005b; Farmer et al., 1996). In fact, ribose has been identified as a key flavor precursor of cooked chicken meat and as little as 2–4 fold addition to raw chicken prior to cooking increased desirable odor and flavor of cooked chicken (Aliani & Farmer, 2005b). It seems likely that ribose plays a greater role in the formation of flavour in chicken than in the red meats due to the proportionately lower concentrations of six carbon sugars in chicken (Farmer, Kennedy, & Hagan, 2009).

Glucose and glucose-6-phosphate are formed by the glycogenolysis and glycolysis pathways, respectively, whilst IMP, ribose and ribose-5-phosphate have been reported to derive from the ATP breakdown pathway (Lee & Newbold, 1963). The progress of these pathways was the subject of biochemical investigations in the 1960s (Davidek & Khan, 1967; De Fremery, 1966; Terasaki, Kajikawa, Fujita, & Ishi, 1965). However, these studies did not investigate the changes in concentrations of sugars such as ribose and ribose phosphate, or how these pathways may contribute to the availability of flavor precursors in the raw meat. More recent studies have determined a range of sugars in beef during ageing (Koutsidis et al., 2008).

The work described in this paper aims to investigate how time post-slaughter (both during processing and shelf-life) affects the formation of ribose and other potential metabolites of ATP in raw meat. This

Abbreviations: ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; AMP, adenosine 5′-monophosphate; IMP, inosine 5′-monophosphate.

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knowledge will increase understanding of the time post-slaughter and storage conditions needed to reach the maximum concentration of flavor precursors for development of desirable cooked chicken flavor.

2. Materials and methods

2.1. Chemicals

D-Ribose-5-phosphate disodium salt, D-glucose-6-phosphate disodium salt hydrate, alpha-D-lactose monohydrate, alpha-L-rhamnose, D-ribose, D-glucose, D-fructose, D-mannose, D-fructose-6-phosphate disodium salt dehydrate, D-mannose-6-phosphate disodium salt hydrate, D-ribulose, D-ribulose-5-phosphate disodium salt, D-xylulose, D-xylulose-5-phosphate sodium salt, adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP), inosine 5′-monophosphate disodium salt (IMP), hypoxanthine (Hx), inosine, purine, cytidine and tetrazolium blue (3,3′-(3,3′-dimethoxy(1,1′-biphenyl)-4,4′-diyl)-bis(2,5-diphenyl-2H-tetrazolium)-dichloride) were purchased from Sigma-Aldrich Ltd. (Poole, UK). Alkaline phosphatase (EC 3.1.3.1) from bovine intestinal mucosa, Dowex 50WX4 resin (strongly acidic cation, 200–400 dry mesh), Dowex WGR-2 resin (weakly basic anion, 20–50 mesh), Dowex Marathon WBA resin (weakly basic anion, 25–50 wet mesh), hexamethyldisilazane, chlorotrimethylsilane, cyclohexane and dimethyl sulphoxide were also purchased from Sigma-Aldrich Ltd. Analytical-grade chloroform and methanol (high-performance liquid chromatography (HPLC) grade) were from Lab-Scan Ltd. (Dublin, Ireland), perchloric acid from May & Baker Ltd. (Dagenham, UK) and HPLC grade ethanol and acetonitrile from Fisher Scientific UK Ltd. (Loughborough UK). Potassium hydroxide, potassium dihydrogen orthophosphate, potassium tartrate, glycine, magnesium chloride 6-hydrate, sodium acetate trihydrate, sodium carbonate, sodium hydroxide, hydrochloric acid were also from Fisher Scientific UK Ltd. All water used in the processes was from an Elgastat Option 4 water purification unit (Elga Ltd., High Wycombe, UK).

2.2. Chicken meat

Whole chilled chickens were provided by one commercial poultry supplier and, within an experiment, were from the same batch and farm. They were a standard Ross 308 genotype and were reared, slaughtered and processed under commercial conditions. A brief outline of the processing procedure is shown in Fig. 1. Two experiments were designed to investigate the effect of time and temperature on the formation of ATP-related compounds in chicken breast post-slaughter. Experiment 1 was designed primarily to study the changes in ATP related compounds as the breast muscle went in to rigor, whilst Experiment 2 studied the changes in metabolites post rigor. For Experiment 2 the chickens were supplied overwrapped as roasting chickens for the retail market. These two experiments were conducted on different occasions with Experiment 1 conducted in summer and Experiment 2 in winter.

2.2.1. Experiment 1. Post-slaughter period (15–120 min post-slaughter)

Chickens were slaughtered using electrical stunning and exsanguination, plucked and eviscerated using the standard commercial process (Fig. 1). The birds were hanged from their legs on a moving chain and all steps were conducted automatically within a plant with temperature control as shown in Fig. 1. The air temperature was 15 °C for 13 min, 7–8 °C "pre chill" for 30 min and 1–2 °C "chill" for 2 h. A total of 18 chickens were sampled, six at each of three different stages (A, B and C, Fig. 1), at approximately 7, 25 and 115 min post-slaughter. Chicken carcasses were brought to an in-plant laboratory within 3 min, and samples were taken and frozen within the following 5 min. This gave sample freezing times post-slaughter of ca. 15, 33 and 123 min. One breast muscle (*M. pectoralis major*) was removed, a strip of chicken muscle was cut (1 cm wide) from the closest edge to the keel bone and divided into approximately 3 g portions. Duplicate samples were immediately placed in plastic clipper-bags (Somerville Packing, Lisburn, Northern Ireland), sealed and placed in a polystyrene insulated box and covered with dry ice at −65 °C. Samples were held at dry ice temperature until return to the Agri-Food and Biosciences Institute (approximately 40 min after the last sample was collected) where they were transferred to storage at −80 °C until use.

2.2.2. Experiment 2. Chilled storage (28–200 h post-slaughter)

Six individual standard chickens were sampled at "stage C" (after chilling) and transferred from the poultry plant to the laboratory (approximately 40 min) in a container filled with ice (0 °C). They were then held at 4 ± 1 °C until the end of the experiment (200 h), which was selected to be similar in time to the end of commercial shelf life, ca. 8 days. Samples of breast meat were taken at 4, 28, 55, 100, 150 and 200 h post-slaughter. At each of these sampling times, samples (each of 3 g) were taken with a scalpel from one breast from each chicken, avoiding cut surfaces from previous sampling dates. A small preliminary study had indicated that the most variation in concentrations of sugars occurred in the anterior and posterior portions of the breast, whereas samples from the central portion had generally similar concentrations of sugars. Therefore, samples were taken from the mid portion as shown in Fig. 2. A

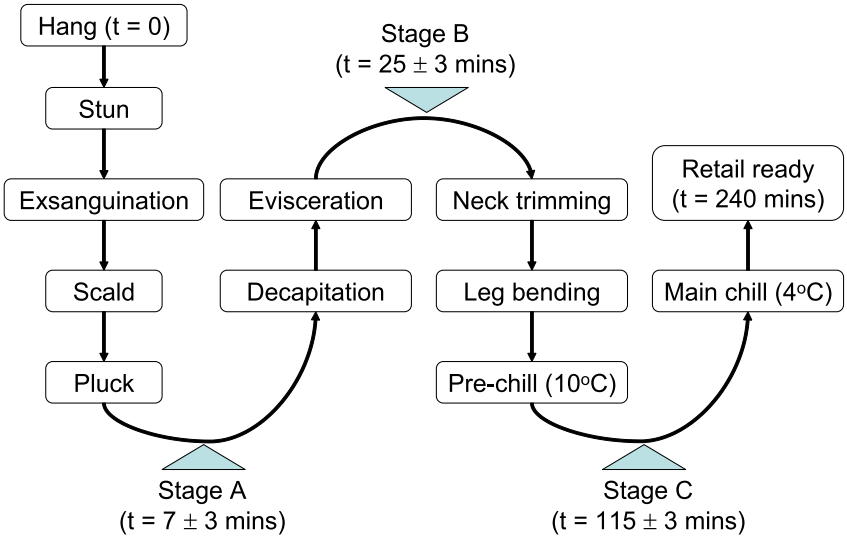

Fig. 1. Summary of poultry slaughtering and processing line showing sampling times for Experiment 1.