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# Precursors of Chicken Flavor. I. Determination of Some Flavor Precursors in Chicken Muscle

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Previous studies suggest that differences in concentrations of natural flavor precursors of the Maillard reaction may affect the odor and flavor of cooked chicken meat. To determine whether such differences occur in the purchased product, chickens from a range of commercial sources were analyzed for selected precursors. These analyses demonstrated that variation occurs both between different commercial sources and between individual chickens from the same source. Coefficients of variation exceeding 30% were observed for inosine 5′-monophosphate, guanosine 5′-monophosphate, and inosine, comparable with those previously determined for reducing sugars and their phosphates. These correspond to concentration ranges of 3-fold and higher, which in some cases may have the potential to affect odor and flavor formation. In contrast, thiamin and amino acids (both protein and nonprotein) show less variation with ranges mainly less than 2-fold.

**KEYWORDS: Reducing sugars; phosphorylated sugars; IMP; GMP; inosine; hypoxanthine; thiamin; amino acids; chicken; HPLC**

## INTRODUCTION

The natural components of raw meat have little aroma (*1*) until they react together during cooking to give the characteristic aroma of cooked meat. These reactions include the Maillard reaction between an amino compound (amine, amino acid, peptide, or protein) and a carbonyl compound (usually a reducing sugar), the thermal degradation of thiamin, the oxidation of lipids, and the interactions between these pathways. Previous studies suggest that the quantities of flavor precursors in raw muscle may be one source of variation in the odor and flavor of red meat and poultry (*2*−*5*).

The concentrations of some flavor precursors in chicken meat have been analyzed previously: ribonucleotides (*6*), thiamin (*7*, *8*), amino acids (*6*, *9*), reducing sugars (*10*), fatty acids, and lipids (*11*, *12*). However, information on the natural quantities of flavor precursors in meat available for consumption is sparse and often contradictory, and there is little data on the variability between individuals of a species or different sources.

In this study, the naturally occurring concentrations of selected precursors of Maillard and related flavor-forming pathways are presented; the natural variability in nucleotides, nucleosides, bases, amino acids, and thiamin is compared with that determined for reducing and phosphorylated sugars reported in a previous paper (*13*). Concurrent research determined the importance of such variation for sensory quality, and this work is reported in a following paper (*14*).

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## EXPERIMENTAL PROCEDURES

**Materials.** Guanosine 5′-monophosphate (GMP), inosine 5′-monophosphate disodium salt (IMP), hypoxanthine (Hx), adenosine 5′-monophosphate (AMP), purine, and inosine were purchased from Sigma (Poole, United Kingdom). All L-amino acids, glutathione (γ-Glu-Cys-Gly; GSH), anserine, carnosine, thiamin hydrochloride, and alkaline potassium ferricyanide were also from Sigma. Potassium dihydrogen orthophosphate, potassium hydroxide, sodium acetate trihydrate, sodium hydroxide, and hydrochloric acid were obtained from BDH (Poole, United Kingdom), methanol [high-performance liquid chromatography (HPLC) grade] was from Labscan Ltd. (Dublin, Ireland), and perchloric acid (PCA, 72% w/w) was from May & Baker Ltd. (Dagenham, United Kingdom). Hydrochloric acid and takadiastase were obtained from Reagecon (Shannon, Ireland) and Fluka Chemicals (Poole, United Kingdom), respectively.

All commercial chickens analyzed were either obtained directly from a local supplier or were purchased from supermarkets in Belfast. The sources/suppliers are denoted A−E. Sources E, B, A, and D in this paper correspond to sources B, C, D, and E in the paper by Aliani and Farmer (*13*), respectively.

All chickens were purchased and analyzed at a time at which they could have been purchased and eaten by the consumer, that is, within their "sell by" date. Thus, the time after slaughter varied between individual chickens. Care was taken to ensure that chickens were not all from the same batch by purchasing chickens from a given source on at least three separate occasions. Chickens were either held at 4 °C for 2 days (for sugar determination) or were frozen at −20 °C for up to 14 days (all other analyses). The left breast (*M. pectoralis major*) and left leg (thigh and drumstick, combined muscles) were cut from each carcass, skinned, trimmed of any visible fat, homogenized separately, and prepared for extraction.

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**Analysis of Nucleotides, Inosine, and Hx.** PCA extraction, followed by neutralization with potassium hydroxide, was used to separate nucleotides from proteins in raw chicken meat. The methodology used was a modified procedure of Mottram (personal communication). Approximately 50 g of raw chicken meat (breast and leg) was homogenized in a food processor (Robot chef 2, Robot-Coupe, Vincennes, France). Duplicate subsamples (3 g), to which 0.5 mL of an internal standard, 40 mM purine (2.5 mg), was added, were homogenized with 6 mL of 0.6 M PCA for 3 min at full speed in 50 mL centrifuge tubes (Apex, Alpha Laboratories, Eastleigh, United Kingdom), using an Ultra-Turrax homogenizer (Janke Kirka and Kunkle Werk, Labortechnik, Staufen, Germany). The precipitated proteins were removed by centrifugation for 5 min at 3900*g* (Heraeus Megafuge 1.0, Kalkberg, Germany) followed by filtration through Whatman No. 54 filter paper under gravity. The pH was then adjusted to pH 5.5 (Orion 520A pH meter, Boston, MA) by dropwise addition of 6 M potassium hydroxide. The resulting precipitate of potassium perchlorate was removed after centrifugation (11 min, 3900*g*) by filtration through Whatman No. 54 filter paper. The aqueous filtrate was held at 4 °C before analysis during the night.

Standard solutions of IMP (25 mM), AMP (15 mM), inosine (25 mM), GMP (25 mM), Hx (10 mM), and the internal standard purine (40 mM) were prepared in 5 mL of 0.01 M potassium dihydrogen orthophosphate buffer at pH 5.5. AMP and Hx required a few drops of 1 N hydrochloric acid to enable them to dissolve.

The final extract was analyzed on an HPLC system equipped with a PC 1000 data system and a variable wavelength UV detector (all from Thermo-Separation Products, Manchester, United Kingdom). An aliquot of extract (20 $\mu$L) was injected for HPLC analysis. The nucleotides were separated on a Prodigy ODS-3 HPLC column (5 $\mu$m particle size, 100 Å pore size, 150 mm × 4.6 mm i.d.) from Phenomenex (Manchester, United Kingdom). A Prodigy ODS-3 guard column was used (3 $\mu$m particle size, 100 Å pore size, 30 mm × 4.6 mm i.d.) also from Phenomenex.

Two solvents were used for elution: solvent A was a methanol/water mixture (60:40), and solvent B was aqueous $KH_2PO_4$ (0.02 M, adjusted to pH 5.5 with 1 M potassium hydroxide). All solvents were filtered through a 45 $\mu$m membrane filter (Millipore) and degassed using helium for 30 min before use. The binary gradient consisted of 3−20% A (97 to 80% B) in 16 min, 20% A (80% B) for 5 min, followed by, at the end of each run, a reverse gradient from 20 to 3% A (80−97% B) in 5 min. UV detection was at a wavelength of 245 nm.

**Analysis of Thiamin.** A portion (10 g) of the homogenized chicken meat was removed, vacuum packed, and stored at −20 °C for up to 15 days prior to analyses. The methods used for extraction and analysis by HPLC method have been reported previously (*15*).

**Analysis of Amino Acids.** The extraction method for "nonprotein" or "free" amino acids was the same as used for extraction of reducing and phosphorylated sugars (*13*). Weighed chicken samples (3 g) were extracted using methanol and then chloroform, as used previously by Mandeville et al. (*16*) for extraction of free amino acids from raw shrimp waste. For amino acid extraction, no resin treatment was employed, and the centrifugate was filtered using Whatman No. 54 filter paper. The resulting filtrate was kept at −20 °C until subjected to chromatography on 40 $\mu$L of the solution. The residue was retained for analyses of the "protein" fraction.

Extraction of amino acids in the protein fraction was conducted on freeze-dried (24 h, Edwards freeze-dryer, Modulo, England) residue obtained after the centrifugation procedure explained above. The freeze-dried samples were kept at −20 °C for up to 2 weeks. Samples of freeze-dried chicken residue (approximately 0.75 g) were accurately weighed (50 mg) and hydrolyzed in 6 N hydrochloric acid for 22 h at 110 °C and then cooled in a refrigerator. The cooled samples were then transferred into a 50 mL volumetric flasks containing the internal standard norleucine (2.5 $\mu$M in 0.5 mL of 0.2 M lithium buffer, pH 2.2).

Chromatographic separation and quantification were achieved on a Biochrom 20 Amino Acid Analyzer equipped with a photodiode detector (Amersham Pharmacia Biotech, Cambridge, United Kingdom). Peak areas were recorded and calculated on a Hewlett-Packard Vectra ES 112 PC using Chemstation software. The program used for the separation and postcolumn derivatization of amino acids was that recommended by the manufacturers (*17*). Five "all ready" lithium citrate buffers, loading buffer, ninhydrin solution, and "ultrasolve" solution were purchased from Amersham Pharmacia Biotech AB (Uppsala, Sweden). The details of buffer formulations are given in the Pharmacia Biotech operating manual (*18*).

The concentration of each amino acid was calculated by reference to the internal standard. The total amino acids in chicken breast muscle were determined by summing the concentrations obtained in protein and nonprotein fractions.

**Determination of Recoveries.** The recoveries of each precursor from the raw chicken meat were determined by analyzing samples of homogenized chicken muscle, which had been spiked with a known amount of precursor. Recoveries were based on the difference between the total amount in the spiked vs the unspiked samples. Reproducibility was assessed by the determination of recovery for 4−6 individual samples. The results for all analyses are reported without correction for recovery.

**Statistical Analysis.** Statistical comparisons of the effect of commercial source or muscle type on the quantities of precursors were conducted using two-factor analysis of variance (ANOVA) with replication and Fisher's least significant differences (LSD) test (*19*).

**Table 1.** Comparison of Mean Recoveries and Reproducibilities for Analyses of Selected Components of Chicken Muscle

| precursor | mg added | $n$[a] | mean (%) | SD[b] | CV (%)[c] |
|---|---|---|---|---|---|
| IMP | 1.5 | 4 | 87 | 12 | 14 |
| inosine | 1.0 | 4 | 104 | 4 | 4 |
| Hx | 0.06 | 4 | 94 | 13 | 14 |
| AMP | 0.2 | 4 | 99 | 3 | 3 |
| threonine | 0.14 | 4 | 68 | 5 | 7 |
| proline | 0.13 | 4 | 98 | 2 | 2 |
| glycine | 0.16 | 4 | 70 | 4 | 6 |
| cysteine | 0.08 | 4 | 40 | 1 | 3 |
| methionine | 0.07 | 4 | 72 | 3 | 4 |
| tyrosine | 0.13 | 4 | 67 | 11 | 17 |
| histidine | 0.14 | 4 | 71 | 2 | 2 |
| thiamin | 0.005 | 4 | 80 | 2 | 3 |

[a] $n$ = number of replicate recovery analyses. [b] Standard deviation. [c] Coefficient of variation.

## RESULTS AND DISCUSSION

**Validation of Methods.** The recoveries and reproducibilities (as indicated by coefficients of variance, CV) are compared for all precursor analyses in **Table 1** and were acceptable for inosine, AMP, and thiamin. The recoveries for IMP and Hx were also good, but reproducibility was less so, as indicated by a CV of more than 14%. Recoveries were also determined for seven selected amino acids from the nonprotein fraction of chicken (**Table 1**), chosen in order to cover a range of important amino acid classes (S-containing, hydroxyl group, aliphatic, and aromatic). The amount added was approximately 70% of the amount of free amino acids in the chicken breast determined in preliminary analyses. The amount of cysteine was arbitrarily chosen (similar to the value obtained for methionine), as very little free cysteine was detected in the chicken breast extract. Recoveries and reproducibility were acceptable for all except cysteine, being ca. 70%, except for proline (98%). The recovery obtained for cysteine was poor at only 40%, although reproducibility was surprisingly good, with a CV of 3.3%. The recovery of cysteine will have been influenced by the nature of extraction method employed and may have been improved by use of an additional step to allow the oxidation of cysteine prior to analysis.

Although the recovery of amino acids from the protein fraction of chicken meat was not determined separately, the total

**Table 2.** Concentrations (mg 100 $g^{-1}$ Wet Weight) of Nucleotides, Nucleosides, and Bases in Breast and Leg (Thigh and Drumstick) of Chickens ($n$ = 6) from Five Commercial Suppliers (A–E)

| | | breast | | | | | leg | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | source | GMP | AMP | IMP | inosine | Hx | GMP | AMP | IMP | inosine | Hx |
| mean | A | 27.1[a] | 3.0 | 77.5 | 32.6 | 7.3 | 20.3 | 1.3 | 44.4 | 21.5 | 14.9 |
| range | | 12–40 | 2.4–3.8 | 72–89 | 25–39 | 5–10 | 9–36 | 1.1–1.6 | 23–76 | 4–29 | 8–30 |
| SD[b] | | 9.8 | 0.5 | 6.4 | 5.7 | 2.3 | 9.6 | 0.2 | 20.9 | 9.0 | 8.0 |
| mean | B | 17.1 | 2.9 | 59.4 | 37.3 | 9.9 | 18.2 | 2.7 | 36.4 | 23.9 | 12.7 |
| range | | 6–30 | 2.5–3.1 | 50–68 | 33–38 | 6–16 | 3–24 | 1.9–3.5 | 29–42 | 18–27 | 11–14 |
| SD | | 11.1 | 0.3 | 6.3 | 2.4 | 3.4 | 8.0 | 0.6 | 4.9 | 3.9 | 1.1 |
| mean | C | 25.0 | 3.9 | 57.3 | 30.5 | 13.1 | 26.4 | 3.7 | 26.7 | 16.8 | 17.9 |
| range | | 6–36 | 2.9–7.7 | 49–70 | 20–45 | 7–20 | 19–34 | 2.8–6.0 | 11–38 | 13–18 | 13–24 |
| SD | | 10.7 | 1.9 | 7.6 | 9.3 | 4.5 | 5.1 | 1.2 | 11.7 | 2.3 | 3.8 |
| mean | D | 12.4 | 3.2 | 74.3 | 29.4 | 9.7 | 20.4 | 3.0 | 39.0 | 22.5 | 14.7 |
| range | | 3–25 | 2.8–3.7 | 62–86 | 13–44 | 5.6–11.8 | 10–30 | 2.3–3.4 | 26–54 | 17–27 | 12–16 |
| SD | | 9.8 | 0.4 | 10.1 | 13.1 | 3.5 | 8.5 | 0.4 | 12.3 | 3.6 | 1.9 |
| mean | E | 2.7 | 9.4 | 149.9 | 51.5 | 18.7 | 1.0 | 6.0 | 76.4 | 58.0 | 27.9 |
| range | | 0.8–4.6 | 8.3–10.0 | 114–174 | 47–56 | 15–22 | 0.4–2.2 | 5.6–6.5 | 42–94 | 50–71 | 25–30 |
| SD | | 3.0 | 0.6 | 30.1 | 4.0 | 2.6 | 0.8 | 0.4 | 18.5 | 9.8 | 2.0 |
| mean (30) | A–B–C–D–E | 16.8 | 4.5 | 83.7 | 36.2 | 11.7 | 17.3 | 3.3 | 44.6 | 28.5 | 17.6 |
| SD | | 12.5 | 2.7 | 37.4 | 11.0 | 5.1 | 11.0 | 1.7 | 22.0 | 16.3 | 6.8 |
| CV(%)[c] | | 74.3 | 60.3 | 44.6 | 30.4 | 43.1 | 63.5 | 50.8 | 49.3 | 57.3 | 38.3 |

[a] Values are means of duplicate analyses. [b] Standard deviation. [c] Coefficient of variation.

**Table 3.** Effect of Source and Muscle on the Concentrations of Nucleotides, Inosine, and Hx (mg 100 $g^{-1}$ Wet Weight); Statistical Results from Six Individual Chickens from Five Commercial Sources[a]

| | source[b] | | | | | muscle[c] | | ANOVA two factors replication | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| compound | A | B | C | D | E | breast | leg | SEM | effect of muscle | effect of source | interaction |
| GMP | 23.7 b | 17.6 b | 25.7 b | 16.4 b | 1.9 a | 16.8 | 17.3 | 3.404 | 0.836 | <0.001 | 0.300 |
| AMP | 2.1 a | 2.8 ab | 3.7 c | 3.1 bc | 7.7 d | 4.5 | 3.3 | 0.282 | <0.001 | <0.001 | <0.001 |
| IMP | 61.0 b | 47.9 ab | 42.0 a | 56.6 b | 113.2 c | 83.7 | 44.6 | 6.096 | <0.001 | <0.001 | 0.001 |
| inosine | 27.1 a | 30.6 a | 23.6 a | 25.9 a | 54.7 b | 36.2 | 28.5 | 2.953 | 0.001 | <0.001 | 0.006 |
| Hx | 11.1 a | 11.3 ab | 15.6 b | 12.2 ab | 23.3 c | 11.7 | 17.6 | 1.546 | <0.001 | <0.001 | 0.262 |

[a] Values sharing the same superscript are not significantly different by Fisher's LSD test ($P < 0.05$). [b] A–E, Different commercial sources. Values are means for both breast and leg from six chickens. [c] Values are means for six chickens from each of five commercial sources.

amino acids obtained in all cases was higher than 20% of wet weight, which was as expected for the protein percentage of chicken meat (approximately 20−25% wet weight; ref *20*).

**Nucleotides, Nucleosides, and Bases in Chicken Muscle.** **Table 2** shows the mean concentrations (mg 100 $g^{-1}$ wet weight) of nucleotides, nucleosides, and bases in breast and leg (thigh and drumstick) of six chickens from five different commercial sources (A−E). **Table 3** shows the results of statistical analyses of the effect of source and muscle on these data by ANOVA.

IMP was generally the most abundant nucleotide in commercially available chicken muscle, with average quantities of IMP in chicken breast and leg muscle of 83.7 and 44.6 mg 100 $g^{-1}$, respectively. Inosine, a breakdown product of IMP, was the second major compound in most chickens (36.2 and 28.5 mg 100 $g^{-1}$ in breast and leg), while GMP and Hx were detected at lower concentrations. AMP was least abundant in most of the chickens analyzed in this study. ATP and ADP were not determined in this study, but more recent studies indicate that these nucleotides would be present only at very low concentrations (<10 mg 100 $g^{-1}$) in chickens available for purchase (Aliani and Farmer, unpublished data). Fujimura et al. (*6*) previously reported the concentration of ATP metabolites in homogenized chicken breast muscle from eight chickens. The concentrations of AMP and IMP reported by these authors were approximately four times higher (10.0 and 330 mg 100 $g^{-1}$, respectively) than the average values obtained in our study, while the concentrations of inosine and Hx were lower at 15.0 and 1.4 mg 100 $g^{-1}$. This greater prevalence of the earlier metabolites of the ATP breakdown pathway is probably due to the fact that Fujimura et al. (*6*) analyzed chickens soon after slaughter rather than at the time of potential consumption, as reported herein, although differences in extraction method or source of the chickens may also have contributed. The role of time postslaughter on these flavor precursors will be the subject of a later paper.

The difference between muscles for four of the metabolites (AMP, IMP, inosine, and Hx) was highly significant (**Table 3**). Breast had higher concentrations of AMP, IMP, and inosine than thigh muscle but lower concentrations of Hx. This may reflect a faster rate of reaction in thigh than breast muscle. "Source × muscles" interactions for AMP and inosine reflect the fact that the differences between breast and leg muscles for these compounds occur in chicken from some sources but not others (**Table 2**).

There was a highly significant difference between commercial sources for all nucleotides (**Table 3**). Source E had significantly higher concentrations of AMP, IMP, inosine, and Hx than the other sources and lower concentrations of GMP. It seems that the postslaughter conditions at commercial source E are most conducive to a high rate of ATP breakdown to give higher concentrations of subsequent metabolites. The reasons for these differences are unclear but did not show any relationship to the remaining shelf life for each chicken. Further studies are needed to investigate the genetic, husbandry, or processing conditions responsible for these differences.

**Table 4.** Concentrations ($\mu$g g$^{-1}$ Wet Weight) of Thiamin in Breast and Leg of Six Chickens from the Same Source (E)

| chicken | breast | leg |
|---|---|---|
| mean | 2.17[a] | 2.38 |
| range | 1.55–2.77 | 1.76–3.05 |
| SD[b] | 0.57 | 0.50 |
| CV%[c] | 26 | 21 |

[a] Values are means of duplicate analyses. [b] Standard deviation. [c] Coefficient of variation.

In addition to differences between sources, there is considerable variation in the concentrations of nucleotides between individual chickens from the same source (**Table 2**). For all nucleotides, the CVs within sources exceeded 30% in both muscles. The CVs for nucleotides are in a similar order to those discussed previously (*13*) for reducing and phosphorylated sugars (30−45%). The variation observed for nucleotides and related compounds suggests that much of the observed variation is due to natural genetic variation between chickens and/or variations in time after slaughter in commercially available chickens. The extent of this variation between individual chickens was substantial; 7-fold differences in GMP concentration were observed between individual chickens from source D. For IMP, variations between individual chickens were small in breast meat but up to 3-fold in leg muscles, while inosine concentrations varied by up to 3-fold between individual chickens in both breast and leg. A previous study (*3*) suggested that a 2−4-fold addition of IMP can increase "meaty" and "roasted" aroma in beef and pork. The sensory experiments described in the following paper (*14*) suggest that these differences are not sufficient to contribute to the odor and flavor differences between individual chickens.

Both reducing sugars and nucleotides and related compounds were suggested by sensory studies (*14*) to be precursors for the formation of flavor in chicken. Thiamin and amino acids had less effect, even when added in substantial quantities. For this reason, fewer analyses for these precursors were conducted, with six individual chickens being analyzed from one source to confirm the reported natural concentrations (*6*−*9*).

**Thiamin in Chicken Muscle. Table 4** shows the amount of thiamin in chicken breast and leg from six chickens from the same source. The CVs (26 and 21% for breast and leg, respectively; **Table 4**) were lower than those observed for sugars (*13*) or ribonucleotides. The concentration ratio between individual chickens with highest and lowest concentrations of thiamin was only about 1.7.

The mean concentration of thiamin was 2.17 and 2.38 $\mu$g g$^{-1}$ wet weight in breast and leg muscle, respectively. This difference between muscles was not statistically significant. These concentrations are generally higher than those reported previously in chicken. Abdulrahman and Abdelbary (*7*) reported lower but similar results in light and dark muscles, 1.51 $\pm$ 0.09 and 1.92 $\pm$ 0.03 $\mu$g g$^{-1}$ wet weight, respectively, from raw broiler chicken meat. Leonhardt and Wenk (*21*) reported 1.4 $\mu$g g$^{-1}$ wet weight thiamin for both breast and thigh, using the method described by Rettenmaier et al. (*22*). Ang (*8*) also reported a lower concentration of thiamin in chicken leg meat: 0.85 $\pm$ 0.03 $\mu$g g$^{-1}$ wet weight, although they employed an extraction method similar to the one used in our studies. The reason for these differences is unclear.

**Amino Acids in Nonprotein or Free Fraction of Chicken Breast. Table 5** shows the concentrations of amino acids in nonprotein fraction of breast muscle from six chickens from the same commercial source. Mean concentrations of free amino acids ranged from 0.03 to 19.8 mg 100 g$^{-1}$ wet weight (**Table 5**). Although the extraction method used was designed to extract free amino acids from the nonprotein fraction of chicken muscle, dipeptides such as anserine (alanine-methylhistidine) and carnosine (alanine-histidine) were also extracted in high concentrations (mean 668 and 353 mg 100 g$^{-1}$ wet weight, respectively; **Table 5**). Of particular interest for flavor formation were the concentrations of the sulfur-containing amino acids. While mean methionine was detected at 4.9 mg 100 g$^{-1}$ wet weight, cysteine/cystine (analyzed together) was only detected (with low recovery) in two of the six samples at very low concentrations (0.05/0.13 mg 100 g$^{-1}$). The concentrations of amino acids from the nonprotein fraction of chicken breast have also been reported by Fujimura et al. (*23*). These authors did not report the presence of any cysteine/cystine, but it is not clear whether they sought it. The values obtained by Fujimura et al. (*23*) for other amino acids are, in most cases, lower than the values presented in this paper (**Table 5**). As these authors did not report the recoveries for their analysis, it is unclear whether this difference is explained by differences in analytical method or due to differences in the source of chickens.

**Table 5.** Concentrations of Amino Acids and Related Compounds (mg 100 g$^{-1}$ Wet Weight) in Nonprotein Fraction from Six Individual Chickens (Source E) Breast Muscle

| amino acid | mean | range | SD[a] | CV (%)[b] | previously reported (*6*) |
|---|---|---|---|---|---|
| phosphoserine | 1.0[c] | 0.6–1.2 | 0.3 | 30 | NR[d] |
| taurine | 6.6 | 5.0–7.0 | 1.4 | 21 | NR |
| urea | 6.8 | 5.0–8.0 | 1.0 | 15 | NR |
| aspartic acid | 4.9 | 3.7–6.5 | 1.2 | 24 | 2.4 |
| hydroxyproline | 2.8 | 1.8–4.0 | 1.0 | 36 | NR |
| threonine | 8.8 | 5.8–10.7 | 1.8 | 20 | 4.0 |
| serine | 12.2 | 9.0–16.0 | 2.4 | 20 | 3.3 |
| asparagine | 8.3 | 5.6–9.5 | 2.0 | 24 | NR |
| glutamic acid | 18.6 | 15.0–23.0 | 2.8 | 15 | 5.3 |
| glutamine | 14.0 | 12.5–19.0 | 2.5 | 18 | NR |
| proline | 8.5 | 4.0–12.0 | 2.7 | 32 | 3.4 |
| glycine | 8.1 | 5.0–10.0 | 1.7 | 21 | 4.2 |
| alanine | 19.8 | 13.0–30.0 | 5.8 | 29 | 3.6 |
| valine | 7.9 | 4.5–10.5 | 2.2 | 28 | 0.7 |
| cystine/cysteine | 0.03 | 0.00–0.13 | 0.053 | 176 | NR |
| methionine | 4.9 | 3.6–6.0 | 0.9 | 18 | 2.9 |
| isoleucine | 5.3 | 3.0–7.5 | 1.5 | 28 | 0.5 |
| leucine | 10.5 | 7.0–14.0 | 2.4 | 23 | 1.4 |
| tyrosine | 7.7 | 5.5–10.0 | 1.5 | 19 | 2.0 |
| $\beta$-alanine | 2.6 | 2.0–4.4 | 1.2 | 46 | NR |
| phenylalanine | 5.3 | 3.5–7.0 | 1.3 | 25 | 1.0 |
| ornithine | 0.2 | 0.1–0.4 | 0.1 | 50 | NR |
| lysine | 11.3 | 8.0–14.0 | 2.1 | 19 | 5.8 |
| histidine | 4.8 | 2.3–7.0 | 1.5 | 31 | 0.5 |
| arginine | 8.7 | 7.0–11.0 | 1.6 | 18 | 2.4 |
| anserine | 668.3 | 630–740 | 40.7 | 6 | NR |
| carnosine | 353.3 | 110–730 | 296.5 | 84 | NR |
| total | 1211 | | | | |

[a] Standard deviation. [b] Coefficient of variation. [c] Values are means of duplicate analyses. [d] NR, not reported.

The sulfur-containing compound, taurine, was also detected in nonprotein fraction of breast muscles (**Table 5**) at a mean concentration of 6.6 mg 100 g$^{-1}$ wet weight. Taurine, an ethylamine-containing sulfonic acid, was reported in the nonprotein fraction of chicken breast and leg muscle by Mecchi et al. (*22*). These authors also reported the presence of methionine and GSH but not cysteine/cystine. No GSH was detected in the nonprotein fraction of breast muscle in the analyses reported herein. Experiments showed that GSH eluted separately to other amino acids but that aqueous GSH added to chicken samples

**Table 6.** Concentrations of Amino Acids (mg 100 $g^{-1}$ Wet Weight) in Protein Fraction from Six Individual Chickens (the Same Source) Breast Muscle

| amino acid | mean | range | SD[a] | CV (%)[b] | previously reported (*9*) |
|---|---|---|---|---|---|
| aspartic acid | 2328[c] | 2200–2400 | 100 | 4 | 2110 |
| threonine | 1113 | 1040–1160 | 54 | 5 | 1030 |
| serine | 967 | 900–1000 | 52 | 5 | 896 |
| glutamic acid | 3325 | 3200–3400 | 99 | 3 | 3362 |
| proline | 807 | 700–980 | 94 | 12 | 896 |
| glycine | 1025 | 960–1090 | 55 | 5 | 1000 |
| alanine | 1407 | 1360–1470 | 46 | 3 | 1300 |
| valine | 1245 | 1100–1350 | 87 | 7 | 1005 |
| cystine/cysteine | 223[d] | 180–260 | 29 | 13 | 181 |
| methionine | 132 | 60–200 | 55 | 42 | 481 |
| isoleucine | 1170 | 1050–1260 | 70 | 6 | 960 |
| leucine | 2045 | 1900–2100 | 81 | 4 | 1740 |
| tyrosine | 928 | 880–960 | 32 | 3 | 771 |
| phenylalanine | 1053 | 1000–1100 | 46 | 4 | 910 |
| lysine | 2283 | 2150–2400 | 93 | 4 | 1958 |
| histidine | 780 | 720–800 | 34 | 4 | 730 |
| arginine | 1650 | 1500–1700 | 84 | 5 | 1424 |
| total | 22482 | | 1109 | 5 | 20754 |

[a] Standard deviation. [b] Coefficient of variation. [c] Values are means of duplicate analyses. [d] Tentative.

before extraction was not extracted by the method used in this work. Thus, any free GSH present in chicken muscle was retained in the protein fraction at the centrifugation step of the extraction procedure.

Many of the amino acids listed in **Table 5** showed a CV of greater than 20%, although concentration ratios between individual chickens were generally, but not always, less than 2. These results indicate that the concentrations of these compounds were affected by differences between individual chickens in genetics and/or extent of proteolysis.

**Amino Acids in Protein Fraction.** The concentration of amino acids in the protein fraction was determined directly by acid hydrolysis and analysis of the residue remaining after removal of the ethanol/water soluble amino acids. This fraction included peptides greater than two amino acids in length but will have been dominated by the muscle proteins. **Table 6** shows the concentrations of amino acids in the protein fraction of breasts from six chickens. The concentrations of individual amino acids ranged between 132 and 3330 mg 100 $g^{-1}$ wet weight (for methionine and glutamic acid, respectively). Of the amino acids and related compounds detected, phosphoserine, taurine, urea, hydroxyproline, asparagine, glutamine, $\beta$-alanine, and ornithine were only detected in the nonprotein fraction and not in the protein fraction.

As expected for these mainly structural proteins, the variation between individual chickens was generally low, with CVs less than 10% for most amino acids, 12 and 13% for proline and cysteine, and 42% for methionine. The high variability for methionine was probably due to the low concentration of this amino acid.

General agreement was obtained between the results reported by Perez-Llamas et al. (*9*) and the results presented herein for most amino acids, including cysteine/cystine. However, Perez-Llamas et al. (*9*) observed greater quantities of methionine in the protein fraction: 481 mg 100 $g^{-1}$ as compared to 132 mg 100 $g^{-1}$ reported in our studies (**Table 6**).

Chickens available for sale to consumers show considerable variation in the concentrations of a number of the key precursors of flavor. The variation in the nucleotides IMP, GMP, and inosine is comparable to that previously determined for reducing sugars and their phosphates, with CVs exceeding 30% and ranges with 2−3-fold difference between highest and lowest concentrations. These differences are due not only to differences in commercial origin but also and importantly due to differences between individual chickens. Concentrations of thiamin and free nonprotein amino acids showed much less variation, while little occurred in the structural amino acids. The observed variability in nucleotides, nucleosides, and sugars has potential importance for the generation of flavor in cooked chicken; this is the subject of the next paper in this series.

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