AMINO ACID PROFILE OF TURKEY HENS FOR THE DETECTION OF MEAT FRAUD: A FEASABILITY STUDY

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Abstract – Adding water or amino acid solutions to fresh poultry without declaration, leads to an increase in weight of the cut. Such fraudulent manipulations cannot be easily detected, without any suitable analytical tools. A way forward may lie in comparing the free amino acid profile of different meat and to create a database. Such database could help in the detection of fraud.
First, a feasibility study was performed to identify variation in the free amino acid profiles of different turkey meat samples with different history: type of muscle, slaughterhouse, market and storage.
It was found that only amino acids with small natural variations are suitable for establishing a reliable database, the amino acids aspartic acid, glutamine, tyrosine, arginine and the dipeptide carnosine were considered not suitable for this purpose. More extensive studies are required in order to select fewer amino acids for a reliable detection method.

Keywords – Authenticity, Meat fraud, HPLC, Turkey.

I. INTRODUCTION

According to a BBC reports, poultry products can be plumped with water and various water binding and taste enhancing ingredients up to 50% of their original weight. However, weight manipulation of fresh meat cuts are forbidden [1] and also the addition to meat products is regulated [2] in Europe. While added proteins of non poultry origin can be detected by methods like PCR, immune-analysis and mass spectrometry, the detection of protein hydrolysates of poultry origin is much more difficult. One possible way of approaching this problem might be to study the free amino acid pattern of the cuts as the procedures will influence the free amino acid contents. Nevertheless, free amino acids in poultry are also highly dependent on factors such as protein source from the feed, age, gender of the animal, muscle type and storage conditions [3-5] . Consequently, individual free amino acid contents does not represent a suitable way for proofing the addition of protein hydrolysates. For this purpose, the alteration of the free amino acid profile may be more robust and may to determine meat alteration. However, due to the high natural variation in the amino acid profile an extensive and systematic determination of the free amino acid contents of various parts of turkey meat with different post and pre-harvest conditions must be performed.

Furthermore, the two dipeptides carnosine and anserine are present in high amounts in meat and normally not part of added protein hydrolysates, and could theoretically serve as internal standards.

The analysis of amino acids was performed using HPLC, generally ion exchange chromatography [6]. As meat is a very complex matrix a reliable sample preparation is necessary [7][8].

In this work, we present a feasibility study for the development and establishment of a database for the detection of added protein hydrolysates in turkey meat.

II. MATERIALS AND METHODS

A. Sampling and sample preparation

The turkey hens (B.U.T. big 6, an average weight of 10320 g and in average 112 days old) were collected from a slaughterhouse in Germany. Samples were also obtained from the market (-M). The fresh butchered turkey hens were dissected as described before [9] and the *Musculus pectoralis superficialis* (PS) as well as the *M. biceps femoris* (BF), sampled from a slaughterhouse (-S) or the market (-M), were used. These samples were

immediately cut in cubes of approximately 1 cm length, packed in aluminium foil and frozen for 60 s in liquid nitrogen before storage at -80 °C. One sample series from a slaughterhouse (30h p.m.) was stored after sampling for 30 h at 4 °C before freezing.

As internal standards, 37 µl norleucine-solution (1 g/l, for calculation) and 113 µl thialysine-solution (1 g/l, for validation) were added to 2.0 g turkey meat in 2 ml of 0.025 M EDTA / 0.100 M Tris buffer. The homogenisation was performed with a Ultra-Turrax T10 (IKA Werke GmbH und CO KG, Staufen, Germany) with 12 000 rpm at 20 °C before filling up the sample to a total volume of 5 ml. The samples were divided into two even parts and processed independently. The proteins were removed by an acid precipitation with 714 µl of 15 % 5-sulfosalicylic acid and rested over 30 min at 4 °C. After centrifugation (5 300 rpm, 30 min, 4 °C) the samples were filtered (0.45 µm), aliquoted and stored at -20 °C. The samples were centrifuged and filtered again as described above directly before the analyses.

B. Analysis of amino acids and dipeptides

The free amino acid content was determined with cation exchange chromatography (3 µm beads) using an amino acid analyser (membraPure GmbH, Berlin, Germany). For the analysis five different lithium ion buffers were applied over a pH-range from 2.9 to 10.4. The flow rate was 180 µl/min. For every six sample measurements, a separate standard (Amino Acid Standards Physiological, Sigma-Aldrich, Steinheim, Germany) was used. The concentration of the amino acids in the standard was 100 µM, with the exception of cystine (50 µM). The analyses were validated with a different and certified standard solution (Amino Acid Mix Solution, Sigma-Aldrich, Steinheim, Germany) regularly.

C. Statistical analysis

All analyses were performed at least in duplicate and at a minimum of four different carcasses per data series. The limit of quantification (LOQ) was defined as ten times the signal to noise ratio. A p-value of < 0.01 was considered as significant different. The calculation of statistical parameters was performed by a Student's t-test using Excel (Microsoft Office Professional Plus 2010, Microsoft Corporation, Redmond, USA).

RESULTS AND DISCUSSION

Especially for fresh meat, the variations in free amino acid profile can originated from the productions and storage conditions. Therefore, parameters such as storage conditions, temperature and time, are influencing the free amino acid contents. Free amino acids are degraded to ketogenic or glucogenic products, but also new free amino acids are formed due to proteolysis of meat proteins.

These opposing effects can lead to higher or lower contents of some free amino acids depending on the conditions.

The free amino acids profile of two different conditions, refrigerated (PS-30h) and bought from the market (PS-M) are listed in table 1.

Table 1 Free amino acid contents of turkey hen chest muscles for different sampling

(PS: *M. pectoralis superficialis*, BF: *M. biceps femoris*, < LOQ: Below limit of quantification, n.q.: not quantified)

Amino acid / dipeptide	PS-30h (mg/100g)	PS-M (mg/100g)
L-Aspartic acid (Asp)	14.38±0.30 [a]	1.29±0.04 [b]
L-Threonine (Thr)	7.98±0.19 [a]	5.46±0.11 [b]
L-Serine (Ser)	12.74±0.23 [a]	5.89±0.14 [b]
L-Asparagine (Asn)	< LOQ	< LOQ
L-Glutamic acid (Glu)	15.71±0.19 [a]	6.28±0.02 [b]
L-Glutamine (Gln)	41.08±2.37 [a]	< LOQ
Glycine (Gly)	5.40±0.08	6.00±0.07
L-Alanine (Ala)	17.29±0.15 [a]	14.03±0.07 [b]
L-Valine (Val)	4.87±0.08 [a]	7.33±0.06 [b]
L-Cystine (Cys-Cys)	< LOQ	< LOQ
L-Methionine (Met)	1.13±0.02 [a]	3.05±0.02 [b]
L-Isoleucine (Ile)	3.09±0.03 [a]	5.13±0.03 [b]
L-Leucine (Leu)	5.19±0.19 [a]	8.75±0.10 [b]
L-Tyrosine (Tyr)	4.42±0.04 [a]	< LOQ
L-Phenylalanine (Phe)	2.88±0.02 [a]	3.55±0.04 [b]
L-Histidine (His)	1.19±0.02 [a]	3.85±0.01 [b]
L-Carnosine (Car)	322.87±11.01 [a]	91.52±5.85 [b]
L-Anserine (Ans)	n.q.	n.q.
L-Lysine (Lys)	4.32±0.10 [a]	2.17±0.03 [b]
L-Arginine (Arg)	5.28±0.17 [a]	< LOQ [b]
L-Proline (Pro)	< LOQ	9.41±0.16

In order to be able to have a robust method for detecting meat manipulations only free amino acids with small natural variations were found

suitable as reference substances. A variation of threefold of the amino acid concentration was set as a cut-off. Therefore, Asp, Gln, Tyr, Arg and the dipeptide Car were not considered appropriate. The other free amino acids showed a more consistent pattern regarding their content and are therefore possible candidates suitable for establishing a database.

To be able to have a generic database, it must be tested with different muscle type, to investigate how muscle type affect free amino acid profiles. The muscle used belong to two different metabolic profile and this could lead to difference in free amino acid profiles. The *M. pectoralis superficialis* consists mainly of type IIb (αW, fast-twitch) with some type IIa (αR, fast-twitch) muscle fibres [10], whereas the *M. biceps femoris* is composed mainly of type IIa muscle fibres with some type IIb and type I (βR, slow-twitch) muscle fibres [10]. The femoral muscles are named as "red meat", whereas the chest muscles are termed as "white meat".

The free amino acid composition of two muscles (PS, BF) of turkey hens was tested and the results are shown in Table 2.

Table 2 Free amino acid contents of different muscles of turkey hens

(PS: *M. pectoralis superficialis*, BF: *M. biceps femoris*, < LOQ: Below limit of quantification, n.q.: not quantified)

Amino acid / dipeptide	PS-S (mg/100g)	BF-S (mg/100g)
L-Aspartic acid (Asp)	6.85±0.28 [a]	12.66±0.66 [b]
L-Threonine (Thr)	7.95±0.19 [a]	14.87±0.15 [b]
L-Serine (Ser)	12.14±0.14 [a]	53.14±0.62 [b]
L-Asparagine (Asn)	< LOQ	< LOQ
L-Glutamic acid (Glu)	16.55±1.18	10.55±0.12
L-Glutamine (Gln)	54.84±2.95 [a]	217.01±3.93 [b]
Glycine (Gly)	5.21±0.07	n.q.
L-Alanine (Ala)	14.78±1.03	n.q.
L-Valine (Val)	4.70±0.06	5.03±0.06
L-Cystine (Cys-Cys)	n.q.	n.q.
L-Methionine (Met)	< LOQ	1.30±0.01
L-Isoleucine (Ile)	3.21±0.04	3.15±0.01
L-Leucine (Leu)	4.76±0.01 [a]	5.71±0.13 [b]
L-Tyrosine (Tyr)	4.41±0.14	4.57±0.09
L-Phenylalanine (Phe)	2.83±0.04	2.99±0.02
L-Histidine (His)	< LOQ	2.67±0.02
L-Carnosine (Car)	333.80±38.17	128.47±12.45
L-Anserine (Ans)	719.75±7.73	n.q.
L-Lysine (Lys)	3.91±0.08 [a]	6.70±0.13 [b]
L-Arginine (Arg)	4.27±0.02 [a]	8.05±0.05 [b]
L-Proline (Pro)	n.q.	n.q.

Greater variances occurred by the free amino acids Ser, Gln as well as Asn and His, which clearly shows that the database must be designed for different muscle types.

III. CONCLUSION

The presented findings demonstrate that free amino acids might be good candidates for establishing a database for the detection of food fraud in meat. The identification of suitable amino acids is important and ten amino acids should be sufficient. Nevertheless, the analysis of a lot more samples covering clearly more parameters is necessary. Additionally, more expansive software is needed for the analysis of such a data volume.

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