PEPTIDE BIOMARKERS AS A WAY TO DIFFERENTIATE CHICKEN AND TURKEY MEAT

Miguel A. Sentandreu[1*], Enrique Sentandreu[1], Paul D. Fraser[2], Carlos B. Amat[3] and Peter M. Bramley[2]

[1]Instituto de Agroquímica y Tecnología de Alimentos (CSIC). Avenida Agustín Escardino 7 , 46980 Paterna (Valencia). Spain

[2]Center for Systems and Synthetic Biology, School of Biological Sciences, Royal Holloway, University of London, Egham TW20 0EX, U.K.

[3]Instituto de Gestión de la Innovación y del Conocimiento (CSIC-UPV). Universidad Politécnica de Valencia - Camino de Vera s/n, Ciudad Politécnica de la Innovación – Edificio 8E 5ª Planta, 46022 Valencia. Spain

[*]Corresponding author (phone: +34 963900022 Ext. 2103; fax: +34 963636301; e-mail: ciesen@iata.csic.es)

***Abstract*—the present work describes the feasibility to develop a proteomic approach capable to differentiate turkey from chicken meat in meat mixes through identification of species-specific peptide biomarkers. The method is robust and simple, involving protein extraction of myofibrillar proteins, enrichment of the target protein using OFFGEL fractionation, trypsin digestion and identification of species-specific peptides by MALDI-TOF mass spectrometry. Apart from its simplicity, this approach has the advantage to be effectively applied for detection of both raw and cooked meat, representing an interesting and serious alternative to methods currently in use for meat speciation such as immunoassays and DNA-based analysis.**

***Index Terms*—meat authentication, OFFGEL fractionation, proteomics, peptide biomarkers**

I. INTRODUCTION

Honest and accurate labeling of food is an essential component of food safety and choice, especially in the case of processed food products where differentiation of the different constituents can be difficult. Legislation must protect the consumer against mis-description, which is commonly carried out with the aim to increase profit. In the case of meat products, the most common cases of adulteration deal with substitution of higher quality with lower value meat, resulting in an increased profit for food producers. Different approaches have been used to determine meat authentication. Immunoassays and DNA-based analysis are currently between the most widely employed technologies (Ballin et al. 2009). Despite their advantages, these techniques are not exempted from limitations, especially when applying to the analysis of processed meat products. The harsh conditions employed during processing, such as cooking of meat, can negatively influence the recognition of the target proteins in the case of immunoassays, or can result in DNA degradation, increasing the chances of having non species-specific fragments (Jonker et al. 2008; Woolfe & Primrose 2004). Recent advances in mass spectrometry analysis applied to proteomics represent an interesting alternative to these techniques through identification of peptides specific of each animal species. In the present work, we describe an approach for differentiation of turkey and chicken meat in mixes through generation of species specific peptides coming from trypsin digestion of myosin light chain 3. The method is robust and reliable, and it can be applied indistinctively to either raw or cooked meats.

II. MATERIALS AND METHODS

One gram of either raw or cooked meat samples were homogenized in 10 mL of 50 mM Tris buffer, pH 8.0. The homogenate was then centrifuged at 10,000 g for 20 min at 4 °C, collecting the precipitate that was further redissolved it in Tris buffer, pH 8.0, containing 6 M urea and 1 M thiourea. The appropriate volume of each one of these extracts was taken in order to fractionate 2.5 mg of total protein in the pH range 4-7 using an Agilent 3100 OFFGEL fractionator. Proteins obtained in the first seven fractions were separated by SDS-PAGE. The protein bands corresponding to myosin light chain 3 were then cut from the gel and subjected to in-gel digestion with trypsin. After this, peptides were desalted using zip-tips™ and finally analyzed using a Bruker MALDI-TOF-MS Reflex III operated in the positive reflectron mode with an acceleration voltage of 20 kV. Identification of peptides was done by peptide mass fingerprinting of myosin light chain 3 for the different animal species using the MASCOT search engine against NCBInr protein database.

III. RESULTS AND DISCUSSION

For differentiation of chicken and turkey meat, myosin light chain 3 (MLC-3) was chosen as the target protein for generation of species specific peptides because of its properties to separate from other myofibrillar proteins in the most acidic fractions after OFFGEL fractionation (Sentandreu et al. 2010). This constituted an efficient enrichment step in this protein, contributing detection of low amounts of a type of meat into a mixture. **Figure 1** shows sequence alignment of chicken MLC-3 with the supposed sequence of turkey MLC-3. This assumption was necessary because turkey MLC-3 is currently not included in protein databases and so its sequence was deduced in view of the degree of identity with respect to myosin light chain 1 (MLC-1) for this species, which is available in protein databases. The fact that both chicken MLC-1 and MLC-3 are available in protein databases helped us, by comparison, to conclude this. The only uncertainty was for the first nine amino acids of MLC-3 because this is the only region in which MLC-1 and MLC-3 seems to be different (**Figure 1**, amino acids marked in red). For this region, we assumed the sequence of chicken MLC-3 as for turkey MLC-3. As we can observe in **Figure 1**, turkey MLC-3 would have a high degree of homology with respect to its chicken counterpart, differing only in 3 amino acids all along the whole sequence. These different amino acids would be placed in positions 20, 47 and 85 within the sequences. Theoretical trypsin digestion of these sequences allowed us to observe that, despite this high homology, it was possible to obtain turkey species-specific peptides based on these differences.

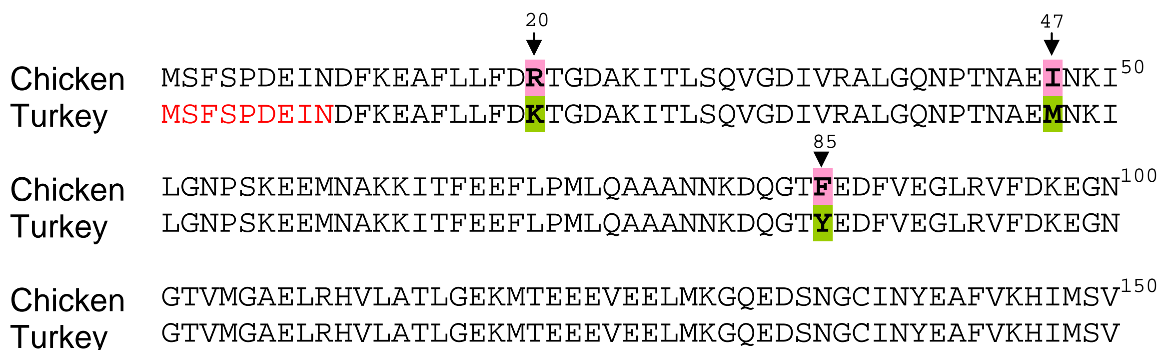

Figure 1: Sequence alignment of chicken myosin light chain 3 with supposed turkey Myosin light Chain 3 based on sequence similarities between myosin light chains 1 and 3. The amino acid differences between the two sequences are indicated by black arrows.

SDS-PAGE of fractions 2 and 3 obtained after OFFGEL separation of chicken (A) or turkey (B) meat extracts contained MLC-3 but only few other protein bands (Results not shown). For these fractions, protein bands corresponding to MLC-3 were cut from the gel, subjected to in-gel trypsin digestion and finally analyzed by MALDI-TOF MS. As can be observed in **Figure 2**, digestion of chicken and turkey MLC-3 generated different ion signals in the m/z range 940-1120. Peptide mass fingerprinting obtained in each case allowed assigning these signals to peptides EAFLLFD**R** ($M+H^+$ 1010.561) for chicken and EAFLLFD**K** ($M+H^+$ 982.332) for turkey species, corresponding to position 13-20 in MLC-3 sequence. The mass difference between the two peptides was consistent with the difference in the amino acid composition of chicken and turkey MLC-3 in position 20 (see **Figure 1**).

Species-specific peptides were also identified in the m/z range 1380-1520 after MALDI-TOF MS analysis, as shown in **Figure 3**. Here, the amino acid difference in position 20 between chicken and turkey MLC-3 generated the ion signals $M+H^+$ 1482.619 for chicken (A) and $M+H^+$ 1454.414 for turkey (B) MLC-3, corresponding to sequences EAFLLFD**R**TGDAK and EAFLLFD**K**TGDAK, respectively. Peptide mass fingerprinting also revealed an ion signal found at m/z 1403.327 in the digest of turkey MLC-3 but not in its chicken couterpart. This corresponded to the turkey-specific peptide ALGQNPTNAEMNK, placed in position 37-49 of MLC-3 sequence. As can be seen in **Figure 1**, this peptide is species-specific because turkey MLC-3 contain one methionine residue in position 47, whereas chicken MLC-3 contains an isoleucine.

Mass (M+H+)	Position in MLC-3	Sequence	Origin
1010.561	13-20	EAFLLFD**R**	*Gallus gallus*
982.332	13-20	EAFLLFD**K**	*Meleagris gallopavo*

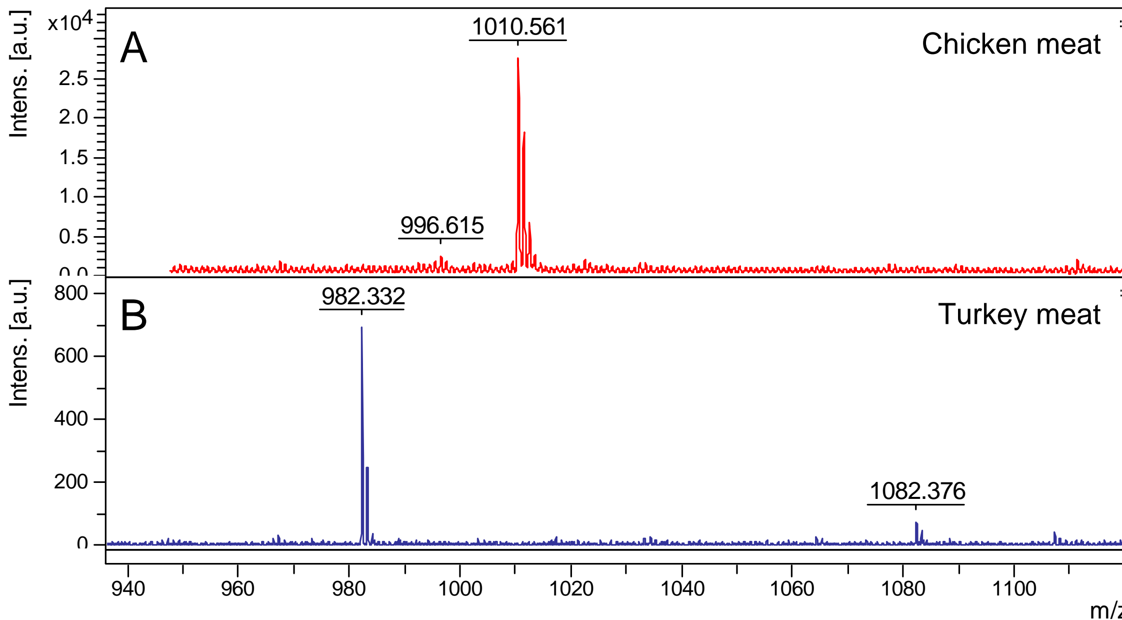

Figure 2: Comparative zoom-in for m/z range 940-1120 of MALDI-TOF MS analysis corresponding to trypsin digests of enriched myosin light chain 3 coming from either cooked chicken (A) or turkey meat (B). Mass and sequence of species-specific peptides, together with animal origin, are indicated in the table. Amino acids differing between the two species appear in bold.

Mass (M+H+)	Position	Modification	Sequence	Origin
1482.619	13-25		EAFLLFD**R**TGDAK	*Gallus gallus*
1454.414	13-25		EAFLLFD**K**TGDAK	*Meleagris gallopavo*
1403.327	37-49	MSO: 47	ALGQNPTNAEMNK	*Meleagris gallopavo*

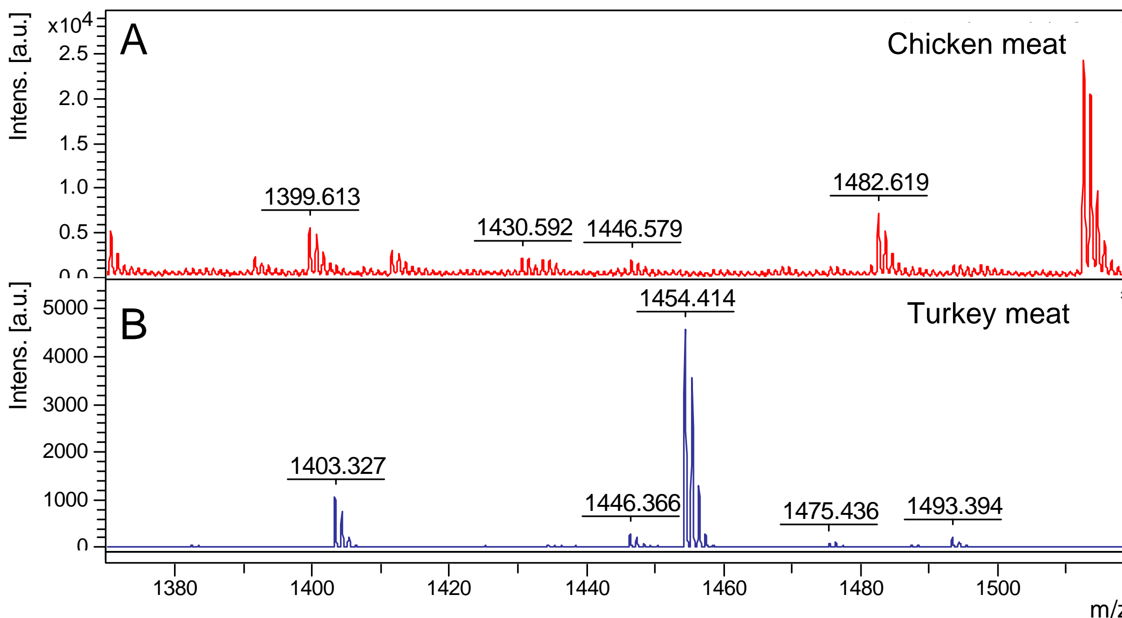

Figure 3: Comparative zoom-in for m/z range 1380-1520 of MALDI-TOF MS analysis corresponding to trypsin digests of enriched myosin light chain 3 coming from either cooked chicken (A) or turkey meat (B). Masses and sequence of species-specific peptides, together with animal origin, are indicated in the table. Differences in the amino acid composition between the two species appear in bold.

Former methods based on protein detection, such as electrophoresis, chromatography or immunoassays, lack good resolving power in closely related species such as chicken and turkey meat, because they are not based on differences at sequence level (R.K.Owusu-Apenten 2002). In addition, they are more affected by denaturation of proteins and so some of them do not work on identification of highly cooked meat, for example. More recently, other alternatives described the development of DNA assays based on polymerase chain reaction (PCR) for differentiation between chicken and turkey meats in processed meat products. Koppel et al. (2008) developed a quantitative PCR assay for the simultaneous identification of pork, beef, turkey and chicken. Even if they obtained good reproducibility and sensitivity, they reported that quantification of the different meats in real samples can be difficult because DNA can be degraded during food processing or during storage. In a similar way, Jonker et al. (2008) developed a sensitive real-time PCR assay for the identification of chicken, turkey and other meat species using small DNA fragments. Despite they reported the adequacy to select small fragments in the analysis of heated meats, they also highlighted the increasing risk of having cross-reactivity between species. The proteomic approach shown in this work represents an interesting alternative to methods based on DNA analysis. The possibility to make the assay quantitative, as it has been recently reported (Sentandreu et al. 2010), gives sense to progress on this proteomic technology.

IV. CONCLUSION

The present work shows proteomics as an interesting alternative to methods currently in use for meat speciation such as immunoassays or DNA-based analysis. Thus, it has been demonstrated the feasibility to differentiate between chicken and turkey meat through the identification of species-specific peptide biomarkers in either fresh or cooked samples. This proteomic approach constitutes a robust and accurate methodology, avoiding some of the major limitations displayed by other methods in the analysis of highly processed meats. From this perspective, the primary amino acid sequence of key peptides, such as those presented here, would be considerably more resistant to food processing than DNA sequences.

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