Assessing chicken meat authenticity within divergent farming systems (organic versus antibiotic-free) using SWATH-MS-based proteomic analysis and chemometrics

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Objectives: Several challenges are facing the meat industry and most importantly those related to claims like the "One Welfare", type of farming system, sustainability, or eco-friendly production. While these soft claims are generally beyond the scope of analytical chemistry, developing methodologies that can identify markers and/or signatures that can help make better decisions and guarantee the intrinsic/extrinsic qualities to consumers are highly welcomed. From an analytical standpoint, several methodologies have been used to authenticate the geographical or the species origin of animal food products, but very few about the type of production (farming) system. Therefore, the development of fast and reliable foodomics strategies using for example proteomics in conjunction with multivariate and machine learning data analyses methods are attracting great interest. Thus, the present work has been de- signed to use for the first time dataindependent acquisition - mass spectrometry proteomics (SWATH-MS: sequential window acquisition of all theoretical fragment ion spectra mass spectrometry) and chemometrics for the discrimination of two farming systems: organic *versus* antibiotic-free used to produce chicken meat.

Materials and Methods: Twenty chickens from the *Ross 308* strain were used in this work, from which 10 were reared under an or- ganic system and 10 under an antibiotic-free inside ground farming. The animals were slaughtered under the same standardised handling system and slaughtering time at Fileni® industry (Cingoli, Italy). Muscle biopsies from *Pectoralis major* (breast) were taken early post-mortem and stored at -80°C until analysis. Total muscle proteins were extracted for shotgun proteomics using 200 mg of frozen muscle in 3.5 mL of freshly prepared extraction buffer containing 8 M urea, 2 M thiourea, 1% DL-dithiothreitol, 2% CHAPS, and 1% Pharmalyte 3−10. The homogenates were centrifuged 30 min at 10,000 × g and at 4°C to remove fat and insoluble proteins. Then, the protocol described by Zhu *et al.* [1] was used for the preparation of the protein bands using one-dimensional SDS-PAGE for quantitative proteomics by means of SWATH-MS [2]. Multivariate orthogonal partial least squares discriminant analysis (OPLS-DA) was performed using the *ropls* R package. Quality metrics, variable importance in projection ≥ 1 and permutation diagnostics (1000 random permutations) were calculated to consider the most influential markers. Pathway enrichment anal- ysis (Gene Ontology (GO) terms) was performed on the discriminatory proteins identified by the OPLSDA using Metascape® as described by Gagaoua *et al.* [3].

Results: The OPLS-DA helps reduce the dimension of the proteome dataset by extracting a subset of most salient biomarkers that can subsequently be used to predict the class of interest - in this instance organic chicken from antibiotic-free farming system. It significantly separated in this trial the organic chicken samples from those of antibiotic-free farming system. It uncovered a panel of 71 proteins (corresponding to 64 gene names) from a total of 695 proteins as drivers of the discrimination. Of the 71 protein bio- markers identified, 42 were elevated in organic chicken samples while the remaining 29 were elevated in antibioticfree samples. The cross validation of OPLS-DA model showed the high levels of an explained variance (R^2Y) of 0.939 and predictability (Q^2Y) of 0.704. To avoid overfitting, the model was validated with permutation test that resulted values of Q^2 and R^2Y of 0.698 and 0.994, respectively. The proteins were found to belong to 19 significantly enriched GO terms mainly dominated by "GO:0006091: generation of precursor metabolites and energy"; "GO:0030239: myofibril assembly", "GO:0006165: nucleoside diphosphate phosphorylation", "GO:0003012: muscle system process" and "GO:0006734: NADH metabolic process". Among the top 5 discriminating proteins that can be validated for the authenticity of the two production systems within the *Ross 308* strain, we cite TPM2 (Tropo- myosin 2), MYH1 (Myosin-1), ACTA1 (α-actin), ACTBL2 (β-actin) and SIN3A (Paired amphipathic helix protein). **Conclusion** This study is the first to apply SWATH-MS and chemometrics to efficiently discriminate by means of protein biomarkers the chicken meat produced under organic from antibiotic-free farming system. The OPLS-DA allowed identifying the main molecular signatures that differ between the two production systems. The identified biomarkers are under validation using Parallel reaction monitoring as a targeted quantities proteomics.

References:

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