VARIATIONS IN CARBON AND NITROGEN ISOTOPE RATIO ASSOCIATED WITH DRY-CURED HAM LABEL

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I. INTRODUCTION

Stable elements Isotope Ratio Analysis (SIRA) is one of the most powerful techniques for assessing the origin and authenticity of food such as meat [1] and meat products [2], but few studies to date are available for processed meats such as dry-cured ham [3,4]. Carbon and nitrogen isotopic abundances in meat products are related to geographical origin, feeding, animal metabolism and meat processing. The variations in $^{13}C/^{12}C$ ratio ($\delta^{13}C$) has been ascribed to the amount of C_3 or C_4 plants in animal's diets, while the changes in $^{15}N/^{14}N$ ratio ($\delta^{15}N$) in meat products was ascribed to genotype, feed and husbandry practices [1,2,3]. In this preliminary approach, 4 European dry-cured hams underwent $\delta^{13}C$ and $\delta^{15}N$ analysis to determine their isotopic signature: Italian PDO (Protected Designation of Origin) Prosciutto di Parma and Prosciutto San Daniele, Spanish TSG (Traditional Speciality Guaranteed) Jamón Serrano, and the "EU" hams, without a protected label.

II. MATERIALS AND METHODS

A total of 44 dry-cured hams were taken at retail points; for each ham type, samples were from different producers and maturation times: PDO Prosciutto di Parma (N=12), PDO Prosciutto San Daniele (N=13), STG Jamón Serrano (N= 9) and EU ham (N=10). Freeze-dried defatted *Biceps femoris* (1.5 mg) with 3 mg of WO_3 were combusted at 1150 °C in an elemental analyser (Vario PYRO Cube, Elementar, UK), and the generated N_2 and CO_2 analysed in the mass spectrometer (PrecisIon, Elementar, UK). The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios were expressed in delta notation (δ ‰) according to $\delta\,‰ = \frac{(R_{sample} - R_{std})}{R_{std}} \times 1000$. R_{sample} is the isotope ratio of the sample and R_{std} is the isotope ratio of the international standard. The isotopic values were calculated against working standards (flour and rice for $\delta^{13}C$ and $\delta^{15}N$, L-valine for $\delta^{13}C$ and glycine for $\delta^{15}N$), calibrated against international reference materials (USGS-42, USGS-61, USGS-74). The instrument performance was checked in proficiency tests. The measurement precision (1σ) on 6-times repeated sample was 0.1 ‰ for $\delta^{13}C$ and 0.3 ‰ for $\delta^{15}N$. Differences in $\delta^{13}C$ and $\delta^{15}N$ values among ham types were performed with One-Way-ANOVA, multiple comparison Duncan test ($P < 0.05$), using SPSS Statistics 28.0 software.

III. RESULTS AND DISCUSSION

Values of $\delta^{13}C$ and $\delta^{15}N$ in dry-cured hams are graphically reported in figure 1. Variations found in $\delta^{13}C$ can be ascribed to the different diets of the originating pigs [2,3]. In countries such as Denmark and Germany, where the majority of raw matter used for the production of EU hams comes from, C_3 plants ($\delta^{13}C \approx -27‰$), growing in temperate climate, are mostly used in animal feed. Conversely, C_4 plants as corn ($\delta^{13}C \approx -11‰$), growing in warm climate, are used to feed animals destined to Serrano ham in Spain; in Italy, the presence of corn in feeding is regulated for PDOs ham production. This leads to $\delta^{13}C$ values lower in EU hams (< -23 ‰), intermediate values for Serrano ham (-20.0‰ and -22.5-‰) and higher values for Italian PDOs hams (-21.0 and -18.0‰). Values of $\delta^{15}N$ in meat may be influenced by pig's breed, slaughtering age and husbandry practices. Heavy, slow growing pigs (used for Italian PDOs) were significantly enriched in ^{15}N compared to fast growing pigs (more used for Serrano and EU hams) [3]. Moreover, the extended ripening of assayed Parma hams (16-24 months) with respect to Serrano (9-12 months) and EU (10-15 months) ones allows the enrichment in ^{15}N due to enzyme-driven proteolysis, leading to an increase in $\delta^{15}N$ values.

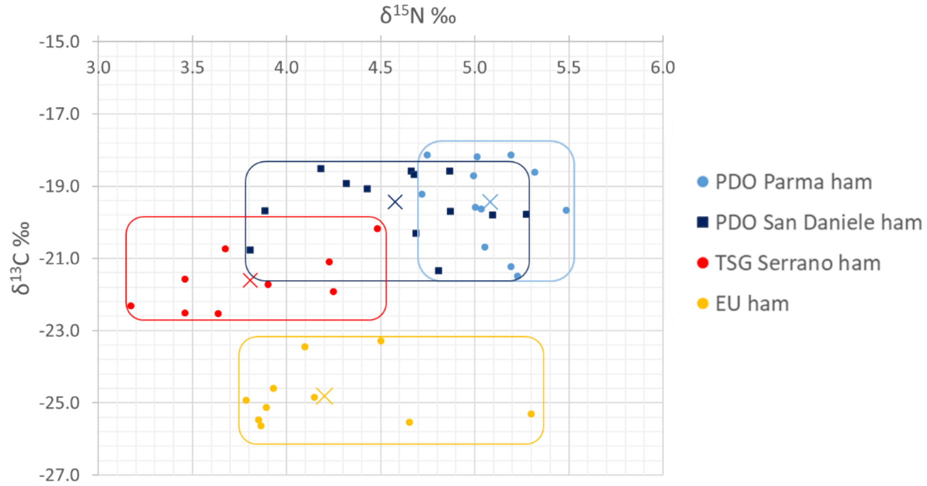

Figure 1. Biplot of $\delta^{13}C$ ‰ and $\delta^{15}N$ ‰ measurements in dry-cured ham samples. The symbol X represents the centroid of each group.

As reported in Table 1, $\delta^{13}C$ discriminates Italian PDO hams from Jamón Serrano and EU hams (*P* < 0.001), while $\delta^{15}N$ seems effective to discriminate each ham type, including Parma from San Daniele (*P* < 0.01). Serrano and EU hams are discriminated both by $\delta^{13}C$ (*P* < 0.001) and $\delta^{15}N$ (*P* < 0.05).

Table 1 Mean values of $\delta^{13}C$ ‰ and $\delta^{15}N$ ‰ of dry-cured hams belonging to the assayed ham types. Comparisons were run by means of ANOVA.

	PDO Parma ham N= 12	**PDO San Daniele ham** N= 13	**TSG Serrano ham** N= 9	**EU ham** N=10	**RSD[1]**	***P-value***
$\delta^{13}C$	-19.43[a]	-19.52[a]	-21.61[b]	-24.81[c]	0.11	0.000
$\delta^{15}N$	5.08[a]	4.58[b]	3.82[d]	4.20[c]	0.13	0.000

[1]Residual standard deviation
Different letters in the same row indicate statistically significant differences (Duncan's test p < 0.05)

IV. CONCLUSION

The stable isotope ratios of carbon and nitrogen are promising tools to assess the origin of dry-cured hams produced in different European countries. The creation of a database of $\delta^{13}C$ and $\delta^{15}N$, possibly including more isotope ratios and extended to other ham samples, can be proposed as an authenticity control procedure in order to support the EU protected labels and improve the European ham industry.

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