Assessment of pork authenticity by means of multi-element analysis #

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In the last years, the increased level of consumer awareness in food choices and the strengthening of the prevention and suppression of food frauds has spotlighted the concept of "authenticity" of a food product. Therefore, genuinity and origin of raw materials has became an increasingly important issue for the food chains, consumers and industries.
In this scenario, in 2011 the European Community started to improve consumer information about the origin of foods, introducing, for some products (eg. beef), the mandatory indication on the label (Regulation 1169/2011). More recently, France and Italy, in order to increase consumer transparency, have extended the requirement of origin declaration to meat and dairy in prepared foods (2016/1137 French Decree) and to milk and dairy products (9th December 2016, Italian Decree), respectively.
In order to trace “scientifically” the geographical provenance of food, specific analytical approaches were developed, including those based on multi-element analysis, by the use of spectroscopy and mass spectrometry techniques.
The study of the origin of a meat-based food through the multi-element analysis is built on the principle that, as the elemental composition of feed reflects that of the soil where the vegetal components were grown, also the elemental composition of animal tissues could reflect a specific elemental profile.
In this work, the possibility to discriminate meat of pigs bred in three different geographical areas was explored through a multi-element analysis performed with both the Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Varian Vista MPX) and Inductively Coupled Plasma-orthogonal-acceleration Time-of-Flight Mass Spectrometer (ICP-oa-TOF-MS, GBC Optimass 9500). In particular, 12 samples of domestic Heavy Pig, bred in Northern Italy and used to be processed into Italian PDO dry-cured hams (A), 7 samples of Nero di Parma, a local breed of black pigs reared in the province of Parma (B) and 8 samples of foreign pork, bred in Germany (C) and used in Italy to be processed into unbranded meat products, were analyzed. Muscle samples, were taken from the outer section of *Biceps femoris* muscle in fresh hams, wrapped in aluminum foil, vacuum packaged and frozen until analysis.
About 1 g of minced muscle and relative blanks were mineralized in triplicate with 4 ml of HNO_3 by a pressure digestion with microwave heating (UltraWAVE, Milestone, Italy) and then, after filtration on 0.45 µm filters (Millex®-HA, Millipore) and appropriate dilutions, subjected to ICP-OES and ICP-TOF-MS analysis for determining 28 elements. Ca, Mg, Na, K, P, Fe, Cr, Ni, Mn and Zn were quantified by ICP-OES, while Li, Al, V, Co, Cu, Ga, As, Rb, Sr, Mo, Ag, Cd, Cs, Ba, Ce, Tl, Pb and U by ICP-TOF-MS. Element concentration data were normalized to dry matter content, in order to compensate for the differences in the moisture degree of meat samples. As, Ag, Ce, Cd, Tl and U were excluded from the statistical analysis, as they showed concentration values under LOD in all or almost all samples.
In a first step, the univariate analysis of variance (One-Way ANOVA procedure, SPSS Statistic V22.0) was applied to compare elements between the groups of investigated pork samples (A, B and C).
Al, presenting an extremely high variability in A group, was excluded from analysis. In total, 19 elements (Ca, Mg, Na, K, P, Fe, Mn, Zn, Li, V, Co, Cu, Ga, Rb, Sr, Mo, Cs, Ba and Pb) were considered.
Table 1 shows the mean values of element concentration (expressed as mg/Kg of dry material or µg/Kg of dry material) and the results of ANOVA test (Tukey HSD post-doc test, $P < 0.05$).

Tab. 1: Mean values of element concentration in pork samples, one-way ANOVA and Tukey HSD post-doc tests.

Element*	A	B	C	p-value
Mg	1090 a	969 b	994 b	0.003
Na	1938 a	2376 b	1965 a	0.000
K	15253 a	13404 b	14205 a	0.003
P	8574 a	7610 b	8001 a	0.017
Fe	20.8 a	30.4 b	16.6 c	0.000
Mn	224 a	283 b	232 a	0.007
Zn	80 a	120 b	62 c	0.000
Co	2.48 a	3.50 b	2.92 a	0.000
Cu	1778 a	2070 b	1743 a	0.007
Pb	8.75 a	18.9 ac	19.2 bc	0.012

** Mg, Na, K, P, Fe and Zn in mg/Kg of dry material; Mn, Co, Cu and Pb in µg/Kg of dry material; different letters along rows mean significant difference (Tukey HSD post-doc test, P < 0.05)*

The test pointed out that Na, K, P, Fe, Mn, Zn, Co and Cu allowed to discriminate ($P < 0.05$) the Nero di Parma samples (B) from samples A and C. On an average, Nero di Parma showed the highest content of Na, Fe, Mn, Zn, Co and Cu and the lowest values of K and P. Moreover, Fe and Zn could discriminate samples belonging to the three groups, because the foreign pork samples (C) exhibited the lowest values of both elements.

At a later stage, Principal Component Analysis (PCA) was performed, based on the elements which proved to be discriminant in one-way ANOVA (Tab. 1). Three components were extracted accounting for 82.7% of total variance (% variance explained by PC1 = 47.8, PC2 = 23.0, PC3 = 11.9). The scatter plot of samples onto the PC1-PC2 plane, showed that some samples were sub-grouped according to breeding origin: the Nero di Parma samples (B) were separated from the A and C ones, which, conversely, overlapped (figure not shown).

In order to foresee if the domestic Heavy Pig samples could be discriminated from the foreign ones, a Linear Discriminant Analysis (LDA) was attempted for explorative purposes, despite the low number of samples. Unlike previous PCA analysis, the discriminant functions were obtained under the constraint to differentiate the three groups using a variable number lower than the number of samples belonging to the smallest group. In this respect, variables less related to each other among those listed in Table 1 (Pearson $r < 0.65$), were retained. Thus, Na, Mg, Co, Cu, Zn and Pb were submitted to Stepwise LDA analysis. Figure 1 shows the results of LDA analysis, which highligths a good separation among the three groups. Mg, Zn and Pb resulted as the most important variables for the discrimination of the breeding area of investigated pigs. A higher number of samples will be analysed in the future to build a reliable model to discriminate pork samples according to geographic origin.

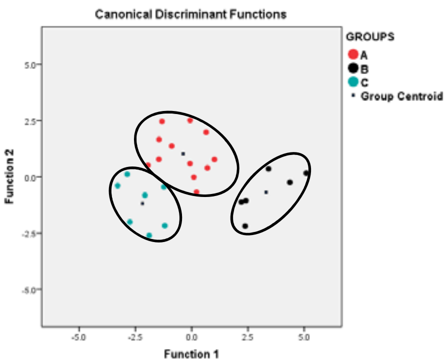

Fig. 1: LDA plot of pork samples bred in different areas.