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Quality of dry cured ham: Methods for authentication of geographical origin, rearing system and technology

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Abstract. Traceability and authentication of meat and meat products are major concern for consumers, producers and retailers. In Europe, several areas produce high quality dry cured hams under Protected Designation Origin. Efficient and objective methods must be developed to assess origin of dry cured hams. This paper is focused on methods to assess geographical origin, rearing systems and processing conditions. Methods to assess geographical origin are based on multi-analyses of both stable isotopes ($^{18}O/^{16}O$, $^{2}H/^{1}H$, $^{15}N/^{14}N$, $^{13}C/^{12}C$, $^{34}S/^{32}S$) and trace minerals (Se, Fe, Sr, Cu, Zn…). Genotypes of pigs can be check by biotechnological methods based on DNA analyses (RFLP, microsatellites). Methods to trace feeding system are based on stable isotope measurement or on quantification of organic components (hydrocarbons, poly phenols, fatty acids, vitamins) in muscles and adipose tissues. Up to now, very few methods have been focused on the authentication of specific processing conditions. Up to now, most of the methods remain at the stage of potential tools and needs to be performed on large numbers of samples to be applied with certainty in routine controls. A proper authentication of dry cured hams requires a combination of methods and multivariate statistical analyses. One of the main challenges for the future is to build wide opened data bases easy to use, aggregating data available on dry cured hams.

Keywords. Authentication – Dry cured ham – Geographical origin – Analytical method.

Qualité du jambon sec : Méthodes pour l'authentification de l'origine géographique, du système d'élevage et de la technologie

Résumé. *La traçabilité et l'authentification des viandes et des produits carnés est un sujet majeur de préoccupation pour les consommateurs, producteurs et détaillants. En Europe, plusieurs régions produisent des jambons secs de grande qualité sous appellation contrôlée. Des méthodes efficaces et objectives doivent être développées pour vérifier l'origine des jambons secs. Cet article est focalisé sur les méthodes destinées à vérifier l'origine géographique, les conditions d'élevage et de transformation. Les méthodes consacrées à l'identification de l'origine géographique reposent sur une analyse multiple d'isotopes stables (*$^{18}O/^{16}O$*,* $^{2}H/^{1}H$*,* $^{15}N/^{14}N$*,* $^{13}C/^{12}C$*,* $^{34}S/^{32}S$*) et de minéraux (Se, Fe, Sr, Cu, Zn…). Le génotype des porcs est vérifié par des méthodes de biotechnologie basées sur l'analyse de l'ADN (RFLP, microsatellites). Les méthodes pour tracer les modalités d'élevage reposent sur la mesure des isotopes stables ou la quantification des composés organiques (hydrocarbures, polyphénols, acides gras, vitamines) dans les muscles et les tissus adipeux. À ce jour, peu de méthodes ont été consacrées à la traçabilité des conditions de transformation. Présentement, la majorité des méthodes restent au stade d'outils potentiels et nécessitent d'être validées sur de grands nombres d'échantillons pour être utilisées en routine. Une identification correcte des jambons secs requiert une combinaison de méthodes et d'analyses statistiques multivariantes. Un des enjeux majeurs pour le futur est de construire des bases de données largement ouvertes agrégeant les données disponibles sur les jambons secs de manière intelligible.*

Mots-clés. *Authentification – Jambons secs – Origine géographique – Méthodes analytiques.*

I – Introduction

Traceability and authentication of meat and meat products are a major concern not only for consumers but also for producers and retailers (Karoui *et al*., 2007). Thus, the globalization of food market allows a worldwide transportation of both raw material and processed meat that make easy malpractices and substitution of high quality raw material and products by lower quality ones. The international exchanges are also involved in the outbreak of worldwide diseases related to meat consumption (BSE) or animal farming (avian influenza, Foot and Mouth disease).

Consequently, for the last 20 years, the European Union has been reinforcing the policy demanding to all the food companies to develop efficient methods to trace food from farm to fork and also from fork to farm (Schwagele, 2005). In parallel, The EU has promulgate regulation "On the protection of geographical indications and designations of origin for agricultural products and foodstuffs" (Council regulation, 2081/92) for the protection of food names based on geographical origin (Protected Designation of Origin – PDO, Protected Geographical Indication – PGI) or on traditional recipe (Certificate of Specific Character – CSC).

The main goals of these regulations are to:

(i) Restore the confidence of consumers in meat and meat products giving right information on products to guide their choices;

(ii) Develop a comprehensive and integrated food safety policy and prevent food crisis;

(iii) Ensure fair trade and right prices for high quality products (Schwagele, 2005).

If regulations are required to prevent voluntary and involuntary mislabeling of food and food products, they are not sufficient. Effective analytical methods are required to deliver objective proofs that the label products were produced to meet the requirements described in specifications of PDO, PGI or CSC.

Numerous methods have been proposed to authenticate geographical origin or specific process of various foods (Perez *et al*., 2007; Muller and Steinhart, 2007; Luykx *et al*., 2008). They can be divided into two groups (Perez *et al*., 2007). The first one includes methods based on fast and non destructive methods using spectroscopic characteristics of products. These methods provide fingerprints of foods and often require complex data analyses. They remain very difficult to relate to the specifications of products. The second one includes methods based on chemical analyses of raw material or end-product. Often more tedious these methods can be easily interpreted because they are based on an extensive scientific knowledge on the relationship between environment, production system and processing conditions on quality traits of end-products. Up to now, more than thousand papers have been published on the main labeled dry cured hams produced in Europe. They describe the relationships between traits of raw material or dry cured products and genotypes (Gandemer, 2002, Tejeda *et al*., 2002), rearing systems of pigs (Lebret *et al*., 1996, Coutron-Gambotti *et al*., 1999, Andres *et al*., 2001) and processing conditions (Gandemer, 2002, Andrès *et al*, 2004). The main requirements written in specifications of labeled dry cured hams are supposed to be involved in the typical traits of dry cured hams of each area of production (Flores, 1997, Gandemer, 2009). Thus, rearing and feeding systems including breed, age at slaughter and consumption of local feeds during fattening largely affect raw material chemical composition (Gandemer, 2002*;* Ruiz et Lopez-Bote, 2002). The conditions of processing affect the chemical, physical and organoleptic traits of dry cured hams through a set of complex reactions of lipolysis, oxidation and proteolysis, kinetics of those largely determined by temperature and length of the different steps of the process (Gandemer, 2002*;* Toldra et Navarro, 2002, Toldra, 2006). In contrast, few papers have been published on composition in micronutrients such as minerals or in the ratio of stable isotope in muscle or adipose tissues from pigs and on the environment where they are reared.

Up to now, most of the available methods have been developed to assess the origin of plant foods (Olive oils, wines…) (Kelly *et al*., 2005*;* Gonzalvez *et al*., 2009). These for meat and meat products authentication are less numerous (Ballin, 2010). Most of them are not yet effective tools but just potential ones because in many cases they have been established on a too small set of animals or dry cured hams often of well known origins.

II – Geographical origin

The ratios of stable isotopes of components that constitute all the biological tissues such as muscles and adipose tissues depend on many factors but some of them are strongly related to geographical origin ($^{18}O/^{16}O$, $^{2}H/^{1}H$, $^{15}N/^{14}N$, $^{13}C/^{12}C$, $^{34}S/^{32}S$)(Karoui and de Baerdemaeker, 2007). Thus, $^{18}O/^{16}O$ and $^{2}H/^{1}H$ ratios in water depend on parameters such as the altitude, the distance to ocean and the climate. $^{15}N/^{14}N$, $^{13}C/^{12}C$, $^{34}S/^{32}S$ ratios depend on organic matter in soil and fertilizers. The amount and composition of trace elements in soil (Se, Fe, Sr, Cu, Zn…) are strongly related to the geological underground or specific pollutions from human activities (mining, accident). These elements are incorporated into animal tissues through food chain (Franke *et al*., 2005).

Measurement of $^{18}O/^{16}O$ and $^{2}H/^{1}H$ in tissue water is an interesting tool for geographical origin assessment because these ratios are strongly correlated to these in drinking water (Karoui and de Baerdemaeker, 2007*;* Heaton *et al*., 2008). Compared to the ratio of these stable isotopes in ocean water, these ratios are lower in altitude or far from the ocean because stable isotopes are discriminated through the successive cycles of evaporation, condensation and precipitation. The ratios of stable isotopes in water are very good indicators of meat origin because they are only slightly affected by feeding systems and main part of the body water come from drinking water. These methods were used with success to discriminate milk products (Heaton *et al*., 2008*;* Karoui and De Baerdemacher, 2007) and beef meat from different continents (Boner and Förstel, 2004; Schmidt *et al*., 2005*;* Horacek and Min). No data is available on pig meat. But discriminating pig meat from European areas of production of high quality dry cured hams could be very difficult because the main areas of production are close to each other, close to the ocean and in mid-mountains. So the isotope ratio in water could be too close to discriminate geographical origin of meats.

In some studies, $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratio in proteins or lipids of meat were used to discriminate beef and lamb meat according to their geographical origin (Karoui and de Baerdemaeker, 2007; Piasentier *et al*., 2003). The principle is based on the fact that plants from tropical countries are mainly C4 plants while those from temperate countries are mainly C3 plants. C3 plants discriminate more ^{13}C and exhibit a lower $^{13}C/^{12}C$ than C4 plants. Consequently, animals eating more C3 plants have a lower $^{13}C/^{12}C$ in their tissues. However, the quantification of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ suffers of serious drawbacks related to feeding systems (see next part) or to agricultural practice such as fertilizers which increase ^{15}N in plants (Schmidt *et al*., 2005*;* Bahar *et al*., 2008).

Determination of various minerals in meat has been shown to be efficient tools for geographic origin authentication of meat. Some interesting results were obtained on poultry, lamb and beef meats (Bahar *et al*., 2008). To be conclusive, it could be assumed that each area of production exhibits a specific profile in some minerals. However, these methods suffer of serious limitations. First, several areas in the world have similar geological undergrounds. Second, some feeds such as cereals and protein sources are commercialized on a worldwide market. Third, some minerals are added in diets of animals through mineral complementation. That is why a multi-elemental analysis coupled with multivariate statistical analysis is required to ensure a good discrimination of geographical origin (Franke *et al*., 2005).

III – Rearing conditions

Rearing conditions (outdoor/indoor, age at slaughter, length of fattening) and feeding systems largely affect pig adipose and muscle tissues. These effects are marked in traditional pig production based on local breed (Iberian, Corsican, Basque …) and fattening diet relied to local food (acorns, chestnuts, grass). Numerous papers describe the chemical traits of pig adipose and muscle tissues as related to many parameters of rearing and feeding in both industrial and traditional pig production (ref). Some of these parameters are of great interest to trace rearing and feeding systems because they are highly variable: lipid content, fatty acid and triacylglycerol composition. Other minor components found in animal tissues, mainly in adipose tissue, are typical of feed source: vitamins, poly-phenols, and hydrocarbons (Prache *et al*., 2007).

1. Breed or genotype

Recent developments in biotechnology open a new field in the traceability and authentication of individuals, lines, genotypes and breeds. The biotechnological methods have been developed very fast for the last 20 years. In theory, these methods are able to give a genetic fingerprint indentifying perfectly each individual and permitting to trace each animal from farm to fork because DNA is specific to each individual. However, the cost of these methods is up to now too high for a routine use (Dalvit *et al*., 2007; Lockley et Bardsley, 2000).

In contrast, these tools should be very helpful to check the genotypes used for dry cured hams production in PDO where specifications refers to local breed or allows some crossbred genotypes (i.e. Duroc X Iberian) and bans industrial pig genotypes. The development of genetic tools for local breed authentication and their crossbred require a large data base including the typical traits of the main breeds and genotypes used in European pig production. Tracing the local breeds is crucial to the survival of the herds and to defends and valorizes the high quality dry cured hams. Several studies have been devoted to differentiate the Iberian pig breed and line and to control the level of Duroc blood in the crossbreds for detecting mislabeled dry cured hams (Alvez *et al*., 2002; Fernandez *et al*., 2004; Ovilo *et al*., 2000; Garcia *et al*., 2006). The tools are based on DNA microsatellites and AFLP fragments allow a good differentiation of Iberian from crossbred Duroc X Iberian but are less efficient to distinguish crossbred Duroc x Iberian (50/50) from these with a lower proportion of Duroc blood.

2. Feeding systems

As mentioned above, some stable isotope ratios such as $^{15}N/^{14}N$ and $^{13}C/^{12}C$ are good tracers of feeding systems. Thus $^{13}C/^{12}C$ ratio in meat is related to the proportions of C4 and C3 plants in the diet. In Europe, the main C4 plant used in animal feeding is maize which is included in the diet to increase energy density in feed. So an increased $^{13}C/^{12}C$ in meat is an indicator of a more intensive feed system (Bahar *et al*., 2005*;* Boner et Förstel, 2004). Similarly, an increased $^{15}N/^{14}N$ ratio in meat is related to an intensive system of feed production because this increase is related to more intensive use of fertilizers. These isotope ratios give interesting results in discriminating ruminant meat fed grass versus maize or reared onto organic system versus more intensive system (Piasentier *et al*., 2003). In Iberian pig, $^{13}C/^{12}C$ ratio is higher in adipose tissue in pigs fed on traditional system (acorns and grass) than in pigs fed on more intensive system (concentrate). The higher is the proportion of concentrate, the higher is $^{13}C/^{12}C$ ratio in the tissue (Gonzalez-Martin *et al*., 1999). This measurement of this ratio could be interesting for discriminating Bayonne hams from these produced in the other areas because maize is largely included in the feed of Bayonne pigs.

3. Lipid composition of adipose and muscle tissues

Lipids and lipid fractions have been often used to distinguish animal according to their rearing conditions. Thus it was established that fatty acid composition of both adipose and muscular tissues is strongly related to these of feeds in pigs because it is a monogastric animal. This is of particular interest to distinguish pigs fed on local feeds such as acorns, chestnuts or grass from these fed on concentrate. Regarding fatty acid composition of raw material, genotype is also a major factor of variation. In Europe, the higher quality dry cured hams are produced from local breeds with a slow growth rate which deposit large amount of fat during the fattening period when they are too old to deposit muscle. Consequently lipids contain a high proportion of monounsaturated fatty acids coming from the conversion of starch from diet into saturated and monounsaturated fatty acids. Both fatty acid and triacylglycerol compositions were used to distinguish pigs according to their breed (Local breeds versus crossbred) or their diet (local feeds versus concentrate). In all the cases, triacylglycerols are more efficient to discriminate pigs because small variations in fatty acid composition are correlated to large variations in triacylglycerol composition (Riaublanc *et al*., 1999). Several authors have succeeded to distinguish Iberian pigs according to the feeding systems based on fatty acid composition of lipids from adipose tissue, intramuscular fat or liver (Flores *et al*., 1988; Ruiz *et al*., 1998; Perez-Palacios, 2009) or on triacylglycerol composition of adipose or muscular tissues (Diaz *et al*., 1996; Tejeda *et al*., 2002; Viera-Alcaide *et al*., 2007). Some minor lipid components can be good indicators of local feed consumption. In various amounts in feeds, they are stored in body fat. Hydrocarbon profiles of adipose tissue were used to distinguish Iberian pigs according to feeding systems. n- alkanes are not efficient (Tejeda *et al*., 2001a) but some peculiar hydrocarbon such as eut-kaurene (Navaez-Rivas *et al*., 2008) and neophytadiene (Tejeda *et al*., 2001b, Perez-Palacios, 2009) coming from grass could be used to discriminate Montanera pigs fed on acorns and grass from other Iberian pigs fed various amounts of concentrate. Tocopherols, namely gamma one, which is in a high amount in acorns (Tejerina *et al*., 2010), could help to discriminate traditional Montanera feeding system from others containing concentrate (Perez-Palacios, 2009*;* Tejerina *et al*., 2010).

4. Age at slaughter

The age of pigs is regarded as one of the main parameters improving meat quality and is included in the specifications of dry cured hams in many areas of production. Up to now, no method allows tracing this physiological parameter.

IV – Processing

The changes in raw matter during dry cured ham processing are largely involved in the typical sensory traits of end products. These changes involved a complex set of chemical and physic-chemical reactions affecting lipids, proteins, water and salt contents (Gandemer, 2002*;* Toldra et Navarro, 2002, Toldra, 2006).. The intensity of these changes largely depends on the conditions of processing used in the main area of production in Europe. Many PDO specifications contain specific requirements on the different steps of the process (length, temperature)(Flores, 1997). The changes in chemical and physic-chemical traits of meat and adipose tissues of hams have been largely described and marked differences were observed according to methods of processing. However, very few papers focus on methods to check that the specific requirements on process written in specifications are respected.

The use of thawed meat is prohibited in high quality dry cured ham production. Several papers are devoted to the differentiation of fresh and thawed raw meat. A review of methods indicates that only a combination of several methods allows discriminating fresh from thawed meat including DNA degradation, enzyme profile in juice extracted from meat and microscopy techniques (Ballin and Lametsch, 2008).

Volatile profiles of dry cured hams depend on the length and the temperature of the main steps of the process as well as the chemical traits of the raw material. Many papers described differences in volatile profiles from hams of different countries or feeding systems (Ruiz zt al., 1999*;* Bolzoni *et al*., 1996*;* Dirinck *et al*., 1997). Some volatiles found in aroma of hams come directly from feeds and are tracers of feeding systems. However, quantification of volatiles is very difficult and results vary greatly according to the method of volatile extraction and from one laboratory to another. So, volatile analyses are not proper tools to discriminate hams.

V – Conclusion

This review shows that the research on the authentication of meat and meat products is in progress. This is a major concern for consumers and producers. However, very little has been done on dry-cured hams. So up to now, we lack of accurate methods to assess that the specifications of dry-cured hams produced under PDO, PGI ou CSC in Europe are strictly applied. Most of methods remain potential tools and are far from their use as standard recognized methods to detect mislabeled products. That is why most of these methods were developed with small sets of samples of well-known origins. These methods must be validated using large numbers of samples of unknown origins and processes including raw meat and dry-cured hams arising from intensive systems of production all around the world. Large opened data bases must be built putting together all the characteristics of dry-cured hams as related to their area and specifications of production. A better characterization of the environment where animals are reared is required to able to mobilize very promising methods based on stable isotope ratios or trace elements quantifications.

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