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NEW TECHNOLOGIES FOR CHARACTERIZATION AND QUALITY ASSURANCE IN PIG

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ABSTRACT

Consumers' concerns regarding the quality and safety of their food supply has increased over recent years. In order to regulate and reinforce control practices, analytical methods guaranteeing correct assignment of production system and consequently quality and prices, are required in food industry. This review discusses reliability of some of these new methodologies, mainly those based on the feeding system identification. Compounds such as fatty acids, triacylglycerols, volatiles, tocopherols, nitrogen stable isotope ratio, etc. are potential tracers in tissues of feeding diets. Most of the technologies based on the quantification of these compounds are feasible tools for assuring quality and differentiating outdoors fed animal from those fed in intensive conditions with feed. However, to distinguish a greater number of feeding types or feeding combinations the use of different techniques or a multivariable analysis is required to assure quality.

Key words: analytical methods / quality assurance / Mediterranean meat products / feeding differentiation

1 INTRODUCTION

Several food safety incidents in Europe have raised consumers' concerns regarding the quality and safety of their food supply. These social scares together with additional concerns over animal welfare and the environmental impacts of food production have increased consumers' demand for organically-produced food, grass-fed animal products, which are free of toxins or medications, and which are considered to be healthier (O'Donovan and McCarthy, 2002; Bredahl *et al*., 2001) and with best quality characteristics (Ruiz *et al*., 2002; López-Bote, 1998). Hence, consumers are willing to pay a higher price for products that provide these desirable (sensory, nutritional and production) attributes (Bredahl *et al*., 2001).

In this sense, Mediterranean meat products that are characterized by sustainable production system of autochthonous pig breeds (Alentejana, Cinta Senese, Black Slavonian, Krškopolje, Iberian pig, *etc*.) in many cases adapted to an outdoors feeding regime, have become of great interest in recent years due to the high quality of the products and their economical importance. However, these outdoor-rearing systems are variable, heterogeneous and in many cases are not well categorized (Pugliese and Sirtori, 2012). Hence, pigs could be reared under different modalities ranging from pigs receiving a total supply of concentrate even outdoors to those reared exclusively in the forest (Pugliese, 2012). Taking into account not only the heterogeneity of these production systems (genetic, ages, feeding, *etc*.) but also the high prices that top quality products can command in the market, has propitiated fraudulent practices based on the production of animals raised in intensive situations and their sale as if they had been fed in free-range conditions.

Consequently, in order to ensure a proper correspondence between quality characteristics and prices, governments and control institutions have tried to establish some quality assurance schemes. Quality assurance schemes may require animals to be bought from farms certified by a recognized assurance scheme that receive inspector visits or may limit geographic origin. Most have extensive documentation requirements, including

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identification of animals/carcasses, feedstuffs or treatments used including slaughter and grading, through a large part of the supply chain (Wood *et al*., 1998; Bredahl *et al*., 2001). Moreover, to regulate and reinforce control practices, analytical methods guaranteeing correct assignment of the production systems and consequently quality and prices, are required in the food industry. Hence, a range of markers for meat authentication are under active investigation. However, in many cases they are only potential tools that have been tested in a small number of samples or in standard or experimental situations in which the origin of the samples was known.

This review discusses reliability of some of these new methodologies, mainly those based on feeding system identification, which together with the genotype is one of the main factors affecting quality of products.

2 METHODS BASED IN LIPID COMPOSITION OF TISSUES

2.1 FATTY ACID COMPOSITION

Fatty acid profile was one of the methods first and widely used to differentiate animal rearing conditions. It is especially interesting in monogastric animals such as the pig because the tissue fatty acid profile is strongly related to the fatty acid composition of the feed. Hence, it has been considered for many years as an effective parameter to discriminate animals fed on outdoors systems from those fed on concentrate (Ruiz *et al*., 1998; Wood *et al*., 2008). This is because the fatty acid profile of pigs fed on local natural resources such as acorns, grass, chestnuts *etc*. (with a particular fatty acid composition) is different from those fed on concentrate. It was considered so reliable that its determination by gas chromatography (GC) was also officially included in Spanish legislation as tool to differentiate the feeding types of Iberian pigs during the fattening phase (REAL DECRETO 1083/2001). However, the inclusion of new ingredients in pig diet formulations resulted in a high number of wrong classifications and the consequent scepticism about its reliability. The use of other detector systems in gas chromatography such as mass detector (GC-MS) has been reported to improve the classification results (López-Vidal *et al*., 2008). In a recent research work carried out in practical situations (Sanabria *et al*., 2013), in which 749 fat samples of Iberian pigs from 38 batches were analysed, it was concluded that the determination of fatty acid profile using GC could still be used but in combination with other analytical methods.

2.2 TRIACILGLYCERIDES

The triacylglycerol fraction analysis by gas chromatography in combination with pattern recognition techniques such as principal component (Viera-Alcaide *et al*., 2007) or linear discriminant analysis (Gallardo *et al*., 2012; Narvaez-Rivas *et al*., 2013) have also been applied to characterize the feeding system. This method has some advantages over fatty acid determination because it is faster and does not need previous treatment or loss of sample because it is performed by direct injection of fat dissolved in hexane (Narváez-Rivas *et al*., 2013). Moreover, it allows the differentiation of extensive *vs.* intensive feeding with high recognition capability (Gallardo *et al*., 2012). The use of artificial neural networks as a pattern recognition technique applied to triacylglycerols identification obtains better classification results than a linear model (linear discriminant analysis) and shows a prediction ability of 97% for differentiation of free-range pigs and those fed mixed-diet in intensive or extensive conditions (Narváez-Rivas *et al*., 2013). However, the inclusion in the model of fat samples from pigs fed on natural resources in combination with a mixed diet reduced the differentiation accuracy.

2.3 VOLATILE COMPOUNDS

Volatile compounds in tissues have also been related to fattening diets and have been studied as tracers of animal feeding systems. Hence, volatile composition of subcutaneous fat has been found to differ in pigs fed free-range or mixed diets in intensive or extensive conditions with access to grass (Narváez-Rivas *et al*., 2011) using purge and trap gas chromatography-mass spectrometry (GC-MS) in combination with chemometrics to obtain suitable classification models. Moreover, in ruminants the persistence of volatile tracers of pasture diet in fat from lambs fed on increasing levels of concentrate have allowed the correct differentiation of different feeding backgrounds (Silvadier *et al*., 2010). On the other hand, the multiple volatile compounds generated by chemical or enzymatic reaction in dry-cured products (Toldrá *et al*., 1998) have also been used as a differentiation tool of pig feeding (Narváez-Rivas *et al*., 2010). However, the main disadvantages in this case are that volatile composition is not only affected by feeding but also by maturation process, genotype, etc. and that the volatile profile for the same product may depend on the concentration and isolation techniques, with solid phase microextraction (SPME) and purge and trap being the most commonly used (Narváez-Rivas *et al*., 2012).

3 METHODS BASED IN MINOR COMPONENTS OF THE UNSAPONIFIABLE FRACTION

3.1 GAMMA AND ALPHA-TOCOPHEROL QUANTIFICATION. STEREOISOMER ANALYSIS

This method is based on the fact that grass is rich in alpha-tocopherol (Rey *et al*., 1997) while acorns are the main source of gamma-tocopherol (Rey *et al*., 1998) and both compounds can be detected in the unsaponifiable fraction of animals fed with these types of feed. The potential utilization of the quantification of these compounds by HPLC to identify feeding background was first reported by Rey *et al*. (1998) who found higher concentrations of gamma- and alpha-tocopherol in muscle from extensively fed Iberian pigs than those fed in confinement with mixed diets. Later research (Rey *et al.,* 2006a,b) also reported the presence of these compounds in subcutaneous fat and raw *biceps femoris* muscle and a relationship between the weight gained or days of stay in free-range conditions and the gamma-tocopherol concentration in muscle and fat. Hence, gamma-tocopherol would be better in distinguishing the feeding system when compared to alpha-tocopherol, which could be provided by supplemented feeds. The possible use of tocopherols quantification as a tool to differentiate the feeding system has also been reported in other Mediterranean pigs such as Cinta Senese (Pugliese *et al*., 2009). Moreover, tocopherol accumulation has also been found in dry-cured hams (Isabel *et al*., 2009; Rey *et al*., 2010). Recently, the feasibility of gamma and alpha tocopherol quantification in practical situations has been evaluated for distinguishing the four feeding categories of Iberian pigs with a success rate close to 80% (Rey *et al*., 2013), with muscle being a better predictor than fat. However, the differentiation of free-range feeding *vs.* fed in intensive conditions can be achieved with up to 98% of feasibility. Taking into account that a high number of samples were used and that these were collected from farms situated in different geographical regions (unknown at the time of analysis), quantification of tocopherols by HPLC could be considered as a useful tool for feeding regimen distinguishing. This analytical method also had more accurate global results than others based in fatty acid, triacylglycerides, neophytadiene, NIRS, chemsensor, or stable isotopes analysis (García-Casco *et al*., 2013).

Other related compounds that could help correct assignment of feeding type are alpha-stereoisomers. Alpha-tocopherol is a chiral molecule that presents eight stereoisomer forms differing in group orientation at the 2', 4' and 8' positions of the phytyl tail. The predominant stereoisomer derived from plant sources is RRR-α-Tocopherol; however, synthetic α-tocopherol (all-rac-α-tocopheryl acetate), which is the commercially available form of vitamin E for feed supplementation, is composed of an equimolar mixture of 8 stereoisomers. Hence, it has been found that alpha-stereoisomer analysis could discriminate between cattle fed on grass from those fed concentrates containing synthetic vitamin E (Röhle *et al*., 2011). Alpha-tocopherol stereoisomers have also been found to be higher in dry-cured hams from Iberian pigs fed free-range when compared to those fed acorns or mixed diets (Rey *et al*., 2010). Hence, its quantification may increase classification capacity of tocopherols.

3.2 HYDROCARBON FRACTION COMPOUNDS

Hydrocarbons, mainly of plant origin, are compounds present in small quantities in animal fats (unsaponifiable fraction) and their presence in tissues has been related to feeding. These are presented in plants in the form of saturated or unsaturated hydrocarbons and are little modified during digestion and metabolism (Rembold *et al*., 1989), being absorbed at low doses by the small intestine (Tulliez and Bories, 1975). Consequently, their identification in tissues has been used by several authors trying to differentiate animal feed sources (Tejeda *et al*., 1999; Tejeda *et al*., 2001b; Petrón *et al*., 2004; Gamero-Pasadas *et al*., 2006; Narváez-Rivas *et al*., 2010 and 2011). Some of the compounds identified in tissues have been n-alkanes and n-alkenes in subcutaneous (Tejeda *et al*., 1999) and intramuscular (Tejeda *et al*., 2001a) fat as well as in the intramuscular fat of dry-cured hams (Petrón *et al*., 2004) from Iberian pigs. Moreover, a branched hydrocarbon (neophytadiene) has been found in intramuscular fat (Tejeda *et al*., 2001b) and in intramuscular lipids from dry-cured hams (Petrón *et al*., 2005) related to animal feed. The analysis of these compounds requires chromatographic techniques because they are present at very low concentrations and their separation from the unsaponifiable fraction is difficult and tedious (Tejeda *et al*., 1999). Gas chromatography coupled to mass spectrometry (GC-MS) has also been applied (Tejeda *et al*., 2001a; Petrón *et al*., 2004). Other possibilities are the use of thin layer chromatography (TLC) (Bernardini *et al*., 1982; Tejeda *et al*., 1999) or the use of solid phase extraction (SPE) to reduce the analysis time. In a recent study developed in unknown Iberian pig samples from different feeding systems, Hernández-Matamoros *et al*. (2013) reported that quantification and identification of the branched hydrocarbons (mainly neophytadiene) does not allow differentiation of pigs fed exclusively acorns and grass in the forest from those fed this diet for a shorter period and of lower quality. However, this

methodology is a good tool for distinguishing between pigs fed with feeds in intensive conditions from others fed for a variable period of time with natural resources.

3.3 OTHER MINOR COMPONENTS (CAROTENOIDS, OTHER PHENOLIC COMPOUNDS)

Other minor compounds such as phenolic derivatives of gallic acid have been identified in acorns (Cantos *et al.*, 2003) and different ellagitannin-derived metabolites have been detected in Iberian pigs after acorn intake; however, no metabolites accumulated in any tissues analysed but were detected in plasma and urine (Espin *et al.*, 2007). By contrast, carotenoids, pigments presented in high concentrations in green herbage, are stored in tissues and could act as biomarkers of grass feeding in calves (Serrano *et al.*, 2006). These authors reported that plasma carotenoid concentration and the reflectance spectrum of fat in the zone of light absorption was higher in those calves fed on pasture when compared to those receiving different concentrate levels.

4 OTHER METHODS

4.1 NEAR INFRARED SPECTROSCOPY − NIRS

Near Infrared technology (NIRS) has shown to be a promising approach for classifying fat samples by feeding regime based on spectral information (De Pedro *et al.*, 1995; Arce *et al.*, 2009; Zamora-Rojas *et al.*, 2012) or on the prediction of compounds such as the four major fatty acids useful in classifying Iberian pig samples (García-Olmo *et al.*, 2009; Pérez Marín *et al.*, 2009). NIRS has several advantages over other techniques (Garrido and De Pedro, 2007) because it provides rapid results, requires little or no sample preparation, is safe for the operator, can be applied to different products and is cheaper than conventional techniques. However, this technique, as many others, only can classify with high percentage reliability pigs fattened exclusively on natural resources from those fed on feed indoors or outdoors (De Pedro *et al.*, 2013).

4.2 CHEMICAL SENSOR

This technology is based on the use of MS-based electronic nose in which the sample unit injects a volatile mixture into an ionization chamber that is detected in a mass detector. The number of variables is reduced by removing noisy and redundant features (Llobet *et al.*, 2007). This technique can be performed using the volatile fraction of a sample or the fatty acid methyl esters in fat samples. Using a mathematical predictive model, it has been described to be a good method for classifying fat samples from pigs fed on intensive feeds *vs.* those fed in outdoors conditions (Carrasco and Duque, 2013). However, despite the multivariate analysis application, differentiation of other intermediate feeding categories was not successful (Carrasco and Duque, 2013).

4.3 STABLE ISOTOPES

Another alternative to differentiate feeding regimes is determination of the $^{13}C/^{12}C$ isotope ratio of the total carbon in fat (González-Martin *et al.*, 1998). The $^{13}C/^{12}C$ ratio depends on the photosynthetic mechanism used by the plant, the Hatch and Slack cycle (C_4) with $\delta^{13}C$ values in the range −12/−14‰, and the Calvin cycle (C_3) with $\delta^{13}C$ values in the range −26/28 ‰ (De Niro and Epstein, 1978). Therefore, it is possible to distinguish between chicks fed with maize- or wheat-based feed (C_4 and C_3 plants respectively) according to the photosynthetic cycle of the plant. Most of the ingredients used in diet formulation are photosynthetic species C_3-cycle plants with $\delta^{13}C$ values ranging between −21‰ (acorn) and −27.5‰ (alfalfa), while corn and gluten have values close to the C_4 species. When isotopes are used to characterize and differentiate pigs as a function of their feeding system, those receiving feed showed significant differences ($\delta^{13}C = -22.14$‰) when compared to those acorn-fed ($\delta^{13}C = -23.87$ ‰) (González-Martin *et al.*, 1999).

A more recent technique is the utilization of a combination of the above method with gas chromatography, which is called GC-C-IRMS (Isotope-ratio-Mass Spectrometry) consisting of the separation of fatty acid methyl esters by gas chromatography and subsequent combustion and mass spectrometry analysis of the $^{13}C/^{12}C$ isotope ratios of the products obtained (Recio-Hernández, 2010), which gives a characteristic isotope profile of each sample. Oleic acid is the main discriminant variable of the feeding system and pigs fed free-range have values of $\delta^{13}C18{:}1 \leq -25.9$‰. These new technologies, in which many different compounds are detected, are also combined with potential statistical treatments based on principal component or discriminant analysis (multivariate analysis). The discriminant analysis using the values of twelve fatty acids by gas chromatography in combination with the four major fatty-acid isotope ratios by CG-C-IRMS improve the prediction ability when samples of very different origin are classified (Delgado-Chavero *et al.*, 2013).

5 CONCLUSION

Most of the technologies presented in this review are feasible tools for assuring quality and differentiate outdoors fed animals from those fed in intensive conditions with feed mixtures. However, to distinguish a greater number of feeding types or feeding combinations the use of different techniques or a multivariable analysis is required to assure quality with reliability.

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