ARTICLE IN PRESS

Meat Science xxx (xxxx) xxx-xxx



Review

Contents lists available at ScienceDirect

Meat Science



journal homepage: www.elsevier.com/locate/meatsci

Meat provenance: Authentication of geographical origin and dietary background of meat

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ARTICLE INFO	A B S T R A C T
Keywords: Authenticity Fraud Hair Isotopes Muscle Validation	The authenticity of meat is now an important consideration in the multi-step food chain from production of animals on farm to consumer consumption of the final meat product. A range of techniques, involving analysis of elemental and molecular constituents of meat, fingerprint profiling and multivariate statistical analysis exists and these techniques are evolving in the quest to provide robust methods of establishing the dietary background of animals and the geographical origin of the meat derived from them. The potential application to meat authentication of techniques such as stable isotope ratio analysis applied to different animal tissues, measurement in meat of compounds directly derived from the diet of animals, such as fatty acids and fat soluble vitamins, and spectroscopy is explored. Challenges pertaining to the interpretation of data, as they relate to assignment of

1. Introduction

Provenance, according to the Oxford dictionary, is defined as "the place of origin or earliest known history of something", "the beginning of something's existence" or "a record of ownership ... used as a guide to authenticity or quality". In the context of food, and meat particularly, provenance therefore encompasses geographical origin, farm production system, and a record of the meat's journey from the farm to the consumer's table. Traceability, defined as "the ability to follow the movement of a food through specified stages of production, processing and distribution" (WHO/FAO, 2007), and traceability systems may be understood as seeking to protect the latter record of a food's journey from farm to consumer. Therefore, food authentication, "the process by which a food is verified as complying with its label description" (Dennis, 1998), is critical to establishing the provenance of food and validating the traceability systems that may be in place to underpin its provenance.

Food provenance is an important consideration for food producers and consumers alike. For consumers, foods of animal origin, such as meat products, may have a particular value associated with the geographical origin or production system from which they derive, e.g. "Protected Designation of Origin (PDO)", "Protected Geographical Indication (PGI)", "Traditional Speciality Guaranteed (TSG)", "organic", "free range" or "grass-fed" (Camin et al., 2017; Daley, Abbott, Doyle, Nader, & Larson, 2010; McCluskey, Wahl, Li, & Wandschneider, 2005). Consumers are willing to pay a premium for these products and in the future they may pay more for meat generally as sustainability issues come to the fore (Henchion, McCarthy, Resconi, & Troy, 2014). Food producers increasingly seek to maximise the market potential of their products based on, for example, the traditional nature or sustainability of the production system or the nutritional profile of their products. A consequence of this desire to "add-value" is concern among food producers and consumers about food fraud, whereby counterfeit substitutes replace authentic foods (Primrose, Woolfe, & Rollinson, 2010).

In the case of animal-derived foods generally, and meat specifically, not only is geographical origin important but so also is the verification of the dietary background of animals because diet can be a distinct feature of certain production systems, e.g. "organic" or "grass-fed" and it can have profound effects on the composition and quality (nutritional and sensory) and the sustainability of production of animal derived food products (Woods & Fearon, 2009). Thus, for example, verification that "grass-fed beef" is in fact from grass-fed animals or that "corn fed" chicken is in fact corn-fed is required (Osorio, Moloney, Schmidt, & Monahan, 2011a; Rhodes et al., 2010). In Ireland, beef for the U.S. market must be from animals with a "More than 80% Grass Diet" (Bord Bia, 2017). The challenge of authentication is even greater when comminuted or re-formed meat products are considered and issues such as speciation, proportion of ingredients used and addition of undeclared ingredients become important (Montowska & Pospiech, 2012). There

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 $\label{eq:https://doi.org/10.1016/j.meatsci.2018.05.008$ Received 28 February 2018; Accepted 10 May 2018 0309-1740/ © 2018 Elsevier Ltd. All rights reserved.

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F.J. Monahan et al.

are already several volumes and review articles on the subject of food authentication (Lees, 2003; Luykx & Van Ruth, 2008; Primrose et al., 2010; Sun, 2008) and on meat authentication specifically (Arvanitoyannis, 2016; Ballin, 2010; Montowska & Pospiech, 2012; Sentandreu & Sentandreu, 2014) available. The current article will deal with meat provenance authentication in the context of fresh (unprocessed) meats mainly, but many of the methods discussed and the examples given have applicability for processed meats too. A particular focus of the article will be on developments relating to determination of dietary background and geographical origin of meats.

1.1. Methods used in meat provenance authentication

A wide range of analytical measurements has found application in meat authentication (Arvanitoyannis, 2016; Ballin, 2010; Danezis, Tsagkaris, Camin, Brusic, & Georgiou, 2016; Montowska & Pospiech, 2012; Nader, Brendel, & Schubbert, 2016). In terms of the geographical origin and dietary background of meat, methods involving the measurement of quantifiable components at both an elemental and molecular level have been applied, including: stable isotopes (Heaton, Kelly, Hoogewerff, & Woolfe, 2008); trace elements (Franke, Hadorn, Bosset, Gremaud, & Kreuzer, 2008; Franke et al., 2007); fatty acids (Alfaia et al., 2009); volatile organic compounds, such as terpenes and phenolics (Priolo et al., 2004); carotenoids (Prache, Priolo, & Grolier, 2003a) and vitamin E (Röhrle, Moloney, Black, et al., 2011). It is well established that these constituents of meat are directly influenced by the diet consumed by animals and therefore they can provide information about the dietary background of animals. In addition, some of the above "markers" can also be extremely useful for assignment of geographical origin if, for example, specific feedstuffs are associated with a particular geographical region (Schmidt et al., 2005) or are influenced by regional climatic conditions or underlying geology (Capo, Stewart, & Chadwick, 1998). As well as measurement of elemental or molecular markers, a "fingerprint" approach can also be taken whereby spectroscopic techniques are used to determine differences in the optical properties of foods derived from different production systems (Downey, 2016; Prache, 2009).

Furthermore, DNA-based methods are used widely in meat authentication by providing a direct link between meat products and the animals from which they derive (Nader et al., 2016). While they do not provide information about the dietary background or farm of origin or production system, they play an important role in traceability systems, linking meat or meat products back to an individual animal or breed and form the basis of several commercial traceability systems, e.g. DNA TraceBACK (Identigen, 2018). DNA-based methods have also been widely used in speciation (Amaral, Meira, Oliveira, & Mafra, 2016) and in meat fraud detection, e.g. in the horsemeat scandal in the European Union in 2013 (FSA, 2013). In addition, molecular approaches may be used to study the impact of production systems on gene expression (Hocquette, Cassar-Malek, Bernard-Capel, & Picard, 2009; Sweeney et al., 2016).

The analytical techniques applicable to food authentication are also well documented: they include chromatography (GC, HPLC), isotope ratio mass spectrometry (IRMS), inductively coupled plasma mass spectrometry (ICP-MS), spectroscopy (IR, NMR, UV, fluorescence, Raman), molecular (DNA and PCR-based) techniques and enzymatic techniques (Downey, 2016; Lees, 2003; Luykx & Van Ruth, 2008; Primrose et al., 2010; Sun, 2008). In the following sections some of the more promising approaches to authentication of the dietary background and geographical origin of meat are discussed.

2. Stable isotope ratio analysis

Stable isotope ratio analysis (SIRA) involves the measurement of ratios of stable isotopes of bioelements, frequently including carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), hydrogen ($^{2}H/^{1}H$), oxygen ($^{18}O/^{16}O$)

and sulphur (${}^{34}S/{}^{32}S$). Isotope Ratio Mass Spectrometry is typically used for SIRA and the data obtained are reported as delta (δ) values, in per mil (∞) units, with $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, $\delta^{18}O$ and $\delta^{34}S$ notation for ratios of ${}^{13}C/{}^{12}C$, ${}^{15}N/{}^{14}N$, ${}^{2}H/{}^{14}H$, ${}^{18}O/{}^{16}O$ and ${}^{34}S/{}^{32}S$, respectively (Kelly, Heaton, & Hoogewerff, 2005). A strong scientific framework exists for isotope ratio measurements in forensic applications (Cerling et al., 2016); meat provenance studies can build on these analytical, biochemical and geological foundations.

It is well established that the stable isotope composition of bioelements in animal tissue is influenced by the composition of the diet consumed by the animal (DeNiro & Epstein, 1978); therefore, stable isotope signatures obtained from an animal's tissues can provide useful information about the diet consumed by that animal. Thus, C and N isotopic compositions of animal products can be related to production system, e.g. C₃ vs C₄ (photosynthetic pathway) crops, pasture vs cerealbased or organic vs conventional systems (Osorio et al., 2011a; Schmidt et al., 2005). In addition, while not direct indicators of geographical origin, $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios can be useful in indirectly determining geographical provenance if a particular feedstuff is typically fed in a particular region or if analysed in combination with other stable isotopes such as hydrogen and oxygen isotopes (Nakashita et al., 2008). The latter two elements are especially useful for geographical origin assignment of food linked to regional climatic conditions (Kelly et al., 2005) because they are strongly latitude dependent and they are also affected by altitude, distance from the sea, total precipitation and seasonality (Rozanski, Araguas-Araguas, & Gonfiantini, 1992). Sulphur isotopic compositions are mainly affected by the geology of the area where animal feed is grown (sedimentary or igneous) (Rossmann et al., 2000) as well as the proximity to the sea, climatic conditions and fertilization practices (Krouse & Grinenko, 1991).

In meat, SIRA has been shown to be particularly useful in the assignment of dietary background (Bahar et al., 2008; González-Martin, González-Pérez, Mendez, Marqués-Macias, & Poveda, 1999; Piasentier, Valusso, Camin, & Versini, 2003) and geographical origin (Boner & Förstel, 2004; Camin et al., 2007; Chesson, Podlesak, Thompson, Cerling, & Ehleringer, 2008; Guo, Wei, Pan, & Li, 2010; Nakashita et al., 2008; Schmidt et al., 2005). The technique has been applied across all species: beef (Bong et al., 2010; Guo et al., 2010; Heaton et al., 2008; Nakashita et al., 2008; Renou et al., 2004); pork (González-Martin et al., 1999); lamb (Perini, Camin, Bontempo, Rossmann, & Piasentier, 2009); poultry (Franke, Hadorn, et al., 2008); fish (Li, Boyd, & Sun, 2016; Vinci, Preti, Tieri, & Vieri, 2013) and even invertebrate species (Gamboa-Delgado et al., 2014).

2.1. Stable isotopes and the dietary background of meat

The underlying reason for large differences in δ^{13} C values in meat from different production systems is frequently the different proportions of C₃ and C₄ plant species in the diets consumed by animals (Smith & Epstein, 1971). For example, beef from cattle consuming a predominantly C_3 (temperate grass silage) diet ($\delta^{13}C = -29.6\%$) was clearly distinguishable from that of animals consuming a predominantly C₄ (maize silage) diet ($\delta^{13}C = -11.8\%$) (Fig. 1) (Bahar et al., 2005). In the same study, the δ^{15} N values of the diets (8.1‰ and 3.3% in the grass and maize silages, respectively) were also clearly reflected in those of the beef. Even in beef from animals consuming diets with a lower isotopic spacing, for example barley vs grass-based diets (both C₃ plant species) in which a dietary spacing of 2 to 3‰ in δ^{13} C values exists, it was possible to discriminate between beef from animals raised on the different diets (Osorio et al., 2011a) (Fig. 2). In Switzerland, Richter, Spangenberg, Willems, Kreuzer, and Leiber (2012) used bulk and fatty acid compound specific C isotope analysis to distinguish between lowland and mountain pasture lambs, with a δ^{13} C difference in the vegetation of 2.5‰ between the two sites. In British chicken, Rhodes et al. (2010) demonstrated the usefulness of SIRA in distinguishing between chicken from birds fed varying levels of maize

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