Food Chemistry 136 (2013) 1543–1550

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Effect of origin, breeding and processing conditions on the isotope ratios of bioelements in dry-cured ham

Matteo Perini [a,b], Federica Camin [b], José Sánchez del Pulgar [c], Edi Piasentier [a,*]

[a] Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Udine, via Sondrio 2, 33100 Udine, Italy
[b] IASMA – Research & Innovation Centre, Fondazione Edmund Mach, Food Quality & Nutrition Area, via E. Mach 1, 38010 San Michele all'Adige, Trento, Italy
[c] Food Technology, Facultad de Veterinaria, UEx, Campus Universitario s/n, 10003 Cáceres, Spain

ARTICLE INFO

Article history:
Available online 1 July 2012

Keywords:
IRMS
Dry-cured ham
Origin
Husbandry system
Pork processing

ABSTRACT

The stable isotope ratios (SIR) of the bioelements ($^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$) of the defatted dry matter and marbling and subcutaneous fat fractions, were assessed on 86 ham samples belonging to six different types, with the aim of ascertaining the effect of origin and production system on 11 isotopic ratios. The ham types were obtained from pigs reared in three regions, examining in every location one different production factor at two levels of expression: pig genotype (local breed *vs.* industrial hybrid) in Friuli (Italy), pig feeding regime (Bellota *vs.* Campo) in Extremadura (Spain) and ham seasoning time (mid *vs.* end) in Emilia (Italy). The isotopic composition of meteoric water and the dietary abundance of C_4 plants allowed to distinguish Italian PDO from Spanish hams. The contrasting treatments within the regional batches generated promising differences in SIR, potentially useful for tracing the whole ham production system, including the processing procedure.

1. Introduction

The stable isotopes ratios (SIR) of bioelements, which depend on botanical, geographical, agronomic and climatic factors, transmitted from water and plants to animal products, have been widely proposed for meat authenticity and origin assessment. As recently reviewed by Schmidt, Rossmann, Rummel, and Tanz (2009), studies on pork are few in comparison with those on ruminant meat species. The pioneering experiments of DeNiro and Epstein (1978) presented the $^{13}C/^{12}C$ (expressed as $\delta^{13}C$ values) of the major biochemical fractions of pork, analyzed as an experimental food of flies. Mitchell, Steele, and Hare (1993) then changed the $^{13}C/^{12}C$ levels in pig tissues by switching the animals from two opposite C_3 or C_4 plant diets. Eventually the isotope ratio mass spectrometry (IRMS) was used around year 2000 to trace Iberian swine production system. González-Martin and colleagues from Salamanca University first differentiated the fresh pork from fattening Iberian pigs according to their feeding regime on the basis of $^{13}C/^{12}C$ in adipose tissue samples (González-Martin, González-Pérez, Hernández Méndez, Marqués-Marcia, & Sanz Poveda, 1999) and then, by joint analysis of $^{13}C/^{12}C$ and $^{34}S/^{32}S$ of liver tissue, they discriminated both fattening diet and pig breed (González-Martin, González-Pérez, Hernández Méndez, & Sánchez González, 2001). Other studies on stable isotopic ratios were carried out on pigs to evaluate the mechanisms influencing the fractionation of body tissues (Nardoto, De Godoy, Ferraz, Ometto, & Martinelli, 2006; Tuross, Warinner, Kirsanow, & Kester, 2008; Warinner & Tuross, 2009, 2010) or individual molecular compounds (Hare, Fogel, Stafford, Mitchell, & Hoering, 1991; Howland et al., 2003; Stott, Davies, Evershed, & Tuross, 1997) and their implication for ecology and archeology. These researches make an important contribution to understanding the production factors (genetic type, age, growth rate, feeding composition, nutritive level, etc.) affecting the isotopic signatures of swine tissues and their relationship with geographical origin and breeding system. However, isotopic fractionation might occur even during pig meat processing and storage (Thiem, Lüpke, & Seifert, 2004). As claimed by Schmidt et al. (2009), who concluded their review stating that IRMS method "has so far not been applied to meat products", further experiments would be necessary in order to fully understand the influence of biochemical pathways on isotopic shifts during the manufacturing processes of meat.

Among meat products, dry-cured ham is a valuable traditional one which originated in southern European countries, where it is often guaranteed by a protected designation of origin (PDO) and represents an important part of the agro-food economy. As an example, PDO dry-cured ham (*prosciutto*) is the main product of the Italian pig industry and more than 80% of pig production is destined for the PDO traditional Italian ham market (Renaville et al., 2010). To obtain unique quality traits, the production of PDO dry-cured hams is subjected to rules established by several

* Corresponding author. Tel.: +39 0432 558190; fax: +39 0432 558199.
E-mail address: edi.piasentier@uniud.it (E. Piasentier).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.06.084

consortia concerning the characteristics of the raw meat (geographical origin, breed of pigs, feeding regime and rearing system) and the processing conditions (salting, curing and ripening; Toldrá, 2002).

Consequently the aim of this paper is twofold: (i) ascertain the effect of various production factors concerning both pig husbandry and thigh processing procedures on stable isotope ratio variability of the most important bioelements and (ii) confirm the efficacy of IRMS as a tool for tracing dry-cured ham origin and authenticity, by using simultaneously 11 SIR data from three ham fractions.

2. Materials and methods

2.1. Experimental design

The hams were made from pigs reared in three geographical areas [Friuli, Emilia (IT), Extremadura (ES)], where different production factors were studied (Table 1). The effect of pig genetic type was examined on 36 hams from heavy pigs of two genotypes (black local breed and Goland industrial white hybrid) kept on the same diet and breeding conditions in Friuli. The influence of feeding regime was analyzed by comparing the isotope ratios of 26 Dehesa de Extremadura PDO hams, from heavy Iberian pigs fattened outdoors on grazed feedstuffs without ("Bellota" ham) or with ("Campo" ham) concentrate supplements (Sánchez del Pulgar et al., 2011). The processing influence was examined on 24 hams from industrial white hybrid, heavy pigs (Italian Large White × Italian Landrace) reared on the same farm and diet in Emilia and seasoned in three plants for two different times.

2.2. Ham samples and tissue fractions

The ham samples were collected in their processing plants at the end of seasoning (types 1–4 and 6, Table 1) or at mid-seasoning (type 5). A section of *Biceps femoris* muscle (BF) with the surrounding subcutaneous fat (SCF) was taken. The two tissues were separated and the two samples were individually vacuum-packed and frozen at −18 °C until the time of analysis, when BF and SCF were cut into small pieces. The BF pieces were dried completely with the aid of a lyophilizer (freeze-drier) and then homogenized with a suitable grinder and freeze-dried again. The resulting dry powder was fractionated into crude fat (FAT), by extraction with petroleum ether for 6 h in a Soxhlet apparatus, and defatted dry matter (DFDM), essentially protein. The SCF pieces were directly extracted with petroleum ether for 6 h in a Soxhlet apparatus to obtain the SCF fraction. Afterwards the DFDM, FAT and SCF fractions (after evaporating the solvent) were stored in an appropriate container in a vacuum desiccator until measurement.

2.3. Measurements by IRMS

Measurement of the $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{2}H/^{1}H$ and $^{18}O/^{16}O$ ratios of ham fractions was carried out as described by Perini, Camin, Bontempo, Rossmann, and Piasentier (2009). The values were expressed in δ‰ against international standards, calculated against working in-house standards and calibrated against international reference materials, as reported by the same authors. The δ^2H_{DFDM} values were corrected according to the "comparative equilibration technique" (Wassenaar & Hobson, 2003).

For the measurement of the $^{34}S/^{32}S$ ratios we used an elemental analyser (EA Flash 1112 ThermoFinnigan, Bremen, Germany) connected to an isotope ratio mass spectrometer (Delta plus XP mass spectrometer, ThermoFinnigan). The DFDM sample (~2.5 mg) was burned at 1000 °C in a quartz tube filled from the bottom with quartz wool (2 cm), elemental copper (14 cm), quartz wool (2 cm), copper oxide (5 cm) and quartz wool (1 cm). The water was removed using a glass trap filled with $Mg(ClO_4)_2$. The isotopic values were calculated against international reference materials: IAEA-SO-5 ($\delta^{34}S$ = +0.5‰) and NBS 127 ($\delta^{34}S$ = +20.3‰), through the creation of a linear equation.

The uncertainty (2σ) of measurements was <0.3‰ for the $\delta^{13}C$ and $\delta^{15}N$ analysis, and respectively <3‰, <0.6‰ and <0.8‰ for the δ^2H, $\delta^{18}O$ and $\delta^{34}S$ values.

2.4. Statistical analysis

The statistical analysis of data was performed using the SPSS Statistics version 17 for Windows (SPSS Inc., Chicago, IL, USA). The data for each stable isotope ratio were summarized as mean and standard deviation values. The effect of ham type on each stable isotope ratio was investigated using ANOVA, followed by the Sidak test for multiple comparison or, in the case of unequal variance (Levene's test) in the ham type samples, using Kruskal–Wallis's test, followed by Dunnett's T3 test for post hoc comparisons. The comparison between the ratios of the same isotope in different ham fractions (e.g. $\delta^{13}C_{DFDM}$ *vs.* $\delta^{13}C_{FAT}$ *vs.* $\delta^{13}C_{SCF}$) was performed by the GLM Repeated Measures procedure, after having verified the variance–covariance matrix sphericity by Mauchly's test. The post hoc multiple comparison tests were then performed to evaluate the significance of the pair differences across the levels of the within-subjects factor (e.g. $\Delta C_{SCF-FAT} = \delta^{13}C_{SCF} - \delta^{13}C_{FAT}$). The associate variance between pairs of isotope ratios was evaluated using the Pearson correlation coefficient, *r*.

Principal component analysis (PCA) was performed to describe dimensionality and explain the variability of the multiple data set comprising all the isotope ratios analyzed in ham fractions, as described in detail by Perini et al. (2009).

3. Results and discussion

3.1. Stable isotope ratios variability

Overall, 11 SIR data were examined in three ham fractions: defatted dry matter (DFDM) and fat (FAT) of *Biceps femoris* and subcutaneous adipose tissue (SCF). Their descriptive statistics are presented in Table 2, divided into four sub-tables, from (a) to (d).

3.1.1. Carbon

The mean $\delta^{13}C$ values of the various ham fractions (Table 2a) are consistent with those reported by González-Martin et al. (1999,

Table 1
Main characteristics of the examined ham types.

Ham type	Pig origin	Productive factor	Factor levels	No. of hams
1	Friuli	Pig genotype	Black local breed	24
2			White industrial hybrid	12
3	Extremadura	Feeding regime	Outdoor + supplement (Campo)	20
4			Outdoor (Bellota)	6
5	Emilia	Seasoning time	Mid (240 days)	12
6			End (405 days)	12