A tentative authentication of Parma dry cured ham using stable isotope ratio analyses

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Introduction

Food characterization is important for the food industry. An increasing number of consumers are concerned about the origin of food products as an indicator of quality. Today many consumers demand high quality products with a clear regional identity.

In 1992 EU legislation came into force providing a system for the protection of regional foods, through the PGI (Protected Geographic Indication) and PDO (Protected Designation of Origin) labels (European Union Regulation (EEU) 2081/92).

The aims of this legislation were: to support diversity in agricultural production, to protect consumers by giving them information on the specific characteristic of the product and to protect product names against fraud and imitation. To achieve this goal, food authorities can rely on paper traceability, and/or physical and chemical analyses.

Parma dry cured ham (PDCH) is a typical Italian product monitored under the Protected Designation of Origin (PDO, Commission regulation (EC) No 1107/96). Its distinctive properties of aroma and taste are due to the long maturing process (12 months).

Aim of the research

To evaluate the usefulness of stable isotope ratio analyses by IRMS, volatile compounds by SPME/GCMS and fatty acids profile for characterizing PDCH samples in order to distinguish them from other types of dry cured ham without designation of origin. SPME was applied to study the volatile compounds in PDCH.

Material and method

Dry cured ham samples

Four Parma dry cured ham (PDCH) samples ripened for one year with protected designation of origin were taken from slices of dry-cured ham supplied by SSICA (Stazione Sperimentale Conserve Alimentari)

Four samples of dry cured ham without denomination of origin were supplied by the dry cured ham industry.

In each slice, the semi-membranous muscle was selected for the study in order to minimize sample variability. All samples were kept frozen at -20°C until the day before analysis.

SPME extraction

Room temperature was selected as the extraction temperature, in order to prevent possible matrix alteration. At the end of the sample equilibration period (1 h), a conditioned (1.5 h at 280°C) 85 µm Carboxen™/polydimethylsiloxane (CAR/PDMS) StableFlex™ fiber (Supelco) was exposed to the headspace of the sample (8 g) for analyte extraction (3 h) by a CombiPAL system injector autosampler.

GC conditions

Analyses were performed with a Thermo Scientific TRACE GC Ultra equipped with a SolGel-WAX™ column (30 m x 0.25 mm x 0.25 µm), coupled to a quadrupole Thermo Scientific MS DSQ. Oven temperature program was: from 35°C, hold 8 min, to 60°C at 4°C/min, then from 60°C to 160°C at 6°C/min and finally from 160°C to 200°C at 20°C/min. Peak identification was based upon MS spectra in comparison with spectra and retention time of standards.

Fatty acid analysis

Total lipid extraction was carried out according to Bligh and Dyer.[5] The preparation of fatty acid methyl esters was carried out through acid-catalysed trans-esterification with methanolic hydrogen chloride (5%) according to Christie.[6] Fatty acid separation and identification were performed on a Thermo Scientific TRACE GC Ultra, fitted with a flame ionization detector (FID) and equipped with a BPX70 column (120 m x 0.25 mm x 0.2 µm). Fatty acids were identified relative to known internal standards.

Isotopic measurements, standards and equations $\delta^{13}C$ and $\delta^{15}N$ values were measured by continuous flow (ConFlo III) elemental analysis isotope ratio

mass spectrometry (CF-EA-IRMS) and $\delta^{18}O$ and $\delta^{2}H$ values were measured by continuous flow (ConFlo III) elemental analysis isotope ratio mass spectrometry (CF-TC/EA-IRMS) using the standards, equations and conditions as described in Moreno et al. [6,7]

Results and discussion

Stable isotope ratio analyses

For isotopic discrimination of samples we analyzed the carbon and nitrogen isotopic ratios of muscle because it integrates isotopic values of food assimilated over a long period (in agreement with some feeding specifications on Parma dry cured ham PDO).

The results showed a good discrimination among PDCH and other typologies based on $\delta^{13}C$ and $\delta^{15}N$.

Additionally we measured $\delta^{18}O$ and $\delta^{2}H$ and the values showed no overlapping and therefore a complete discrimination was achieved.

The explanation of these results is related to the geographical origin of the samples because oxygen and hydrogen have a strong influence on pedoclimatic conditions.

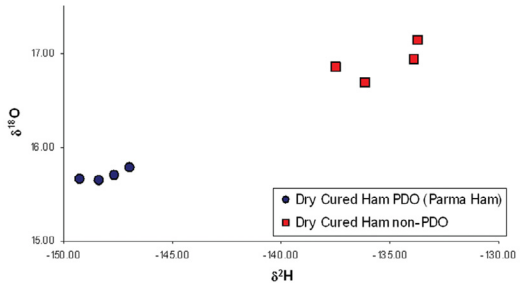

Figure 1. Analysis of $\delta^{18}O$ and $\delta^{2}H$

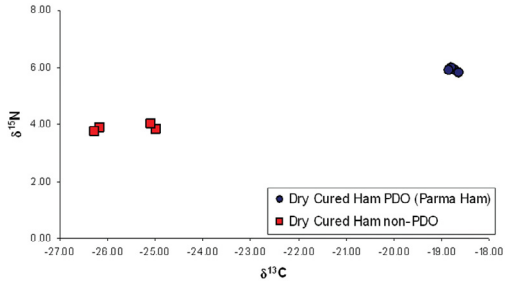

Figure 2. Analysis of $\delta^{15}N$ and $\delta^{13}C$

SPME development and sampling

Different SPME fiber coatings and extraction times were tested to study the volatile profile of dry cured ham samples in order to determine the most suitable condition for further analysis.

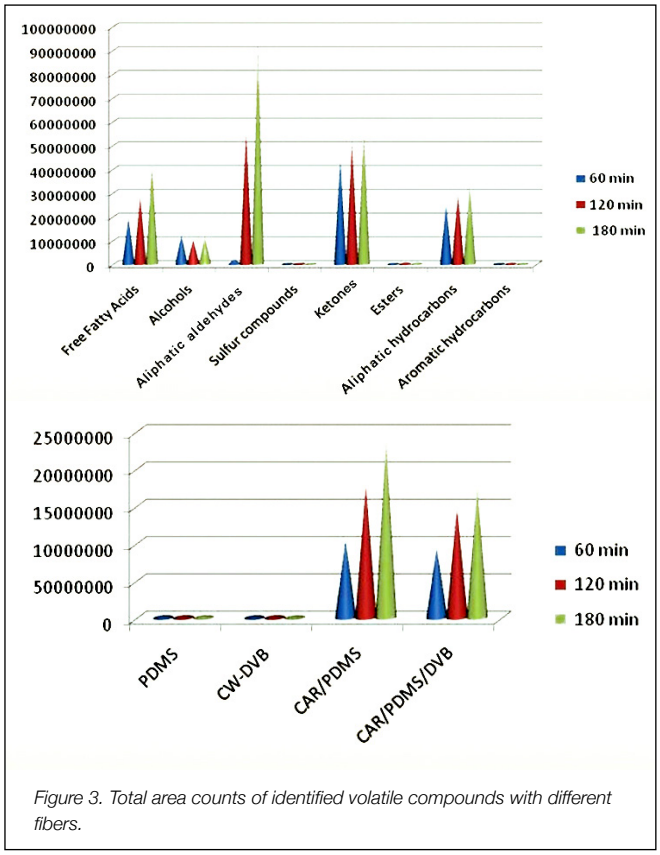

Figure 3. Total area counts of identified volatile compounds with different fibers.

Room temperature was selected as extraction temperature in order to prevent any artefact formation. The best results were obtained exttracting 180 min with CAR/PDMS (85 µm thickness) fiber.

Volatile compounds and fatty acids

89 volatile compounds were identify and quantified in dry cured ham samples. The data was clustered in five series of compounds in order to evaluate the differences between PDCH and ham without designation of origin.

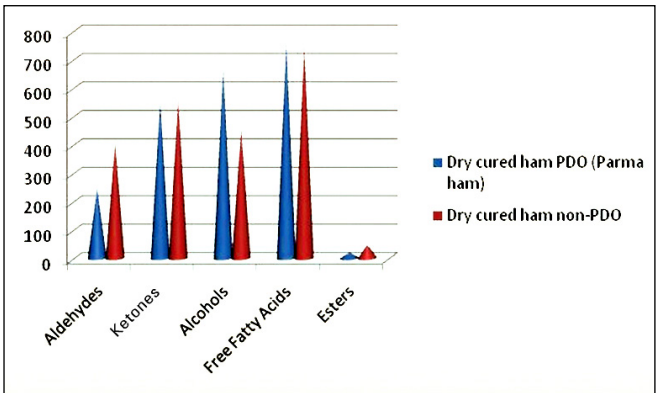

Figure 4. Volatile compounds classes extracted in PDHC samples and ham without denomination of origin (ng/g).

The total amount of aldehydes detected in muscle showed significant differences between the two groups studied. Higher concentrations of hexanal were found in dry cured ham without designation

of origin. This fact can be related to the presence of higher concentrations of unsaturated fatty acids in the diet. Hexanal is formed by the oxidation of either esterified or free linoleic acid and play an important role in the ham flavour because of its low odour threshold.

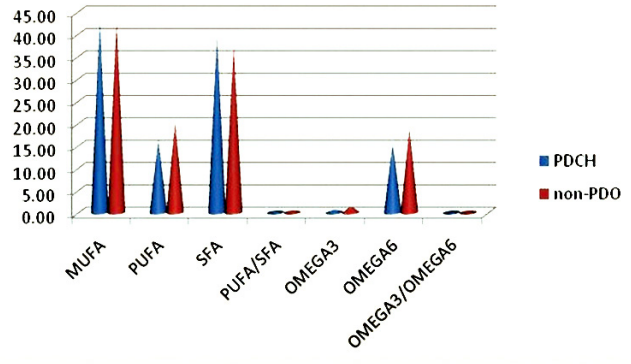

Figure 5. The fatty acid composition (% of methyl esters) of Parma dry cured ham samples and cured ham non PDO.

A meaningful difference was observed in PUFA concentration between PDO and non-PDO samples. This fact can be explained by the use of some oil in animal feed formulation. It is important to highlight that the PDO scheme only admits the use of cereal.

Conclusion

$\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{2}H$ analyses have been shown to enable discrimination among samples of muscle belonging to the PDCH and other origins.

IRMS proved to be a very effective and straightforward technique in combination with SPME-GC in the establishment of criteria to fight against common PDCH fraud.

Rel	Compounds	PDCH	SEM	non-PDO	SEM	SL
	Hydrocarbons					
MS,KI	pentane	106.87	17.73	89.72	41.28	*
MS,KI	2,2,4,6,6 pentamethyl heptane	10.65	3.84	0.00	0.00	N.S.
MS,KI	hydrocarbon	3.24	1.13	0.00	0.00	N.S.
MS,KI	toluene	4.23	0.80	17.74	3.57	*
MS,KI	2,3,3 trimethyl heptane	16.58	2.91	0.00	0.00	*
MS,KI	2,2,6 trimethyl octane	3.91	0.85	0.00	0.00	*
MS,KI	hydrocarbon	5.64	0.99	0.00	0.00	*
MS,KI	hydrocarbon	3.30	0.60	0.00	0.00	*
MS,KI	o-xylene	0.51	0.25	29.39	4.73	*
MS,KI	3,4 dimethyl nonane	0.31	0.12	0.00	0.00	N.S.
MS,KI	1,2 dichloro benzene	0.12	0.06	7.53	1.22	*
MS,KI	nitro benzene	0.00	0.00	2.12	0.66	*
	total	**155.36**		**146.49**		
	Aldehydes					
MS,KI	2-methyl butanal	17.61	1.83	10.12	0.93	*
MS,KI	3-methyl butanal	97.39	13.33	65.52	10.05	*
MS,KI	hexanal	120.38	18.22	277.91	56.06	*
MS,KI	2-hexenal	0.38	0.26	2.11	0.33	*
MS,KI	heptanal	1.21	0.58	6.32	1.61	*
MS,KI	octanal	0.00	0.00	13.03	1.94	*
MS,KI	trans-2-heptenal	2.68	0.39	2.99	0.53	N.S.
MS,KI	nonanal	4.77	0.89	28.01	4.88	*
MS,KI	2-octenal	0.11	0.04	0.30	0.20	N.S.
MS,KI	benzaldehyde	2.38	0.36	1.65	0.43	N.S.
	total	**246.91**		**407.95**		
	Ketones					
MS,KI	2-propanone	287.98	27.44	161.73	7.83	*
MS,KI	2-butanone	74.19	5.58	21.83	3.90	*
MS,KI	2-pentanone	121.58	10.22	224.92	20.93	*
MS,KI	2-heptanone	22.75	2.65	29.29	4.01	N.S.
MS,KI	3-hydroxy-2-butanone	28.74	4.06	105.16	11.08	*
MS,KI	2-octanone	3.24	0.61	4.24	1.37	N.S.
MS,KI	1-hydroxy-2-propanone	18.47	1.63	0.00	0.00	*
MS,KI	6-methyl-5-hepten-2-one	1.63	0.64	3.58	1.12	N.S.
MS,KI	2,3 octanedione	2.25	0.57	9.39	1.90	*
MS,KI	2-hydroy-3-pentanone	0.00	0.00	1.12	0.34	*
MS,KI	2-undecanone	0.16	0.07	0.00	0.00	N.S.
	total	**560.98**		**561.26**		
Rel	**Compounds**	**PDCH**	**SEM**	**non-PDO**	**SEM**	**SL**
	Alcohols					
MS,KI	2-propanol	0.00	0.00	18.54	1.65	*
MS,KI	ethanol	323.56	12.14	125.83	53.32	*
MS,KI	2-methyl-1-propanol	48.75	18.37	0.00	0.00	*
MS,KI	alcohol	17.71	9.75	0.00	0.00	N.S.
MS,KI	2-pentanol	17.96	2.74	7.32	0.58	*
MS,KI	1-butanol	8.58	0.83	13.83	1.57	*
MS,KI	1-penten-3-ol	38.21	3.31	61.94	3.40	*
MS,KI	3-methyl-1-butanol	138.97	28.02	50.19	8.42	*
MS,KI	alcohol	1.41	0.15	0.84	0.36	N.S.
MS,KI	3-methyl-3-buten-1-ol	0.61	0.24	0.00	0.00	N.S.
MS,KI	1-pentanol	42.95	3.45	123.50	17.51	*
MS,KI	2-methyl-3-buten-2-ol	0.77	0.44	1.31	0.18	N.S.
MS,KI	1-hexanol	18.12	2.92	28.68	4.42	*
MS,KI	2-butoxy ethanol	1.56	0.31	0.00	0.00	*
MS,KI	1-octen-3-ol	15.23	3.56	25.58	3.05	N.S.
MS,KI	benzeneethanol	0.41	0.21	0.00	0.00	N.S.
	total	**674.80**		**457.56**		
	Free Fatty Acids					
MS,KI	acetic acid	366.63	34.87	335.27	40.00	N.S.
MS,KI	propanoic acid	10.20	0.81	14.57	1.09	*
MS,KI	formic acid	0.60	0.09	0.44	0.07	N.S.
MS,KI	2-methyl propanoic acid	46.17	10.21	20.58	1.87	*
MS,KI	butanoic acid	108.87	13.60	118.72	17.41	N.S.
MS,KI	3-methyl butanoic acid	118.87	30.20	98.32	12.09	N.S.
MS,KI	pentanoic acid	14.61	1.95	17.59	1.63	N.S.
MS,KI	hexanoic acid	94.28	13.89	137.59	8.71	*
MS,KI	heptanoic acid	1.20	0.20	1.72	0.29	N.S.
MS,KI	octanoic acid	2.96	0.44	2.48	0.26	N.S.
MS,KI	nonanoic acid	0.88	0.21	0.00	0.00	*
MS,KI	decanoic acid	0.25	0.10	0.00	0.00	N.S.
	total	**765.52**		**747.28**		
	Esters					
MS,KI	acetic acid ethyl ester	12.13	0.74	44.70	9.52	*
MS,KI	3-methyl butanoic acid methyl ester	2.22	0.86	0.00	0.00	*
MS,KI	hexanoic acid methyl ester	3.53	0.54	2.91	0.91	N.S.
MS,KI	ester	1.69	0.64	0.00	0.00	N.S.
	total	**19.58**		**47.61**		
	Sulfur compounds					
MS,KI	dimethyl sulfide	21.50	6.28	0.00	0.00	*
MS,KI	dimethyl disulfide	3.58	0.66	4.47	0.71	N.S.
MS,KI	dimethyl sulfoxide	5.57	1.22	3.38	1.82	N.S.
MS,KI	dimethyl sulfone	0.86	0.34	0.79	0.36	N.S.
MS,KI	sulfur compound	0.01	0.01	0.00	0.00	N.S.
	total	**31.52**		**8.65**		
	Nitrogen compounds					
MS,KI	pyridine	0.00	0.00	3.39	1.27	*
MS,KI	nitrogen compound	0.00	0.00	1.78	0.25	*
MS,KI	2,6 dimethyl pyrazine	5.47	0.79	0.00	0.00	*
MS,KI	1-H pyrrole	0.92	0.04	1.01	0.30	N.S.
MS,KI	nitrogen compound	0.20	0.08	0.00	0.00	N.S.
MS,KI	acetamide	0.37	0.06	0.54	0.17	N.S.
MS,KI	formamide	1.28	0.17	0.45	0.10	*
	total	**8.25**		**7.17**		
	Lactones					
MS,KI	2-methyldihydro-3(2H)-Furanone	1.00	0.18	0.75	0.26	N.S.
MS,KI	5-methyldihydro-2(3H)-Furanone	6.97	0.86	6.46	0.45	N.S.
MS,KI	5-ethyldihydro-2(3H)-Furanone	3.87	0.53	6.02	0.34	*
MS,KI	5-propyldihydro2(3H)-Furanone	0.25	0.11	0.56	0.03	*
MS,KI	5-butyldihydro-2(3H)-Furanone	0.52	0.12	0.91	0.07	*
MS,KI	5-pentyldihydro-2(3H)-Furanone	0.00	0.00	0.96	0.22	*
	total	**12.61**		**15.65**		
	Terpenes					
MS,KI	D-limonene	8.10	4.49	2.61	0.34	*
	total	**8.10**		**2.61**		
	Furans					
MS,KI	2-pentyl furane	2.20	0.48	4.17	0.98	*
MS,KI	furan	0.00	0.00	0.17	0.10	*
MS,KI	furan	0.02	0.01	0.00	0.00	N.S.
	total	**2.22**		**4.34**		
	Miscellaneous					
MS,KI	phenol	0.30	0.04	0.34	0.03	N.S.
MS,KI	4-methyl phenol	0.36	0.04	0.68	0.15	*
	total	**0.66**		**1.02**		

Table 1. Volatile compounds extracted in PDHC samples and ham without denomination of origin (non-PDO) (ng/g).

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