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ASSESSMENT OF POSSIBLE USE OF CARBON ISOTOPE RATIOS IN AMINO ACIDS FOR MEAT PRODUCT GEOGRAPHICAL ORIGIN IDENTIFICATION

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SUMMARY

Possible use of compound-specific isotope analysis (GC-C-IRMS) was assessed using 255 meat samples from 24 countries. Upon results of isotope ratio ($\delta^{13}C$) determination in amino acids after gas chromatographic separation samples were classified using Support Vector Machines and linear discriminate analysis. Prognosis accuracy was 65–75%. Despite the fact that the use of compound analysis only provides low identification accuracy there's a possibility to considerably increase reliability of classification in combination with other data by means of extending dimension by the number of variables.

Key words: mass-spectrometry, identification, isotopic relations, GC-C-IRMS.

INRODUCTION

Information on the product origin is a feasible argument which influences the issue of food product export from some countries. It's hard to overestimate the importance of the tool providing reliable and objective information on product origin. As for economy and safety it enables control of import from countries with unfavorable ecological, sanitary and epidemiological situation upon which restrictions were imposed and countries under economic sanctions. One way or another, knowledge of the product origin ensures quality and safety of food products and finally influences the price and demand.

Different analytical methods widely used for food product origin identification have been currently developed. They are: isotopic ratio of light elements, element analysis, nuclear magnetic-resonance, chromatography profiling, near and mid infrared spectroscopy methods [2, 7].

Despite the fact that isotope-ratio mass spectrometry is a powerful method providing comprehensive information on geographic origin of the test object [5, 13], there are more publications on applying different combinations of specified above test methods. **GC-C-IRMS** method is not widely spread in laboratory practice. There are only several examples of its successful use for determining carbon isotope ratios in fatty acids [8]. This approach was also extended for determining $\delta^{13}N$ [4], $\delta^{18}O$, $\delta^{2}H$ [3], and $\delta^{34}S$ [1].

The purpose of this work was to assess the possibility of performing compound specific isotope analysis of amino acids by method based on gas chromatographic profiling followed by determination of carbon isotope ratios for meat geographic origin identification.

MATERIALS AND METHODS

Measurement tools. To determine isotope ratios $\delta^{13}C$ of derived volatile amino acids a platform (Fig.1, P. 7) was used, including gas chromatographer Trace GC Ultra (Termo Fisher, Germany) with an open split directing flows into mass spectrometer ISQ (Termo Fisher, Germany) and isotope ratio mass spectrometer Delta V Advantage (Termo Fisher, Germany) through GC Isolink interface (Termo Fisher, Germany) equipped with an oxidation reactor.

CONFLO IV interface (Termo Fisher, Germany) was used for automatic dissolution of gas products formed as a result of oxidation of separated amino acid derivatives in oxidation reactor and reference gas supply.

Gasses and reagents: liquid carbon dioxide, 99.8% – 10 l gas cylinder; gaseous helium 6.0, 99,9999% – 40 l gas cylinder; trifluoroacetic anhydride >99% (Panreac), methanol >99,9% (Sigma-Aldridch), dichloromethane >99,9% (Sigma).

Amino acid derivative formation

Hydrolysis. 10 mg sample of dehydrated and defatted homogenized meat and 0.5 ml of HCl 6M were added into a vial, then the vial was sealed with a fluoroplastic cover

and left in a drier for four hours at 150 °C. Then it was blown dry with nitrogen at 60 °C.

Etherification. After hydrolysis was over 500 µl of HCl 4M in methanol (CH_3OH) were added to the vial with solid residue. Then it was hermetically sealed with a fluoroplastic cover and left in a drier for 90 min at 110 °C. Then the solvent remains were blown dry with nitrogen at 40 °C.

Derivatization. After etherification was over 500 µl of CH_2Cl_2 and 40 µl of TFAA were added into the vial which was hermetically sealed with a fluoroplastic cover and left in a drier for 1 hour at 90 °C. Then it was blown dry with nitrogen at room temperature, 1ml of 10% ethylacetate solution in hexane was added, the vial was shaken and 200 µl were collected, dissolved in 400 µl of hexane and analyzed using chromatography.

The separation was performed using quartz capillary column DB-5 (Agilent, USA), length – 30 m and inner diameter - 0.25 mm (stationary phase film thickness – 0.25 µm). Separation and detection conditions are demonstrated in Table 1.

Software. Primary results were processed using ISODAT 3.0 software (Termo Electron, Germany). Statistical data analysis was performed using free software for statistical calculations– R version 3.1.2 using the following software packages: caret 6.0-41 [10], e1071 1.6-4 [11], ggplot2 1.0.0 [15], kernlab 0.9-19 [6], lattice 0.20-29 [14], MASS 7.3-35 [9], pROC 1.7.3 [12].

Table 1
Conditions of separation and detection of volatile amino acid derivatives

Parameters	GC-MS	IRMS
Injected volume	µl	-
Injection method	Without flow separation	-
Injector's temperature	230 °C	-
Column temperature mode	40–250 °C, rate 7.0 °C/min 250–280 °C, rate10.0 °C/min	-
Carrier gas flow rate	1 ml/min	-
Detector temperature	200 °C	-
Transfer-line temperature	250 °C	-
Ionization energy	70 eV	-
Oxidation reactor temperature	-	1030 °C

RESULTS AND DISCUSSION

Meat samples were collected in 2013-2014 by the Rosselkhoznadzor territorial administrations in veterinary and sanitary check points for quality and safety tests. 225 samples were collected from 7 types of meat from 24 countries (Table 2). Due to non-homogenity of the sampling pattern and a small number of samples for the covered territory currently the test results make no indication of the meat origin with an accessible confidence level but, herewith, they are quite sufficient for adequate and consistent assessment of applying compound-specific amino acid analysis for geographic origin identification.

Statistical processing. Carbon isotope ratios in amino acids were determined using compound-specific analysis GC-C-IRMS. In comparison to the conventional approach where the result of $\delta^{13}C$ determination is mean value of all complex matrix components GC-C-IRMS enables determining $\delta^{13}C$ value for a specific molecule of the substance, thus, taking into account finer fractionation processes that will be different for different types of organisms. 23 components of 13 amino acids were identified in a chromatography profile of the protein hydrolizate fractionation: asparagine (Asn), valine (Val), leucine (Leu), glycine (Gly), lysine (Lys), methionine (Met), proline (Pro), serine (Ser), tyrosine (Tyr), threonine (Thr), tryptophane (Trp), phenylalanine (Phe), cysteine (Cys).

Due to close chemical bond between amino acids there is clear correlation between the variables (Fig. 2, P. 8). To increase the classifier's resistance to incoming data PCA transformation and compression were applied. For selecting and comparing linear and non-linear classifications the following algorithms were used: support vector machine (SVM) with radial basis functions as the kernel function; linear discriminant analysis SVM (LDA) as the most popular linear classifier.

Prognosis accuracy for LDA and SVM was 65–75%. Despite the fact that accuracy of identification using compound-specific analysis only for geographical origin determination remains not high this result was obtained by determining just one criteria ($\delta^{13}C$) in the space of highly correlated variables (amino acid fractions). So, in combination with other data compound-specific analysis will enable to considerably increase reliability of the classification extending the dimension by the number of variables.

CONCLUSION

Despite quite a small sample size for the covered territory the prospects of amino-acid compound-specific analysis followed by carbon isotope ratios determination as an additional tool to basic approaches used for meat product origin identification were demonstrated.

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Table 2
Test sample

Country	Code*	Meat type							Number of samples per country
		Pork	Beef	Chicken	Goose	Turkey	Horse meat	Mutton	
Argentina	ARG	0	0	0	0	0	5	0	5
Austria	AUT	5	2	0	0	0	0	0	7
Belgium	BEL	5	0	0	0	0	0	0	5
Belarus	BLR	13	1	0	0	0	0	0	14
Brazil	BRA	9	34	8	0	1	0	0	52
Canada	CAN	2	0	0	0	0	0	0	2
Denmark	DNK	6	4	0	0	0	0	0	10
Spain	ESP	1	6	0	0	0	0	0	7
Finland	FIN	0	0	3	0	0	0	0	3
France	FRA	4	0	0	0	0	0	0	4
Germany	DEU	11	0	0	0	0	0	0	11
Hungary	HUN	0	0	0	2	0	0	1	3
Italy	ITA	0	1	0	0	0	0	0	1
Lithuania	LTU	0	7	0	0	0	0	0	7
Moldova	MDA	0	0	0	0	0	1	1	2
Mexico	MEX	0	4	0	0	0	0	0	4
Netherlands	NLD	1	0	0	0	0	0	0	1
New Zealand	NZL	0	0	0	0	0	0	4	4
Poland	POL	0	6	0	0	0	0	0	6
Paraguay	PRY	0	1	0	0	0	0	0	1
Russia	RUS	0	11	6	0	0	0	18	35
Ukraine	UKR	7	46	3	0	0	0	0	56
Uruguay	URY	0	1	0	0	0	0	1	2
USA	USA	0	6	7	0	0	0	0	13
The number of samples by type of meat		64	130	27	2	1	6	25	255

* According to ISO 3166-1.

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