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STUDY ON THE MEAT ISOTOPIC COMPOSITION FOR ORIGIN IDENTIFICATION

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ABSTRACT

Russian consumer and governmental authorities are equally concerned to know where food products come from. This requires more accurate and specialized methods for the evaluation of geographical location. The following methods are used: chemometrics, histological and histochemical, genomic and proteomic, microbiological, immunochemical and mass spectrometric. Method of stable isotope analysis is becoming increasingly promising nowadays for the identification of meat and meat products‘ place of origin. The isotope ratios of the four elements - carbon, nitrogen, oxygen and hydrogen, are mainly determined. The method is successfully used to identify a country of origin of wines, juices and water. The aim of the research was to study the stable isotope ratios for pork and beef samples purchased in Moscow supermarkets (Russian Federation). The country of production of meat samples was determined according to specifications and/or labels. The geography of countries of meat samples origin includes Europe, both America continents and Australia. Databases collected by the All-Russian Scientific Research Institute of the Brewing, Non-Alcoholic and Wine Industrywere used for the analysis and interpretation of the results. Values of $^{13}C/^{12}C$, $\delta^{13}C$, $^{18}O/^{16}O$, $\delta^{18}O$, $^{2}H/^{1}H$, $\delta^{2}H$ for 30 pork and beef samples from 13 countries were obtained. Differences in stable isotope ratios were found depending on place of origin. The data correlated with the oxygen isotope characteristics for wine, which were in the range from 2.5 to 4.5 ppm. According to the $^{13}C/^{12}C$, $\delta^{13}C$ results, the assumption was made about a false indication of the region for the beef sample. Despite the fact that beef was labeled as a product of Lithuania, the region of origin was most probably defined as Germany. The studies carried out showed the possibility to identify the region of raw meat origin by the stable isotope ratio.

Keywords: region of origin; food; authenticity; stableisotope ratio; meat

INTRODUCTION

Nowadays, intentional fraud and falsificationof foods cause concerns of the governments in many countries of the world as they can create a threat to the health safety of the population and lead to a certain economic losses. To undertake effective corrective actions against food fraud, precise methods of analysis that ensure appropriate repeatability and reproducibility of the research results are necessary.

Many countries have legislative requirements that oblige manufacturers to indicate a region or country of food origin on a label or in accompanying documents. The information about the country of origin of a product or raw materials can be especially important in case of severe restrictions associated with safety, for example, in disease outbreaks or when producing products of protected designation of origin (PDO). Modern analytical physico-chemical and microbiological methods of investigation allow establishing food origin with a certain precision. As a rule, these methods are directed towards revealing a relationship between the analyzed samples and the indicators that characterize one or another region. These indicators include the quantity or ratio of micro-, macro-elements, heavy metals, radioelements, stable isotopes, comparison of DNA **(Lo and Shaw, 2018; Kawaguchi et al., 2018)**, microflora of a sample **(Nguen et al., 2008)** and a reference. The differences in the content of ^{90}Sr in cheeses produced in different European countries were found. Radioisotope analysis allows establishing the $^{234}U/^{238}U$ ratio, which can be used in origin identification **(Peres et al. 2007)**. The geological and technogenic factors affect distribution of toxic, micro- and macro-elements. The difference between green tea leaves from China, India and Japan was found **(Brzezicha-Cirocka, Grembecka and Szefer, 2016)**. The similar research was performed earlier on fresh fruit from Europe, Asia, Africa and America regarding the content of Ca, Mg, K, Na, P, Co, Mn, Fe, Cr, Ni, Zn, Cu **(Grembecka and Szefer, 2013)**.

As for animal-derived products, it should be noted that the microelement content in animal meat depends on different factors, such as feed consumption, drinking water, soil polution and composition, which, in turn, depends on the geographical origin **(Ballin, 2010)**. The content of different elements was determined in the poultry meat and dried beef samples **(Franke et al., 2008)**, milk and cheese **(Osorioet al., 2015)**.

Tretyakov et al. (2012) found it possible to use the fingerprinting technique based on the data of mass-spectrometric analysis to identify geographical origin falsification for food products such as meat and red caviar as the ratio of macro- and micro-components is unique.

The main elemental constituents (H, C, N, O и S) of bio-organic material have different stable isotopes (^{2}H, ^{1}H, ^{13}C, ^{12}C, ^{15}N, ^{14}N, ^{18}O, ^{17}O, ^{16}O, ^{36}S, ^{34}S, ^{33}S,^{32}S and others). The isotope ratio can be measured precisely using the isotope ratio mass spectrometry (IRMS) **(Camin et al., 2016)**. The directions of analysis of the stable isotope composition used for foods can be the following:

- isotope ratio for one element in more than one substance (fraction) of a product (intermolecular isotope correlation);
- analysis of the prevalence of isotopes of one element in a molecule (intramolecular isotopic heterogeneity **(Zyakun, 2015)**;
- determination of the isotope ratios for more than one element in a certain substance (multi-element stable isotope analysis);
- combination of theabove-mentionedmethods **(Rossmann, 2001)**.

Schellenberg et al. (2010) applied IRMS to determine the geographical origin of honey based on the stable isotope ratios of carbon, nitrogen, hydrogen and sulphur; potato, for which it was established that δ^{18}O was the most discriminating variable in three geographical groups of samples **(Longobardi et al., 2011)**; sesame seeds and sesame oil **(Horacek, et al., 2015)**, butter **(Rossmann et al., 2000)**, seafood **(Ortea and Gallardo, 2015)**.

The investigations of the isotopic composition of milk were carried out with the aim to identify and reveal variations by the region of origin **(Camin et al., 2016 Brescia et al., 2005)**. It was concluded that milk from the regions, where pastures prevailed, usually showed relatively negative δ^{13}C values, while in the regions where agriculture dominated, the δ^{13}C values were more positive; the δ^{15}N values, as was noted earlier, depend on the soil conditions, intensity of the agricultural use and climate **(Kornexl, Werner, Roßmann, and Schmidt, 1997)**. In the milk samples from Australia, the high content of ^{18}O and ^{34}S was found compared to the known values of dairy products from the European countries **(Crittendenet al., 2007)**.

Analysis of the stable isotopes of light elements is actively used to control quality of grapes and wines **(Rossmann, 2001; Christoph N., Rossmann A., Schlicht C., and Voerkelius, 2006; Zyakun, 2013)**, as well as juices. As a result of the investigations, **Rummelet al. (2010)** differentiated the orange production regions depending on the geographical, climatic and lithological differences.

The aim of this study was to test the method for determination of the C, O and H stable isotopes, as well as their ratios in raw meat and to detect the prospects of its use for identification of regional origin.

Scientific hypothesis

Geographical, climatic, botanical, zoological and zootechnical factors influence the ratio of stable isotopes, which in turn are incorporated into the animal tissue via eating, drinking, breathing and exchange with the environment. A possibility to ascertain a region of origin by the stable isotopic composition can become a reliable hurdle for falsifications in the conditions of global trade.

MATERIAL AND METHODOLOGY

Thirty pork and beef samples originated from 13 countries were purchased in Moscow supermarkets irrespective of the muscle type based on the data of **Harrison et al. (2010)**, who demonstrated that the carbon isotope ratio in different muscles of a carcass differs insignificantly.

The country of origin was determined by the label. Databases of water and wine isotopes collected at theAll-Russian Scientific Research Institute of the Brewing, Non-Alcoholic and Wine Industry were used to correlate the results obtained.

To determine the carbon isotope ratio, the meat sample was weighed, put into the aluminum capsule for combustion in the elemental analyzer at 1000 °C with following purification. Then CO_2 enters the isotope ratio mass spectrometer via the feeding devices, where the analysis of the isotopic composition takes place.

To determine the oxygen and hydrogen isotope ratios, the meat sample was homogenized; the aqueous fraction was separated and filtrated. The obtained sample of the meat aqueous fraction was transferred to the vial, which was placed into the GasBench II sample preparation device to carry out isotopic equilibration and the following measurement of the isotopic characteristics of oxygen and hydrogen of the aqueous component.

The tool base for getting data on the isotopic composition characteristics was a mass spectrometric complex Delta V Advantage of Thermo Fisher Scientific company (USA), providing precise analysis of the prevalence ratio. The isotopic characteristics were measured and compared against the international sample V-PDB.

Method is based on the determination of stable carbon isotope composition in the analyzed sample as compared to the international VPDB standard ($\delta^{13}C_{VPDB}$), by mass spectrometry.

$\delta^{13}C_{VPDB}$ value was calculated by the formula:

$$\delta^{13}C_{VPDB} = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample} - \left(\frac{^{13}C}{^{12}C}\right)_{VPDB}}{\left(\frac{^{13}C}{^{12}C}\right)_{VPDB}} \cdot 1000,$$

where $\left(\frac{^{13}C}{^{12}C}\right)_{VPDB}$ – carbon isotope ratio in the international standard sample, ‰;

$\left(\frac{^{13}C}{^{12}C}\right)_{sample}$ - carbon isotope ratio in the a test sample, ‰.

Stable oxygen and hydrogen isotope ratios, $\delta^{18}O_{VSMOW2}$ and δD_{VSMOW2}, were determined using the research complex of Delta V mass-spectrometer, sampling system GasBench II, and computer system with Isodat 3.0. programme. Method is based on oxygen isotope balance in the aqueous component of the analyzed sample and oxygen in CO_2 within CO_2- He.

$\delta^{18}O_{VSMOW2}$ value was calculated by the formula

$$\delta^{18}O_{VSMOW2} = \frac{\left(\frac{^{18}O}{^{16}O}\right)_{sample} - \left(\frac{^{18}O}{^{16}O}\right)_{VSMOW2}}{\left(\frac{^{18}O}{^{16}O}\right)_{VSMOW2}} \cdot 1000,$$

where $\left(\frac{^{18}O}{^{16}O}\right)_{VSMOW\,2}$ - oxygen isotope ratio in the international standard sample, ‰;

$\left(\frac{^{18}O}{^{16}O}\right)_{sample}$ - oxygen isotope ratio in a test sample, ‰.

Method is based on hydrogen isotope balance in the aqueous component of the analyzed sample and hydrogen within H_2- He.

δD_{VSMOW2} value was calculated by the formula:

$$\delta D_{VSMOW2} = \frac{\left(\frac{^{2}H}{^{1}H}\right)_{sample} - \left(\frac{^{2}H}{^{1}H}\right)_{VSMOW2}}{\left(\frac{^{2}H}{^{1}H}\right)_{VSMOW2}} \cdot 1000,$$

where $\left(\frac{^{2}H}{^{1}H}\right)_{VSMOW2}$ - hydrogen isotope ratio in the international standard sample, ‰;

$\left(\frac{^{2}H}{^{1}H}\right)_{sample}$ - hydrogen isotope ratio in a test sample, ‰.

International IAEA standards,VSMOW2; SLAP2; GISP were used as reference.

Statisic analysis

Statistical analyses results are expressed as mean of triplicate trials. Data were analyzed by one - way analysis of variance (ANOVA) on the means of values (p <0.05). STATISTICA 10.0 software was used in this study for the statistical analyses.

RESULTS AND DISCUSSION

Natural and technogenic particular qualities cause existing differences in the isotope content in geographical areas. Today in the world practice, stable isotope ratios of four elements - carbon, nitrogen, oxygen and hydrogen, in meat and meat products are used to identify a place where an animal was raised. Carbon and nitrogen isotope characteristics are used to determine the feed diet, and oxygen and hydrogen isotopes - to determine the geographical location of a product **(Rossmann, 2001; Kelly, Heaton and Hoogewerff, 2005; Camin et al., 2007)**.

Multi-element (C, O, H) stable isotope ratio analysis proved **(Table 1)** that pork and beef from the same geographical region showed different $\delta^{13}C$ values. For example, meat samples from the same species, but from different countries of production, differ in $\delta^{13}C$ value. Pork the the farms in the European part of Russia, regardless of the breeding type, shows close $\delta^{13}C$ value (2), which reflect close breeding and feeding conditions. The data obtained correspond with those of **Schmidt et al. (2005)** and **Camin et al. (2016)**.

Table 1 Beef and pork $\delta^{13}C$ values.

Meat	Country of origin (as in the label)	$^{13}C/^{12}C$, $\delta^{13}C$, ‰ (‰ ±SD)
Pork, sample 1	Russia	(-23.17) ±0.1
Pork, sample 2	Russia	(-23.16) ±0.1
Pork, sample 3	Russia	(-23.17) ±0.1
Pork, sample 4	Russia	(-23.15) ±0.05
Pork, sample 5	Canada	(-18.67) ±0.05
Pork, sample 6	Denmark	(-26.18) ±0.1
Pork, sample 7	Brasil	(-18.18) ±0.1
Pork, sample 8	Spain	(-21.40) ±0.1
Pork, sample 9	Czech Republic	(-24.14) ±0.1
Beef, sample 1	Lithuania	(-16.57) ±0.05
Beef, sample 2	Lithuania	(-19.58) ±0.1
Beef, sample 3	Lithuania	(3) (-26.33) ±0.1
Beef, sample 4	Germany	(-26.52) ±0.1
Beef, sample 5	Germany	(-26.21) ±0.05
Beef, sample 6	Mexico	(-16.47) ±0.1
Beef, sample 7	Russia	(-17.60) ±0.1
Beef, sample 8	USA	(-10.96) ±0.1
Beef, sample 8	Czech Republic	(-19.00) ±0.05

Table 2 Stable biophilic isotope (carbon, oxygen, hydrogen) ratios in beef and water samples from different geographical areas.

Meat (Country of origin)	$^{13}C/^{12}C$, $\delta^{13}C$, ‰	$^{18}O/^{16}O$, $\delta^{18}O$, ‰	$^{2}H/^{1}H$, $\delta^{2}H$, ‰
Beef (Italy)	(-17.26)	(-7.80)	(-47.73)
Beef (Brazil)	(-10.90)	0.94	23.4
Beef (Nicaragua)	(-15.63)	(-2.51)	(-4.94)
Beef (Australia)	(- 22.68)	2.63	13.71
Beef (Czech Republic)	(-19.00)	(-5.78)	(-32.75)

In the cow diet in Latin America **(Table 2)**, C4-type plantspredominate – maize and sugarcane. Jahren et al. **(2008)** described similar correlations: $\delta^{13}C$ ranges for corn (C4-plant) from -14 to -11‰. Alfa-alfa, wheat, sunflower (C3–plants) give $\delta^{13}C$ ranges from -28 to -22‰. The statistical processing of the results presented in **Table 2** shows that the results were significantly different (p <0.05) by all three groups of stable isotope ratios.

Carbon isotope ratioin beef from Australia and Russia indicate the predominance of C3-type plants in feeds. Plants of C3 type are, for example, most small seeded cereal crops (wheat, barley, rye), most trees and lawn grasses and also oat; soybean, sugar beets, potato and other plants widely used to feed animals. While in Italy and the Czech Republic apparently mixed feeds which contain plants both C3 and C4 types are mainly used. For example, if to compare data for beef and pork from the Czech Republic, the differences in $\delta^{13}C$ are evident: -19.00‰ for beef and -24.14‰ for pork. But if to compare water stable isotope ratio $^{18}O/^{16}O$, $\delta^{18}O$, no difference is noted. The ratio is in the range (-5.70… – 5.78)‰ for both types of meat. Cows and pigs in this country have different feeds but drink the same water. Earlier, one of the authors showed that the oxygen isotope composition of the Czech natural waters falls within the range from -5.5 to -6.5 per mil **(Zyakun et al., 2013, 2015).**

The results of our research correspond to those of **Horacek and Min (2010)** and **Nakashita et al. (2008)** who showed similar results while comparing the beef carbon, nitrogen and hydrogen isotope ratios from South Korea, USA, Mexico, Australia and New Zealand and showed the main differences in the $\delta^{13}C$ and $\delta^{2}H$ values which reflect the plant and water regional specificity.

The most "heavy" in the isotope ratio appeared to be the values for meat which came from Australia.T hese data are correlated with the data on the water component of Australian wine. The oxygen value in meat, $\delta^{18}O$, was 2.63 ‰ and in wine, the isotope characteristics of oxygen were in the range from 2.5 to 4.5‰. Similar data of significant differences between countries in the $\delta^{18}O$ values were obtained by **Franke et al. (2008).**

Interestingly, according to the label, beef 1, 2 and 3 were beef samples originated from Lithuania. But the differences between samples 1,2 and beef sample 3 were significant (p <0.01). The carbon isotope content of beef 3 ((-26.33) ±0.1‰) was much closer to those of beef 4 and 5, which labeled as coming from Germany. The differences between beef sample 3 from Lithuania and beef samples 4, 5 from Germany were not significant. For pork from the European part of Russia $\delta^{13}C$ values are about -23,1‰. The differences in the results obtained show a possible misinformation in the lable of beef tested. Further tests should be done and a database reflecting the isotope characteristics of the regions should be developed and constantly updated.

CONCLUSION

Stable isotope ratio analysis was tested for its suitability as a means for geographical location assignment for beef and pork and have shown significant correlation. **Camin et al. (2016)** defined possibility to identify the place of origin for poultry, milk, butter, cheese, fish, and shellfish via isotope ratios of bioelements.

It is necessary to emphasize the importance of the study on the stable isotopes of oxygen, hydrogen and carbon in conjunction, which is justified by the peculiarities of the animal diet and the isotopic composition of local drinking water. The obtained results were positive and indicated that the comparison of the stable isotope ratios of carbon, hydrogen and oxygen is applicable as a potential tool for identifying the origin of meat, produced not only in different countries, but in different regions of Russia as well.

It is evident that for the practical application of this method, it is necessary to create a database on the content of the stable isotopes in different agricultural raw materials and water by world regions. When this database is available, the method can be used to prevent regional fraudulence in the food industry. The existence of an effective method for determining the region of origin for raw meat will fight unscrupulous companies, reduce the likelihood of the implementation of hazardous factors and maintain the system to protect brand and regional labels.

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