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A study of stable isotope composition of chosen foodstuffs from the Polish market

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Abstract. In the present work the carbon and nitrogen isotopic composition of food products bought in the retail trade in Warsaw is demonstrated. The research was carried out using meat (pork, chicken), hen eggs and honey. These products originated from the conventional and ecological farms. The values of the isotopic ratios are expressed as δ notation and correspond to the international standards (V-PDB for $\delta^{13}C$, and air for $\delta^{15}N$) according to the following general formula: $\delta(‰) = [(R \text{ sample} / R \text{ standard}) - 1] \times 1000$, where R represents the ratio between the less abundant and more abundant isotopes, in particular $^{13}C/^{12}C$ and $^{15}N/^{14}N$. The results received in our laboratory, were compared with results presented in the literature for similar products. The results of the study show that the N and C stable isotope ratios in the tested products can be applied to verify their authenticity.

Key words: stable isotope • honey • meat • hen eggs • food authenticity

Introduction

The isotope ratio mass spectrometry (IRMS) methods play a very important role in food authenticity and origin control. The geographical authenticity of food products determination is necessary to protect consumers from fake products. Stable isotope analysis for the control of declared origin is already being routinely applied by the European Union (EU) countries for fruit juice, wine and honey [4, 8, 9, 11–14, 17–19]. The potential of origin and authenticity verification for other foodstuffs like milk and milk products, olive oil and meat (pork, beef, lamb) is also demonstrated [1–3, 7, 10]. The paper includes the measurements of the carbon and nitrogen isotopic composition of food samples derived from Warsaw retail trade. Elaboration of the isotopic methods for foodstuffs authenticity and origin control was the aim of the study.

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Materials and methods

The samples origin

The research was performed using hen eggs, meat (pork, chicken) and honey. The samples of products originated from the conventional and ecological farms and were derived from Warsaw retail trade according to labelling.

On the egg shells, in pursuance of the EU regulations [5] within the range of requirements for qualities and markings, should be placed the following information – the code of the producer which consists of: a) the code of the system of hens breeding: 0 – organic laying hens, 1 – free range, 2 – barn, 3 – caged; b) the code of the state (e.g. PL for Poland); c) the veterinary number of identification (8 numbers). The meat products from ecological farms are labelled by specific marks [6]. The collected meat samples were stored at –20°C until their preparation for analysis. The investigated honeys were produced in apiarian farms situated in Mazury – a geographical region of Poland.

Preparation of samples

The meat samples (5 g) were sliced into thin pieces packed into a special glass tube and freeze-dried for 24 h using our own construction vacuum installation. Fat from the dehydrated muscles was removed in a Soxhlet apparatus using petroleum ether [15]. Then, the defatted material was milled and packed into plastic containers and stored at room temperature. A similar procedure for removal of water from both components of egg (yolk and albumen) was applied. 5 g samples of yolk or albumen were packed into a glass tube and freeze-dried for 24 h using a vacuum installation. The dehydrated materials were milled and transferred into plastic containers and stored at room temperature until measurements. Proteins from honey were isolated according to the AOAC Official Method 998.12 and our own procedure [19]. Briefly, a 10–12 g sample of honey was placed in a 50 ml centrifuge tube with addition of 4 ml of distilled water and mixed. In another tube about 2 ml of 10% sodium tungstate solution was mixed thoroughly with 2 ml of 0.7 N sulphuric acid. The solution was added to the centrifuge tube and mixed with the solution containing the sample of honey. The tube was shaking in a water bath at 80°C until visible flocks were formed (3–4 min). If no visible flocks forms, or if the supernatant remains cloudy 0.7 N acid in 2 ml increments was added and heating was repeated. Then, the sample was centrifuged at 1500× g per 5 min and the supernatant was removed. The received sediment was washed using 50 ml of distilled water, mixed and centrifuged. The washing procedure was repeated at least five times, until the supernatant was clear. The precipitated protein was dried in an oven at 60°C over 5 h and transferred to a small tube before analysis.

Measurements of isotopic composition

For every six samples (1.0 to 2.0 mg each) of the tested materials, two reference commercial standards for $\delta^{15}N$ and $\delta^{13}C$ determination (B 2155: $\delta^{15}N$ – 4.5 ± 0.1, $\delta^{13}C$ – 26.2 ± 0.1; USGS 40: $\delta^{15}N$ 5.94 ± 0.08, $\delta^{13}C$ – 26.98 ± 0.13) were used. The samples and standards were weighed in tin capsules for further measurement. The C and N isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) was performed by an elemental analyzer (Elemental Analyzer Flash 1112 NCS – Thermo Finnegan, Italy) which is interfaced to a DELTA^{plus} isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany). In one measurement the values of carbon and nitrogen are simultaneously obtained. The standard deviations of the obtained results were 0.3‰ and 0.2‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. The values of the isotopic ratios correspond to the international standards (V-PDB for $\delta^{13}C$ and air for $\delta^{15}N$) and are calculated according to the formula:

– for carbon

$$\delta^{13}C_{vsPDB} = \frac{\left[\frac{^{13}C}{^{12}C}\right]_{SAMPLE} - \left[\frac{^{13}C}{^{12}C}\right]_{STANDARD}}{\left[\frac{^{13}C}{^{12}C}\right]_{STANDARD}} * 1000‰$$

and

– for nitrogen

$$\delta^{15}N_{vsAIR} = \frac{\left[\frac{^{15}N}{^{14}N}\right]_{SAMPLE} - \left[\frac{^{15}N}{^{14}N}\right]_{STANDARD}}{\left[\frac{^{15}N}{^{14}N}\right]_{STANDARD}} * 1000‰$$

Results and discussion

Hen's eggs

The eggs were derived from different laying regimens: caged, barn, free range and organic farms. Carbon and nitrogen isotope values of egg components (yolk and albumen) were examined. The obtained values for tested eggs are shown in Table 1 and Figs. 1a and 1b.

Egg yolks from caged and barn hens have $\delta^{15}N$ values in the range of 4.37‰ and 5.02‰, and $\delta^{13}C$ values in

Table 1. $\delta^{13}C$ and $\delta^{15}N$ of egg components (yolk and albumen) from various commercial laying regimens

Laying regimen	Yolk		Albumen	
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Organic	–22.64	6.83	–20.98	5.52
Organic	–22.67	6.02	–21.46	4.86
Free range	–25.62	5.41	–23.95	4.06
Free range	–26.33	5.19	–23.61	4.38
Barn	–28.55	5.02	–24.96	4.09
Cage	–26.94	4.51	–24.09	3.68
Cage	–26.78	4.37	–24.06	3.74
Cage	–22.89	4.80	–20.72	3.59
Cage	–23.28	4.78	–20.82	3.90

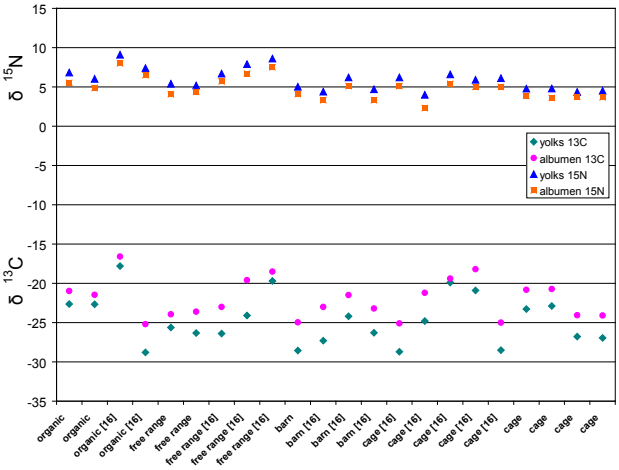

Fig. 1a. Isotopic ratio of carbon $^{13}C/^{12}C$ and nitrogen $^{15}N/^{14}N$ of the egg components (yolk and albumen) for various laying regimens. Data from the literature [16] and obtained at the Stable Isotope Laboratory (INCT).

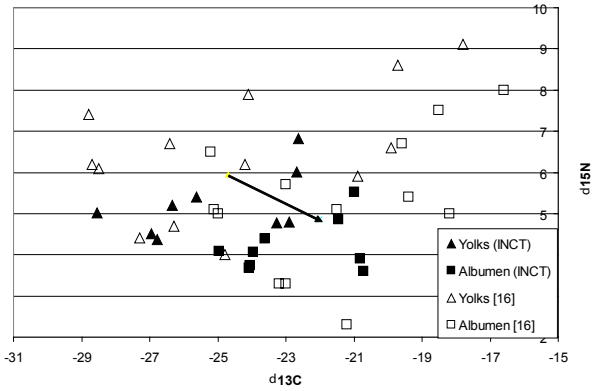

Fig. 1b. Isotopic ratio of carbon $\delta^{13}C/^{12}C$ vs. nitrogen $\delta^{15}N/^{14}N$ of the egg components (yolk and albumen). Data from literature [16] and obtained at the Stable Isotope Laboratory (INCT). Arrow represents a mean isotopic shift between yolk and albumen.

the range of –28.55‰ and –22.89‰. Egg albumens from caged and barn hens have $\delta^{15}N$ values in the range of 3.59‰ and 4.09 ‰ and $\delta^{13}C$ values in the range of –24.96‰ and –20.72‰. Egg yolks from free range and organic eggs have $\delta^{15}N$ values between 5.19‰ and 6.83‰ and $\delta^{13}C$ values between –26.33‰ and –22.67‰. Egg albumens from free range and organic hens have $\delta^{15}N$ values between 4.06‰ and 5.52‰ and $\delta^{13}C$ values between –23.95‰ and –20.98‰. The results from this study suggest that is not possible to separate the egg farming regimens using $\delta^{13}C$ values of the egg components: yolk and albumen. Isotopic drift for carbon and nitrogen between albumen and yolk is observed as is shown in Fig. 1b. The $\delta^{15}N$ values of both egg components show opportunities as an indicator to differentiate eggs for various laying regimens. The results obtained in this study are similar to the results presented by Rogers [16] for eggs produced in New Zealand (Fig. 1a). Rossmann [17] presents carbon and nitrogen isotopic ratios of chicken eggs from commercial farms in Germany, Italy, the United Kingdom and some smaller farms in Bavaria. The study shows that most eggs from small farms in Bavaria have high $\delta^{15}N$ values (from 6 to 11‰), typical of protein sourced from higher trophic levels, in contrast to larger commercial farms, which have lower $\delta^{15}N$ values.

Meat

The use of the isotopic analysis to characterization and estimation of authenticity of meat from different animals (Irish beef, lamb from Spain, France, Iceland, Italy, Greece, Iberian pork), depending on the diet of animals, weather condition and country of origin is described in the literature [1, 7, 10]. The isotopic characterization of carbon and nitrogen in the domestic meat (conventional and organic production) are presented in the paper. The samples of joint of pork and chicken breast were tested. The results are shown in Table 2 and Fig. 2.

The small difference in the $\delta^{13}C$ value of examined products depending on the production system (conventional, organic) was observed (0.7 ± 0.25 for chicken meat and 0.9 ± 0.11 for pork meat) after analysis of

Table 2. Isotopic composition of carbon and nitrogen for conventional and organic meat samples

The description of samples	Not defatted samples		Defatted samples	
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Conventional pork	–24.59	3.32	–24.17	3.46
	–24.58	3.40	–24.19	3.38
	–24.56	3.29	–24.05	3.44
	–24.48	3.36	–24.06	3.36
	–24.47	3.51	–24.20	3.47
	–24.38	3.96	–24.11	4.12
Organic pork	–23.56	4.87	–23.32	4.81
	–23.80	4.66	–23.34	4.77
	–23.54	4.86	–23.19	4.99
Conventional chicken	–22.77	3.17	–22.52	3.37
	–23.13	2.33	–23.23	2.21
	–23.16	2.34	–23.15	2.18
Organic chicken	–22.63	2.69	–22.54	2.74
	–22.05	2.62	–21.97	2.27
	–22.25	2.36	–22.14	2.45

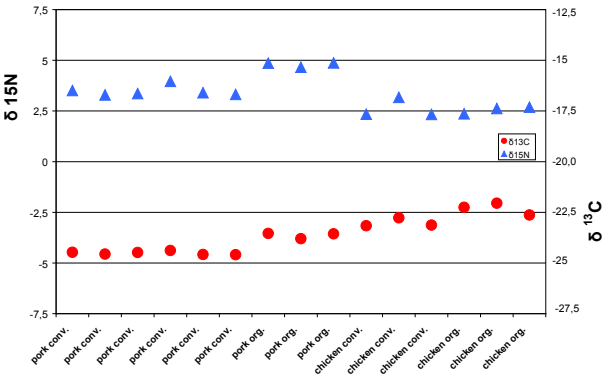

Fig. 2. Isotopic composition of nitrogen and carbon for organic and conventional meat samples.

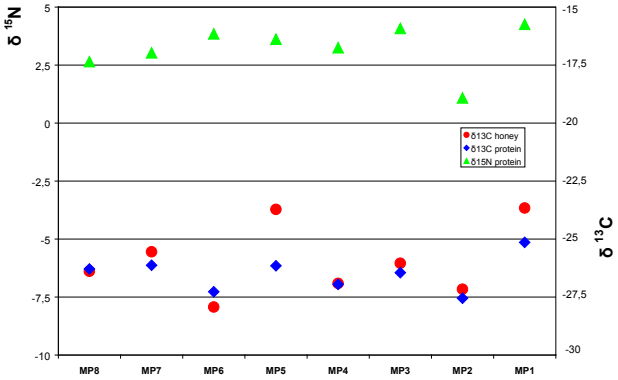

Fig. 3. The carbon and nitrogen stable isotopes ratios of Polish honey and honey protein.

the results. The $\delta^{13}C$ and $\delta^{15}N$ values for meat samples defatted and no defatted are comparable for both types of the meat (pork, chicken). The greater differences in $\delta^{15}N$ values are observed for the organic and conventional pork (1.33 ± 0.18). In general, organic products have more positive $\delta^{15}N$ than the conventional products. The isotopic research of the meat produced in Poland should be continued to confirm this observation. The range of works should be extended to isotopic analysis of fodders in conventional and ecological farms.

Honey

Honey is composed mainly of carbohydrates (fructose and glucose) and proteins. The additional sugars are frequently added to sweeten honey. The stable carbon isotope ratio analysis (SCIRA) is used to detect the addition of C4 (corn or cane) sugars in honey at a concentration above 7%. The C4 sugar content in honey is calculated using the following formula:

$$\text{C4 sugars (\%)} = 100 \times [\delta^{13}C_p - \delta^{13}C_h]/[\delta^{13}C_p - (-9.7)]$$

where: $\delta^{13}C_p$ and $\delta^{13}C_h$ are $\delta^{13}C$ values for protein and honey, respectively, and –9.7 is the average $\delta^{13}C$ value for corn syrup.

The carbon and nitrogen stable isotope ratios in honey and honey proteins from different floral types such as lime tree, rape, buckwheat, multiflorous and honey-dew are presented in Table 3 and Fig. 3.

Multielement stable isotope ratios (H, C, N, and S) of honey from different European regions are reported by Schellenberg *et al*. [18]. This paper includes investigations using honey produced in regions with different climatic and geological condition. Honey samples from 20 European regions including 30 samples from Poland were collected predominantly during two harvesting years 2005 and 2006. The $\delta^{13}C$ and $\delta^{15}N$ mean values of the honey proteins for Polish honey were –26.2 ± 0.4 and 3.9 ± 0.9, respectively. The results obtained in Stable Isotope Laboratory, Institute of Nuclear Chemistry and Technology (SIL, INCT, Warsaw) show that samples coded as MP3/Linden, MP5/Linden, MP7/Honeydew and MP8/Multiflorous have $\delta^{13}C$ and $\delta^{15}N$ values of the honey proteins in the range of that calculated by Schellenberg. The samples coded as MP1/Multiflorous, MP2/Rape, MP4/Multiflorous and MP6/Buchwhet have the analyzed $\delta^{13}C$ and $\delta^{15}N$ values of the honey proteins besides the definite area. These divergences result probably from the fact that the examined honeys originated only from one selected region of Poland and were produced in another season (2011 year). So far, research related to geographical origin of honey from one whole country using isotope ratio mass spectrometry methods was not reported. There is the only study where the determination of the geographical origin of Slovenian black locust, lime and chestnut honey was carried out by the analysis of some physico-chemical parameters and stable carbon and nitrogen isotope ratios [8]. On the other hand, the difference in $\delta^{13}C$ value for protein and honey for the samples MP1/Multiflorous and MP5/Linden is greater than 1‰ which proves that the honey is enriched with C_4 plant sugars such as high fructose corn syrup (HFCS). The addition of C4 plant sugars, calculated basing on the cited formula, is about 9.4% for the MP1/Multiflorous honey and about 14.7% for the MP5/Linden honey.

Table 3. The carbon and nitrogen stable isotope ratios of Polish honey and honey proteins

Code sample/honey type	$^{13}C_{honey}$	$^{13}C_{protein}$	$^{15}N_{protein}$	Adulteration (%)
MP1/Multiflorous	–23.66	–25.14	4.26	9.4
MP2/Rape	–27.16	–27.55	1.09	pure
MP3/Linden	–26.04	–26.45	4.08	pure
MP4/Multiflorous	–26.91	–26.95	3.25	pure
MP5/Linden	–23.72	–26.15	3.62	14.7
MP6/Buckwheat	–27.93	–27.27	3.85	pure
MP7/Honeydew	–25.55	–26.13	3.03	pure
MP8/Multiflorous	–26.39	–26.29	2.65	pure

Conclusion

Stable isotope mass spectrometry is a useful technique to distinguish the food regarding its different geographical or botanical origin. The isotopic characterization of the carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) in some domestic food products such as eggs, meat and honey was given in this work. The results of our study are indicative of continuation the research in order to create database of the isotopic composition of domestic products for future applications in the authenticity and origin control using IRMS methods.

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