

Application of Stable Isotope Ratio Analysis for Origin Authentication of Pork

Kyong Su Kim*, Jae Sung Kim, In Min Hwang, In Seon Jeong, Naeem Khan, Sun Im Lee¹, Dong Bok Jeon¹, Yang Hoon Song², and Kwan Suk Kim²

Department of Food and Nutrition, Chosun University, Gwangju 501-759, Korea

¹Department of International Tea Culture, Chosun University, Gwangju 501-759, Korea

²Life and Environment Sciences, Chungbuk University, Cheongju 361-763, Korea

Abstract

Origin authenticity of the animals used as food has always been a major concern to consumers around the world. In the past twenty years, a stable isotope ratio has been used for origin authentication. In this study, pork samples, both local and imported, were collected from the major markets from all around South Korea and analyzed for stable isotope ratios of nitrogen ($\delta^{15}\text{N}\%$) and carbon ($\delta^{13}\text{C}\%$), using Isotope Ratio Mass Spectrometry (IR-MS). A total of 599 samples with 335 Korean and 264 imported from 13 countries within America and Europe were investigated in accordance to the standard established methods for isotope ratio analysis. The results showed a significant variation related to the origin of the samples, explaining the difference in the feeding styles of the pork in each country. The stable isotope ratio values of carbon ($\delta^{13}\text{C}\%$) were found in the decreasing order of: America ($-15.55\pm 1.01\%$)>Korea ($-19.62\pm 0.89\%$)>Europe ($-24.79\pm 1.35\%$). Canada was having $\delta^{13}\text{C}$ ratio of $-22.87\pm 0.92\%$, which is very low in the region of America and very close to Europe (-23.78 to -27.17%). For nitrogen $\delta^{15}\text{N}\%$ the order was: America ($4.92\pm 0.71\%$)>Europe ($4.54\pm 0.66\%$)>Korea ($3.69\pm 0.54\%$), with a slight variation among countries in each region studied. From the results it was concluded that the stable isotope ratio of the pork samples from different countries provide enough information about the origin and is therefore a potential tool which can be employed for origin authentication.

Key words: origin authentication, IR-MS, stable isotopes ratio, pork, Korea

Introduction

Under the umbrella of World Trade Organization (WTO), the trade volume of food and merchandise are increasing day by day. Each country in the world has distinct latitude and climatic conditions and therefore having special qualitative and quantitative characteristics in their food and food products. Accordingly, consumers always demand for reliable information about the food origin which is an important part in their food selection (Boner and Forstel, 2004; Hargin, 1996). However, presently some of the food suppliers do not fully describe the origin of food animals and other stuffs; even on wide spread transportation, for allied high profits. In European Union, each county have made food name chimes, by various

labeling systems, for the protection of food origin on geographical basis (EEC, 1992).

Organic matter analysis to determine the food origin, involve application of techniques like near-infrared spectroscopy (Lee *et al.*, 2006), x-ray fluorescence spectrometry (Jeong and Lee, 2008), electronic nose (Lee, 2006), capillary electrophoresis (Kim *et al.*, 2003) and nuclear magnetic resonance (NMR) (Kim *et al.*, 2009). Recently mineral analysis through inductively coupled plasma mass spectrometry (ICP-MS) (Kang *et al.*, 2001), along with genetic methods (Choi *et al.*, 2004), and isotope ratio analysis (Jardine *et al.*, 2003; Peterson and Fry, 1987) have also been tried for origin authentication. Out of these methods, isotope ratio analysis, for determination of the food origin, has been proved to be the most useful method and many studies are currently underway around the world.

Isotopes of an element have different number of neutrons and they behave differently in phenomena such as metabolism, vaporization and natural circulation and by

*Corresponding author: Kyong Su Kim, Department of Food and Nutrition, Chosun University, Gwangju 501-759, Korea. Tel: 82-62-230-7724, Fax: 82-62-224-8880, E-mail: kskim@chosun.ac.kr

examining the stable isotope ratio of the area's environment and the origin can be inferred (Camin *et al.*, 2007; Peterson and Fry, 1987). Stable isotopes are those isotopes of an element that do not decay through radioactive processes over time. Several elements have more than one stable isotopes in nature which include hydrogen (H^2 , H^1), boron (B^{11} , B^{10}), carbon (C^{13} , C^{12}), nitrogen (N^{15} , N^{14}), oxygen (O^{18} , O^{16}), sulfur (S^{34} , S^{32}), strontium (Sr^{87} , Sr^{86}) and lead (Pb^{207} , Pb^{206}) (Ghidini *et al.*, 2006). A significant fraction of stable isotopes always occur in food and food products and therefore isotope ratio analysis has been described as a useful tool of origin authenticity for the last more than 20 years (Simon *et al.*, 2005). Stable isotope ratio of hydrogen (δ^2H) and oxygen ($\delta^{18}O$) provides information about climatic conditions of the region and that of sulfur ($\delta^{34}S$) provides information about surface geology of the area (Balling and Rossmann, 2004). The carbon ($\delta^{13}C$) value tells about feeding habits, specific to a region (Kim *et al.*, 2003; Kim *et al.*, 2009; Lim *et al.*, 2008) and nitrogen ($\delta^{15}N$) value explains about plants, available nitrogen in the soil and atmosphere which are also dependent upon geographical and climatic conditions (Anklam, 1998; Yuta *et al.*, 2012).

Origin authentication is associated mostly to meats because of their usual long distance between producers and consumers and often resulting in crisis of origin (Jeong and Lee, 2008). The meat concerning several studies have been focusing on the distinction of stable isotope (^{13}C , ^{15}N , ^{18}O , ^{34}S , ^{87}Sr) ratio analysis (Camin *et al.*, 2007; Hegerding *et al.*, 2002). Stable isotope ratio analysis has been used to determine the origin on wine (Day *et al.*, 1995), milk (Renou *et al.*, 2004), honey (Anklam, 1998), and butter (Balling and Rossmann, 2004), and cattle beef (Yuta *et al.*, 2012).

The present study was aimed to analyze the stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in pork samples of various countries available in South Korean markets to evaluate differentiation between animal feeding and geographical origin and to apply for authentication of origin.

Materials and Methods

Samples collection

A total of 599 pork samples of 14 countries from South Korea, Europe and America were collected from all around Korean markets. These included 335 local and 264 intentionally imported varieties to South Korea as shown in Table 1. All these samples were sourced through the

National Agricultural Products Quality Management Service, Republic of Korea. Only muscle tissues were collected directly from producers and to analyzed for stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), using EA-IR/MS.

Samples preparation

Meat samples were cut into thin slices and removed the fat contents. Then 10 g of each sample was added with 100 mL toluene and lypholized for 2 h at atmospheric pressure, using azeotropic distillation apparatus to obtain pure protein and remove fat and water. The purified samples were then washed with fresh toluene and then used a rotary evaporation system (Rotavapor R-215, BUCHI, Switzerland) to separate solvent. It was then dried for 5 h at 80°C in oven (HB-502M, Han Back, Korea). The samples were then homogenized and stored at -20°C, prior to analysis.

Isotope ratios determination

The samples were analyzed by using continuous flow-through inlet system-isotope ratio mass spectrometry (Choy *et al.*, 2009). The elemental analyzer (EA 3000, EV, Italy) was connected with a continuous flow-through inlet system-isotope ratio mass spectrometer (CF isotope ratio mass spectrometer, Isoprime, GV Instruments, UK).

First the instrument was calibrated, using sucrose (CH-6, IAEA) $\delta^{13}C = -10.4 \pm 0.2\%$ and ammonium sulfate (N1, IAEA) $\delta^{15}N = +0.4 \pm 0.2\%$, as internal standards for $\delta^{13}C$ and $\delta^{15}N$, respectively. The prepared samples (0.1-1.0 mg) wrapped in tin capsules for oxidation-reduction, at 1,050°C and 650°C for perfect combustion organic matter in the element analyzer.

The combustion tube was filled with tungsten oxide and cobalt (II/III) oxide to support the oxidation and second tube filled with copper for reduction. The gases (CO_2 and N_2) were separated by a gas chromatographic column and introduced into the CF-IR/MS. The detail condition of EA-IR/MS for analyzing $\delta^{13}C$ and $\delta^{15}N$ are presented in Table 2. Tin capsule for analyzing isotope ratio were bought from Euro Vector (Milan, Italy). The degree of purity for carrier gas (He) and reference gases (N_2 and CO_2) were over 99.999%. Isotope ratio of carbon and nitrogen were converted into $\delta\%$, according to the following equation.

$$\delta \text{ value } (\%) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1,000$$

δ -value is the isotope ratio of the sample expressed in

delta unit (‰) relative to the reference material. R_{sample} is the isotope ratio of measured sample in particular $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ and R_{standard} ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) is the isotope ratio of International standard material: V-PDB (Vienna Pee Dee Belemnite) for $\delta^{13}\text{C}$ and atmospheric N_2 for $\delta^{15}\text{N}$.

Statistical analysis

The data was analyzed using one-way analysis of variance (ANOVA) to examine statistical significance of the difference in the mean concentrations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among the countries studied. A probability level of $p = 0.05$ was considered statistically significant. The SPSS system for windows of V18.0 software was employed for statistically analysis (SPSS Inc., USA).

Table 1. Stable isotope ratios carbon ($\delta^{13}\text{C}\%$) and nitrogen ($\delta^{15}\text{N}\%$) composition of the pork defatted dry mass (DDM) protein from different geographical regions

Region	Country	Number of Samples	$\delta^{13}\text{C}[\%]_{\text{V-PDB}}$ Mean \pm SD ¹⁾	$\delta^{15}\text{N}[\%]_{\text{AIR}}$ Mean \pm SD
South Korea		335	-19.62 ^{e2)} \pm 0.89	3.69 ^{ab} \pm 0.54
America	Canada	7	-22.87 ^d \pm 0.92	5.65 ^e \pm 0.53
	USA	30	-14.78 ^a \pm 1.34	5.42 ^{de} \pm 0.58
	Mexico	7	-14.81 ^a \pm 0.46	4.81 ^{cd} \pm 0.56
	Chile	74	-15.93 ^{ab} \pm 0.63	4.72 ^{cd} \pm 0.67
Europe	Austria	20	-24.20 ^{ef} \pm 1.27	4.42 ^{bc} \pm 0.51
	Netherlands	30	-25.57 ^f \pm 1.20	4.58 ^e \pm 0.47
	Denmark	10	-25.24 ^f \pm 0.22	3.62 ^a \pm 0.15
	France	22	-24.35 ^{ef} \pm 1.05	4.96 ^{de} \pm 0.50
	Belgium	35	-24.80 ^{ef} \pm 1.33	4.46 ^e \pm 0.73
	Finland	3	-27.17 ^{ef} \pm 0.52	5.73 ^e \pm 0.31
	Poland	15	-24.77 ^{ef} \pm 1.52	4.53 ^c \pm 0.85
	Hungary	6	-23.78 ^{de} \pm 1.48	4.42 ^{bc} \pm 0.58
	Spain	5	-23.44 ^{de} \pm 1.09	4.68 ^{cd} \pm 0.26

¹⁾Mean \pm standard deviation

^{2)a-e}Values with different letters within a column differ significantly ($p=0.05$).

Table 2. Operating conditions of EA-IR/MS for analyzing $\delta^{13}\text{C}\%$ and $\delta^{15}\text{N}\%$ values of the pork defatted dry mass (DDM) protein from different geographical regions

Parameters	Operating conditions
Combustion furnace	1,050°C
Reduction furnace	650°C
GC packed column oven	115°C
Reference capillary column	L=1.5 m, I.D.(f)=100 mm
Sample capillary column	L=2.5 m, I.D.(f)=75 mm
Run time	385 sec
He carrier flow	115 mL/min

Results

The stable isotope ratio measurements of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) obtained for pork samples of 14 countries are given in Table 1. These can be clearly divided into 3 major groups, Europe, America and South Korea, on the basis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ composition values as shown in Fig. 1.

Stable isotope ratio values of carbon ($\delta^{13}\text{C}\%$)

As shown in Table 2, the $\delta^{13}\text{C}$ values for the nine European countries studied ranged between $-27.17\pm 0.52\%$ (Finland) to $-23.44\pm 1.09\%$ (Spain). The values of $\delta^{13}\text{C}$ in the same region were found very close for Netherlands ($-25.57\pm 1.20\%$) and Denmark ($-25.24\pm 0.22\%$), Austria ($-24.20\pm 1.27\%$), France ($-24.35\pm 1.05\%$), Belgium ($-24.80\pm 1.33\%$) and Poland ($-24.77\pm 1.52\%$) and Spain ($-23.44\pm 1.09\%$) and Hungary ($-23.78\pm 1.48\%$). Among the four countries of American region studied, the $\delta^{13}\text{C}$ values for USA, Mexico, Chile and Canada were $-14.78\pm 1.34\%$, $-14.81\pm 0.46\%$, $-15.93\pm 0.63\%$, and $-22.87\pm 0.92\%$ respectively. The average $\delta^{13}\text{C}$ value for 335 samples of South Korea was found to be $-19.62\pm 0.89\%$ (Table 1 and Fig. 1).

Stable isotope ratio values of nitrogen ($\delta^{15}\text{N}\%$)

The $\delta^{15}\text{N}$ values ranged between $3.69\pm 0.54\%$ for South Korea to $5.73\pm 0.31\%$ for Finland. The countries of American region gave $5.65\pm 0.53\%$ and $5.42\pm 0.58\%$ for Canada and USA, while $4.81\pm 0.56\%$ and $4.72\pm 0.67\%$ for Mexico and Chile respectively. Among the European countries, the $\delta^{15}\text{N}$ values were $3.62\pm 0.15\%$ (Denmark), $4.42\pm 0.51\%$ (Austria), $4.42\pm 0.58\%$ (Hungary), $4.46\pm 0.73\%$ (Belgium), $4.53\pm 0.85\%$ (Poland), $4.58\pm 0.47\%$ (Nether-

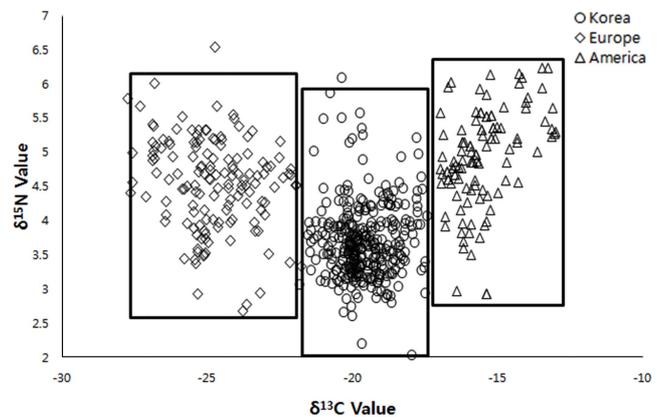


Fig. 1. Two dimension graphical analysis of stable isotope ratios carbon ($\delta^{13}\text{C}\%$) and nitrogen ($\delta^{15}\text{N}\%$) of the pork defatted dry mass (DDM) protein from South Korea and other regions.

land), $4.68 \pm 0.26\%$ (Spain), $4.96 \pm 0.50\%$ (France), and $5.73 \pm 0.31\%$ (Finland) (Table 1 and Fig. 1).

Discussion

Meat always proved to be quite a complex material for isotope analysis. Mostly the interpretation of results is very difficult for several reasons. The fodder is not often grown locally and therefore the link to local geology is not straightforward. Also different tissues of an animal have different turnover rates and it is quite possible to get various ratios within one sample. Additionally chemical composition of animal protein always makes the isotope analysis very difficult (Rummel *et al.*, 2012).

To cope with these possible hurdles, samples were sourced through National Agricultural Products Quality Management Service, Republic of Korea. The samples were collected directly from the producers and therefore can be considered as authentic. Also within the muscle tissues of an animal, the isotopic ratios of carbon and nitrogen should not differ significantly. The results obtained for both $\delta^{13}\text{C}\%$ and $\delta^{15}\text{N}\%$ are evaluated separately as follow.

Evaluation of pork meat defatted dry mass (DDM) $\delta^{13}\text{C}\%$ measurements

It is well known that stable isotopes of carbon (^{12}C and ^{13}C) in food animals is dependent upon the type of food they take, which is directly related to geographical location of the country (Camin *et al.*, 2004). In those areas of the world where animals are fed with more photosynthetic food (C_3 plant materials), rich in ^{12}C isotope, their bodies are loaded with more ^{12}C isotope than ^{13}C and therefore when analyzed will have low stable isotope ratio value of carbon ($\delta^{13}\text{C}\%$).

On the other hand, animals fed on non-photosynthetic foods like grains (C_4 plant materials), have comparatively high ^{13}C content and therefore will show high stable isotope ratio value of carbon ($\delta^{13}\text{C}\%$) (Schewertl *et al.*, 2005). Studies on cattle tissues such as hairs (Minson *et al.*, 1975), dairy products like milk (Kornexl *et al.*, 1997), butter (Rossenmann *et al.*, 2000) and cheese (Camin *et al.*, 2004) confirms that $\delta^{13}\text{C}\%$ value is dependent upon the proportion of C_3 and C_4 plant materials in the diet of animal, prior to slaughter. It has been demonstrated that in beef samples when the cattle is exposed to C_4 -maize silage feedings for prolonged periods, in excess of 12 mon, high $\delta^{13}\text{C}\%$ is obtained (Bahar, 2006; Boner and Forstel, 2004).

The feeding plant materials to animals cannot be the same in different regions of the world, as each part has unique climatic conditions and thus samples from different geological locations will have specific $\delta^{13}\text{C}\%$, which can be used for origin authentication. According to the results given in Table 1, a clear distinction can be made while looking to the $\delta^{13}\text{C}\%$ values of different countries.

Evaluation of pork meat defatted dry mass (DDM) $\delta^{15}\text{N}\%$ measurements

Stable isotopes of nitrogen (^{14}N and ^{15}N) in the food animals depend upon the atmosphere, types of plants, climatic conditions and soil texture of region where they grown up. It has been demonstrated in the past that if same animals are feed the same food but in different regions having different climatic conditions, the $\delta^{15}\text{N}\%$ values will be different (Kornexl *et al.*, 1997; Piasentier *et al.*, 2003). Therefore $\delta^{13}\text{C}\%$ values always do not give any clear picture for origin authentication, especially when the feeding habits are only slightly different. For such cases the $\delta^{15}\text{N}\%$ evaluation is very fruitful to confirm the origin authentication, as both carbon and nitrogen are the important components of organic matters (Ghidini *et al.*, 2006).

Stable isotope ratio measurements of nitrogen ($\delta^{15}\text{N}\%$), for the defatted dry mass (DDM) of 599 pork samples from 14 countries studied, are given in Table 1, and shown in Fig. 1. It can be noted that there is slight variation among the $\delta^{15}\text{N}\%$ values for countries of Europe and American regions. For Korean samples the $\delta^{15}\text{N}\%$ value obtained was $3.69 \pm 0.54\%$, which is adding the authentication of Korean pork from Europe and America regions. For American region, the $\delta^{15}\text{N}\%$ values of Canada ($5.65 \pm 0.53\%$) and USA, ($5.42 \pm 0.58\%$) are very close and slightly different from Mexico ($4.81 \pm 0.56\%$) and Chile ($4.72 \pm 0.67\%$).

This slight variation in the same region may be due to the difference in their atmosphere, soil fertility, plants nature and over all climatic conditions. It can be noted here that the $\delta^{13}\text{C}\%$ value for Canada was very low among the countries of American region and was making confusions about origin authentication, as discussed above. Now after evaluating the $\delta^{15}\text{N}\%$, the said confusion is solved. This lead us to suggest that both $\delta^{13}\text{C}\%$ and $\delta^{15}\text{N}\%$ values were required for clear picture of origin authentication.

From the nine European countries studied, the $\delta^{15}\text{N}\%$ values for seven ranged between $4.42 \pm 0.51\%$ to $4.96 \pm 0.50\%$. This shows their similar climatic conditions and soil fertility, being in the same group. The $\delta^{15}\text{N}\%$ for Denmark was found the lowest ($3.62 \pm 0.15\%$), within the

statistical probability variation calculated ($p < 0.05$) indicating difference from other countries of the same group and relatively close to South Korea. Finland showed $5.73 \pm 0.31\%$, comparable to Canada and USA.

Conclusions

A total of 599 pork samples from South Korea and 13 countries of America and Europe were analyzed for stable isotope ratios of carbon ($\delta^{13}\text{C}\%$) and nitrogen ($\delta^{15}\text{N}\%$), using Elemental Analyzer Isotope Ratio Mass Spectrometer (EA-IR/MS). Results showed significant variation, related to the origin of samples, due to the difference in feeding styles of pork in each country. On the whole stable isotope ratio study gave enough information and hence found a potential tool for origin authentication. Values of carbon ($\delta^{13}\text{C}\%$) and nitrogen ($\delta^{15}\text{N}\%$) for samples from South Korea were found in between the American region and Europe, explaining its specific regional feature. Thus pork of South Korea can be distinguished in the local market from other internationally supplied ones by the application of stable isotope ratios of carbon and nitrogen.

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