Application of Stable Isotope Ratio Analysis for Origin Authentication of Pork

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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 (\(\delta^{15}N\)) and carbon (\(\delta^{13}C\)), 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 (\(\delta^{13}C\)) were found in the decreasing order of: America (-15.55±1.01‰)>Korea (-19.62±0.89‰)>Europe (-24.79±1.35‰). Canada was having \(\delta^{13}C\) ratio of -22.87±0.92‰, which is very low in the region of America and very close to Europe (-23.78 to -27.17‰). For nitrogen \(\delta^{15}N\) the order was: America (4.92±0.71‰)>Europe (4.54±0.66‰)>Korea (3.69±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

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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, D), boron (B11, B10), carbon (C13, C12), nitrogen (N15, N14), oxygen (O18, O16), sulfur (S34, S32), strontium (Sr87, Sr86) and lead (Pb207, Pb206) (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 (δD) and oxygen (δ18O) provides information about climatic conditions of the region and that of sulfur (δ34S) provides information about surface geology of the area (Balling and Rossmann, 2004). The carbon (δ13C) value tells about feeding habits, specific to a region (Kim et al., 2003; Kim et al., 2009; Lim et al., 2008) and nitrogen (δ15N) 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 (δ13C, δ15N, δ18O, δ34S, δ87Sr) 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 (δ13C) and nitrogen (δ15N) 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 (δ13C) and nitrogen (δ15N), 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 lyophilized 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 (CH6, IAEA) δ13C=−10.4±0.2‰ and ammonium sulfate (N1, IAEA) δ15N=+0.4±0.2‰, as internal standards for δ13C and δ15N, 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 (CO2 and N2) were separated by a gas chromatographic column and introduced into the CF-IR/MS. The detail condition of EA-IR/MS for analyzing δ13C and δ15N 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 (N2 and CO2) were over 99.9999%. Isotope ratio of carbon and nitrogen were converted into δ‰, according to the following equation.

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

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