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Short communication

Determination of organic milk authenticity using carbon and nitrogen natural isotopes

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ABSTRACT

Natural stable isotopes of carbon and nitrogen (^{12}C, ^{13}C, ^{14}N, ^{15}N) have abundances unique to each living creature. Therefore, measurement of the stable isotope ratio of carbon and nitrogen ($\delta^{13}C = {}^{13}C/{}^{12}C$, $\delta^{15}N = {}^{15}N/{}^{14}N$) in milk provides a reliable method to determine organic milk (OM) authenticity. In the present study, the mean $\delta^{13}C$ value of OM was higher than that of conventional milk (CM), whereas the mean $\delta^{15}N$ value of OM was lower than that of CM; nonetheless both $\delta^{13}C$ and $\delta^{15}N$ values were statistically different for the OM and CM ($P < 0.05$). Furthermore, the values of $\delta^{13}C$ and $\delta^{15}N$ were found to differ statistically with the collection date and the milk brand ($P < 0.05$). The combination of $\delta^{13}C$ and $\delta^{15}N$ values was more effective than either value alone in distinguishing between OM and CM. The results of the present study, which is based on preliminary data from a limited sample size and sampling period, could be highly valuable and helpful for consumers, the food industry, and/or government regulatory agencies as it can prevent fraudulent labelling of organic food. Further studies include additional analyses of other milk brands and analyses over longer time periods in order to accurately determine OM authenticity using stable isotopes of carbon and nitrogen.

1. Introduction

The principal goal of an organic farm practice is to achieve ecologically and/or socially optimal natural systems by enhancing biodiversity, biological cycles, and biological activity in soil (Samman et al., 2008). In general, consumers who are interested in using organic products are willing to pay a premium price because of the high nutritional value of organic products and limited availability of natural resources (Bahar et al., 2008; Molkentin, 2013; Samman et al., 2008). However, whether or not organic products have a higher nutritional value than conventionally grown products is a rather controversial topic and may depend on the organic food (Samman et al., 2008). The International Federation of Organic Agriculture Movements (IFOAM) and the Food and Agriculture Organization (FAO)/World Health Organization (WHO) Codex Alimentarius Commission has established standards for organic production, labelling, and certification. They also provide the guidelines for international harmonization of the requirements for organic products (Samman et al., 2008).

Milk is considered a common nutritional food, and its annual production in Korea was about 2.1 megatons in 2012. Owing to increased health awareness and interest in organic foods, the market for organic milk (OM) has increased more than 65% per year in Korea (Korea Dairy Committee, 2011). OM is generally produced without the use of pesticides, synthetic fertilizers, bovine growth hormones (BGH), and antibiotics. OM authenticity is usually determined by measuring residual pesticides, antibiotics, and/or BGH using GC/MS or LC/MS. However, these current methods have limitations with respect to the analytical method used and proficiency of the experimenter in individual laboratories, influencing the accuracy and precision. This made it hard to develop a reliable and robust method for the determination of OM authenticity (Ulberth, 2004).

Carbon (C) and nitrogen (N) are the most abundant elements on earth, and their natural stable isotopes (^{12}C, ^{13}C, ^{14}N, ^{15}N) have abundances unique to each living creature. Therefore, measurement of the C and N stable isotope ratio in milk provides a promising method to distinguish between OM and conventional milk (CM), because C and N isotope ratios in living creatures can be related to those found in nature (Kelly, 2003; Kim, Chuang, Kelly, & Clifford, 2011). C and N isotope ratios ($\delta^{13}C = {}^{13}C/{}^{12}C$, $\delta^{15}N = {}^{15}N/{}^{14}N$) in milk can vary greatly depending on the diet of the dairy cow (Molkentin, 2013). For example, the measurement of $\delta^{13}C$ can hypothetically identify the authenticity of beef or organic milk products and/or their geographical origin. This can be done by relating the varying ratios of C3 (i.e. grass) to C4 (i.e. maize) from the photosynthetic plants consumed in the dairy cow's diet (Badeck, Tcherkez, Nogués,

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Piel, & Ghashghaie, 2005; Bahar et al., 2005; Knobbe et al., 2006; Molkentin, 2013). In addition, the measurement of $\delta^{15}N$ can also discriminate organic farm products from conventional products. Synthetic N fertilizers have a lower $\delta^{15}N$ value (−6 to 6‰) compared to manure and fertilizers (1–37‰) allowed on organic farms (Bateman & Kelly, 2007). Therefore, since OM production does not use chemically synthesised N fertilizers, which are to produce corn, glass clover, wheat, or rye, in a dairy cow's diet, the $\delta^{15}N$ value in OM was observed to be higher than that in CM (Camin et al., 2011).

Development of a reliable and robust analytical method to determine OM authenticity would be highly valuable and helpful for consumers, the food industry, and/or government regulatory agencies. In particular, $\delta^{13}C$ and $\delta^{15}N$ measurement of milk could reduce the risk of milk being unfairly labelled as OM, thus protecting the consumers' rights and enabling fair trade (Molkentin, 2013). Therefore, in the present study, four different commercial brands of OMs and CMs were collected from local markets in Korea over four months (March to June 2013), and the values of $\delta^{13}C$ and $\delta^{15}N$ were measured by stable isotope ratio mass spectrometry (IRMS) to determine OM authenticity. Furthermore, we also report monthly variations in the values of $\delta^{13}C$ and $\delta^{15}N$ for each OM and CM brand.

2. Experimental

2.1. Milk collections and sample preparation for isotope ratio measurement

The four common/popular commercial brands of OM and CM tested in this study were purchased from a local market (near Konkuk University) in Seoul, Korea. The same four brands were purchased each of the 3 random times that a purchase was made over a period of four months (March to June 2013). The collected OM and CM samples were measured for the carbon (C) and nitrogen (N) isotope ratios. Each milk sample of interest was transferred to a pre-weighed tin capsule meant for liquid samples (3.5 × 17 mm, IVA Analysentechinik e. K., art No. 184.9918.26, Germany). The sample was dried at room temperature (~25 °C) for at least 3 day and then weighed again once the sample had dried completely. Based on the total C (51.5 ± 5.6%) and total N (3.7 ± 0.3%) contents in milk previously measured by a CN Elemental Analyzer (NA Series 2, CE Instruments, Italy), the 4 μL and 8 μL of the milk samples were used for C ratio ($\delta^{13}C = {}^{13}C/{}^{12}C$) and N ratio ($\delta^{15}N = {}^{15}N/{}^{14}N$) measurements, respectively.

2.2. Measurement of $\delta^{13}C$ and $\delta^{15}N$ using stable isotope ratio mass spectrometry (IRMS)

The C and N isotope ratios were determined using a continuous-flow stable isotope ratio mass spectrometer (IRMS, IsoPrime-EA, Micromass, UK) linked to a C and N analyzer (NA Series 2, CE Instruments, Italy). The C and N isotope compositions ($\delta^{13}C$ and $\delta^{15}N$) were calculated as follows: $\delta\ (‰) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where R is the ${}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$ ratio of the sample of interest and its respective standard. Therefore, the ${}^{13}C$ and ${}^{15}N$ enrichment was expressed relative to those of well-known laboratory reference standards (the Pee Dee Belemnite (PDB) for $\delta^{13}C$, atmospheric (AIR) N_2 for $\delta^{15}N$) (Hauck, 1982). Multiple replicate analyses indicated that standard deviations for the $\delta^{13}C$ and $\delta^{15}N$ measurements were <0.1‰ and <0.2‰, respectively.

2.3. Statistical analysis

Statistical analyses were performed using the general linear model of the statistical analysis program SAS (version 9.2; SAS Institute Inc., Cary, NC, USA). The experimental design was completely randomized with triplicate. All measurements were conducted in triplicate in the present study. The least significant difference (LSD) test was performed at the 0.05 probability level.

3. Results

Table 1 shows the differences in the carbon and nitrogen isotope ratios in OM and CM consumed in Korea. The mean carbon isotope ratio ($\delta^{13}C$) was higher for OM (−22.39 ± 0.63‰) as compared to CM (−23.60 ± 0.24‰), whereas the mean nitrogen isotope ratio ($\delta^{15}N$) was lower in for OM (5.00 ± 0.49‰) as compared to CM (5.70 ± 0.61‰). Both $\delta^{13}C$ and $\delta^{15}N$ values were found to be statistically different for the OM and CM samples ($P < 0.05$).

Fig. 1 indicates the monthly variation in the $\delta^{13}C$ and $\delta^{15}N$ values of the OMs and CMs. The $\delta^{13}C$ and $\delta^{15}N$ ratios of three OM and CM brands each were measured in March 2013, and the measurements were done in triplicate for each milk sample ($n = 9$ for OM and CM). One more OM and CM brand each was added, and a total of four OM and CM brands were measured for their $\delta^{13}C$ and $\delta^{15}N$ ratios in April and June 2013 ($n = 12$ for OM and CM). Although we observed monthly variations in the $\delta^{13}C$ and $\delta^{15}N$ values for OMs and CMs over a four-month period (Fig. 1a), the $\delta^{13}C$ value of OMs for a given month was 5–10% higher than that of CMs for the same month. In contrast, we found that over the same four-month period, the $\delta^{15}N$ value of CMs for any given month was 2–30% higher than that of OMs for the same month (Fig. 1b). Monthly variation in the $\delta^{13}C$ and $\delta^{15}N$ values mainly resulted from the dairy cow's diet. Interestingly, unlike the $\delta^{13}C$ values, the $\delta^{15}N$ values tended to increase over the collection time of the milk in the present study.

Furthermore, the $\delta^{13}C$ and/or $\delta^{15}N$ ratios in OMs and CMs differed based on the milk brand (Table 1). The mean $\delta^{13}C$ was statistically different for the different OM brands and CM brands, respectively ($P < 0.05$); in contrast, the mean $\delta^{15}N$ was not affected by the brand of the CM. Among the four milk brands tested in this study, brand B had a relatively minute difference in the $\delta^{13}C$ and $\delta^{15}N$ ratios between the OM and CM. This could be due to the use of different dairy cow diets and/or use of different N fertilizer types. In addition, the mean $\delta^{13}C$ of all OM brands was statistically higher than that of the CM brands, whereas the mean $\delta^{15}N$ was higher for the CM brands, with the exception of brand B, ($P < 0.05$) (Table 1). With the exception of milk brand B, all the other OM brands could be effectively distinguished from the CM when both $\delta^{13}C$ and $\delta^{15}N$ values were considered simultaneously (Fig. 2).

4. Discussion

Measurement of several isotopic ratios (${}^{13}C/{}^{12}C$, ${}^{15}N/{}^{14}N$, ${}^{18}O/{}^{16}O$, ${}^{2}H/{}^{1}H$, ${}^{34}S/{}^{32}S$) has been applied for the determination of food authenticity by revealing the geographical origin and organic products (Camin et al., 2011; Kim, Cruz, Fadel, & Clifford, 2012). C and N are, in general, the most abundant elements in living creatures, and their isotope ratios are unique to each creature. Therefore, the measurement of $\delta^{13}C$ and $\delta^{15}N$ can be useful to determine organic food authenticity.

Organic farming for milk production does not typically use corn; however, in some cases it is used as a minor component of the cow's diet to enhance milk production (Boner & Forstel, 2004). Moreover, OM production cannot involve the use any pesticides, synthesised fertilizers, antibiotics, or growth hormones, and as a result, its $\delta^{13}C$ and $\delta^{15}N$ differs from those of CM. The $\delta^{13}C$ ratio between OMs and CMs can be used to discriminate between the diets of different dairy cows. Corn, or its by-products (straws,