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Discrimination of organic milk by stable isotope ratio, vitamin E, and fatty acid profiling combined with multivariate analysis: A case study of monthly and seasonal variation in Korea for 2016–2017

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ABSTRACT

This study examined the monthly and seasonal variations of $\delta^{13}C$, $\delta^{15}N$, fatty acids (FAs), and vitamin E in organic milk (OM) and conventional milk (CM) collected in Korea during 2016–2017, discriminating OM authenticity with chemometric approaches. Compared to CM, the mean $\delta^{13}C$ and $\delta^{15}N$ values were lower in OM, whereas the mean α-tocopherol and nutritionally desirable FA contents were higher in OM. Furthermore, $\delta^{13}C$, $\delta^{15}N$, and FA contents vary significantly with the season in OM, whereas α-tocopherol does not show a specific seasonal trend in either OM or CM. Chemometric approaches provided reliable chemical markers, notably C18:3n-3, C18:2n-6, and $\delta^{13}C_{bulk\text{-}milk}$, for accurate OM discrimination according to sampling season. Our findings elucidate milk nutritional quality issues and also provide valuable insight into the control of fraudulent OM labeling in Korea, with potential application in other countries.

1. Introduction

Milk is a well-known health-promoting food that contains essential proteins, fats, carbohydrates, vitamins, and minerals, and milk-fat accounts for 18–24% of the required human dietary fat intake (Huth, DiRienzo, & Miller, 2006). Since the past decade, the annual total crude milk production in Korea has remained at a stable average of 2.1 ± 0.09 megatons; however, over the past three years, the country produced an overstock of approximately 10% of raw milk because of the decrease in milk consumption. In contrast, despite the premium price of organic milk (OM), the domestic OM market share increased 12-fold over the last decade, accounting for approximately $60 million USD in 2016 (Ministry of Agriculture Food and Rural Affairs, 2016). This consumption pattern may be associated with the improved awareness of quality-of-life issues such as environmental conservation and health benefits of organic agro-products (Aryal, Chaudhary, Pandit, & Sharma, 2009; Samman et al., 2008).

OM is typically produced by dairy cows raised on organic feed without the application of antibiotics, synthetic antimicrobials, or hormones. The Codex Alimentarius Commission and the International Federation of Organic Agriculture Movements (IFOAM) have set international guidelines and have also internationally harmonized requirements for the production, processing, labelling, and marketing of organically produced agro-products and foods (Samman et al., 2008). As a result, producers in many countries have used similar approaches to producing OM. In Korea, based on the Act on Promotion of Environmentally-Friendly Agriculture and Fisheries and Management of and Support for Organic Food, OM regulations stipulate that at least 85% of all feeds are to be sourced organically, and the recommended forage-to-concentrate ratios are 6:4 or 7:3. Moreover, the dairy cow must feed not only on silage, but also on forage such as fresh pasture or organically sourced hay. Use of conserved silage, concentrate, and/or cereal are limited to milk production and quality control during winter when there is shortage of fresh dairy feed. Organic dairy farms should have a grassland of approximately 4000 m^2 or a forage crop cultivation area of approximately 1300 m^2 per herbivore, including dairy cows. The barn housing should provide approximately 17 m^2 per dairy cow, and at least twice as much outdoor grazing area should be secured. In addition, no feeds should contain genetically modified organisms. Meanwhile, according to the organic equivalence arrangement between Korea and the USA, becoming effective since July 2014, as long as certain products certified as organic in either country meet certain

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requirements, these may be labeled as organic in both countries without additional certification processes. A similar arrangement between Korea and the EU became effective in February 2015.

Accurate determination of an organic product is becoming increasingly important to protect consumers from deception and fraud, especially as the availability of organic products is increasing in the global market. However, to date, no accurate method for discriminating an organic product has been introduced, despite much effort from the organic industry. Therefore, the development of a reliable analytical method for accurate OM certification is in high demand among consumers, the food industry, and regulatory bodies (Ulberth, 2004).

Because the different dairy farming management system used in OM and conventional milk (CM) farm can affect milk components, the unique dairy feeding regime used in OM farms may play a critical role in OM discrimination against CM (Benbrook, Butler, Latif, Leifert, & Davis, 2013; Butler, Stergiadis, Seal, Eyre, & Leifert, 2011). Therefore, OM authenticity has been established by comparing certain chemical or isotope variations in milk using chromatography, spectroscopy, or mass spectrometry. According to previous reports (Badeck, Tcherkez, Nogués, Piel, & Ghashghaie, 2005; Knobbe et al., 2006; Molkentin, 2013), owing to different photosynthetic isotopic fractionations, the corn-based feeding strategy commonly used by CM farms resulted in higher $\delta^{13}C$ in CM than that in OM. With 10% increased corn content in a cow's diet, $\delta^{13}C$ shifted approximately 1‰ in milk-casein (Camin, Perini, Colombari, Bontempo, & Versini, 2008). In addition, $\delta^{13}C_{fat}$ in OM was always lower, at a maximum of −28‰, compared to that in CM, which was −26.6‰ or higher. Furthermore, α-linolenic acid (ALA) content has shown a distinct negative correlation with the $\delta^{13}C_{fat}$ value in milk (Molkentin, 2009), and the synthetic N fertilizers used to feed plants can induce lower $\delta^{15}N$ values in conventional products compared to that in organic products (Bateman, Kelly, & Jickells, 2005). However, the application of leguminous plants or atmospheric N_2 fixing plants at little more than 0‰ of the $\delta^{15}N$ level could also induce lower $\delta^{15}N$ values even in organic animal products (Yoneyama, 1995). Furthermore, phytonutrients such as nutritionally desirable fatty acids (FAs), vitamin E, and/or carotenoids have been observed at higher levels in OM than that in CM, and these have been reported as potential markers to determine the OM authenticity (Butler et al., 2008; Slots et al., 2009; Średnicka-Tober et al., 2016).

In spite of the continual expansion of the OM market in Korea, to our knowledge, the authenticity of OM produced in Korea is yet to be clearly characterized. Therefore, this study compared $\delta^{13}C$, $\delta^{15}N$, FA, and vitamin E variations between OM and CM produced in Korea from April 2016 to March 2017, and then conducted a statistical analysis using chemometric approaches in order to reveal OM authenticity. Our 1-year case study can elucidate monthly and seasonal variations of chemical markers for the discrimination of OM authenticity produced in Korea, for potential application to other countries after further validation.

2. Materials and methods

2.1. Chemicals

The solvents used for vitamin E and fatty acid analyses were of analytical or high performance liquid chromatography (HPLC) grade. Methanol, ethanol, and isooctane were purchased from Fisher Scientific Korea, Ltd. (Seoul, Korea); hexane was from J.T. Baker (Phillipsburg, NJ, USA), and benzene, heptane, and potassium hydroxide (KOH) were obtained from Junsei (Tokyo, Japan). 2,2-Dimethoxypropane (DMP) and dichloromethane were received from Sigma-Aldrich Korea (Seoul, Korea). Ascorbic acid was purchased from Sanchun Chemical Co. (Gyeonggi-Do, Korea); sulfuric acid (H_2SO_4) and sodium sulfate anhydrous were from Daejung Chemical & Materials Co., Ltd. (Gyeonggi-Do, Korea). Authentic chemical standards (STDs), including vitamin E, a mixture of 37 standard fatty acid methyl esters (FAME, CRM47885), and pentadecanoic acid (P6125) were purchased from Sigma-Aldrich, Korea.

2.2. Samples

On the basis of our prior studies (Chung, Kim, Park, Oh, & Kim, 2016; Chung, Park, Yoon, Yang, & Kim, 2014), the popular OM and CM brand produced from the same manufacturer was chosen and collected monthly from April 2016 to March 2017. The raw OM samples have been obtained from the dairy farm in Hoengseong-gun, Gangwon-do, Korea (37° 27′N, 128° 05′E, 314 m a.s.l.), where it showed the annual precipitation of ∼850 mm and the monthly average temperature of −2.2 °C to 27 °C for the sampling period. And, the raw CM samples were obtained from the other dairy farm in Chungju-si, Chungcheongbuk-do, Korea (37° 03′N, 127° 50′E, 108 m a.s.l.), showing the annual precipitation of ∼930 mm and the monthly average temperature of −2.5 °C to 26.8 °C for the same period. Then, the raw milk samples collected were quickly moved to the factory of the manufacturer located at Eumseong-gun, Chungcheongbuk-do, Korea (36° 57′N, 127° 27′E, 99 m a.s.l.) for the cleanness, homogenization, and pasteurization. In the last week of every month, the manufacturer has directly provided the 0.2-L samples of pasteurized OM and CM with five replicates ($n = 5$ per each), respectively. The OM producing system was in compliance with the Act on the Promotion of Environment-Friendly Agriculture and Fisheries and the Management of and Support for Organic Foods (Ministry of Agriculture Food and Rural Affairs, 2013), and was also certified by the IFOAM. The OM samples measured in this study were certified by an inspection body with specialized equipment, personnel, and facilities, as designated by the National Agricultural Products Quality Management Service. A total of 120 milk samples were frozen at −70 °C, and then lyophilized at −40 °C for approximately 5 days prior to the $\delta^{13}C$, $\delta^{15}N$, vitamin E, and FA analyses.

2.3. $\delta^{13}C$ and $\delta^{15}N$ analyses by IRMS

Each OM and CM sample was weighed to approximately 2 mg in a tin capsule (3.5 mm × 17 mm, IVA Analysentechinik e. K., Dusseldorf, Germany) for simultaneous $\delta^{13}C$ and $\delta^{15}N$ analyses because pre-tests revealed that milk powder samples contained approximately 51% total carbon (%C) and approximately 4% total nitrogen (%N). Thereafter, the wrapped samples were placed in a desiccator until $\delta^{13}C$ and $\delta^{15}N$ analyses by IRMS. $\delta^{13}C$ and $\delta^{15}N$ were measured by an Elementar Vario EL Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany), interfaced to an Isoprime VisION IRMS (Isoprime Ltd., Stockport, UK, a division of Elementar Analysensysteme GmbH, Hanau, Germany). Samples were combusted at 1000 °C in a reactor packed with tungsten (VI) oxide, and then oxides were removed by reduced copper at 650 °C in a reduction reactor. A helium carrier then passed through a water trap containing magnesium perchlorate and phosphorous pentoxide. Subsequently, the N_2 and CO_2 produced from the samples were separated by a Carbosieve GC column (65 °C, 65 mL·min^{-1}) prior to the entering the IRMS. The N_2 peak was analyzed first and CO_2 was retained in an adsorption trap, which was then heated to 165 °C, releasing the CO_2 to the IRMS (Chung, Park et al., 2017). The final $\delta^{13}C$ and $\delta^{15}N$ values in samples interest are given in parts per thousand (‰) relative to international reference standards using the following formula:

δ, ‰ = $[(R_{unknown} - R_{standard})/R_{standard}]$, where R is the C and N stable isotope ratio (i.e., $^{13}C/^{12}C$, $^{15}N/^{14}N$) of the unknown samples and the international reference standards, namely the Vienna PeeDee Belemnite for carbon and atmospheric N_2 for nitrogen, respectively. In this study, laboratory reference materials (bovine liver, nylon 5) calibrated against international reference materials (IAEA-N1, IAEA-N2, IAEA-N3, USGS-40, or USGS-41) were analyzed together with the milk samples. Laboratory reference $\delta^{13}C$ and $\delta^{15}N$ values were −21.69‰ and 7.72‰ for the bovine liver, and −27.72‰ and −10.31‰ for the