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Quantitative analyses of essential fatty acids in cereals and green vegetables by isotope dilution-gas chromatography/mass spectrometry

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Abstract

An analytical method for the accurate determination of essential fatty acids in cereals and green vegetables is established based on isotope dilution-gas chromatography/mass spectrometry (ID-GC/MS). In this approach, ¹³C-labeled fatty acids were added as internal standards to the samples prior to the sample preparation. Repeatability and reproducibility for the analyses of the essential fatty acids extracted from homogenized cereals and green vegetables were examined to validate the ID-GC/MS method. The repeatability and reproducibility were within 2% relative standard deviation. The developed method provides higher-order measurement results with the relative expanded uncertainties of 1–2% and 2–4% for cereals and green vegetables, respectively. Based on the fatty acid measurements, the developed ID-GC/MS method can be used as a candidate reference method for the quantitation of essential fatty acids in cereals and green vegetables. The developed method was also applied to analyze several commercially available products including cereal and vegetable powders.

Keywords: Essential fatty acid, Isotope-labeled fatty acid, Isotope dilution-gas chromatography/mass spectrometry, Cereal, Green vegetable

Introduction

Essential fatty acids are present in various foods as metabolites and intermediates of diverse biological processes as well as the main source of energy in human beings. (Harwood, 1988; Innis, 2008; Holman, 1998; Arbuckle et al., 1994; Neuringer et al., 1984) For humans, dietary intake is the only way to obtain essential fatty acids such as α -linolenic acid (n-3 fatty acid) and linoleic acid (n-6 fatty acid). (Innis, 1991) The low intake of essential fatty acids has a negative effect on human health. (Innis, 2008; Uauy & Dangour, 2006) It is reported that n-3 fatty acid deficiency leads to decreased

visual and neural development in infants and children, and the deficiency of n-6 fatty acid causes poor growth and reproductive failure. (Innis, 2008; Connor, 1999) Therefore, reliable fatty acid measurements of foods are required for adequate intake of fatty acids. Additionally, the quantitative analysis of fatty acids is needed to characterize the nutritional quality of food.

Cereals and vegetables are our staple foods that provide essential dietary nutrients including fatty acids. Analysis of fatty acids in these foods has been investigated using various analytical techniques. (Yoshida et al., 2011; Yasumatsu & Moritaka, 1964; Komaitis & Melissari-Panagiotou, 1990; Lee et al., 2000) of which gas chromatography (GC) approaches have been the most widely utilized methods. Although GC analysis requires esterification and methylation processes to form fatty acid methyl esters (FAMES) to increase the

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volatility and decrease the polarity of compounds for GC measurements, GC coupled with mass spectrometry (MS) provides the most definitive information for the identification and quantification of fatty acids. (Lee et al., 2019a; Takemoto et al., 2003; Cruz-Hernandez et al., 2013) In many cases, a single FAME as an internal standard is added to the sample for the quantitative analysis of fatty acids, (Takemoto et al., 2003; Cruz-Hernandez et al., 2013) which presents limitations in an appropriate correction of recovery during the derivatization process as well as instrument sensitivity owing to the different performances of the reactions and measurements of various fatty acids. The Comité Consultatif pour la Quantité de Matière (CCQM) identified that an isotope dilution mass spectrometry (IDMS) approach has a potential to be a primary method in chemistry. (Quinn, 1997; Richter, 1997) Therefore, in this study, an isotope dilution-gas chromatography/mass spectrometry (ID-GC/MS) method is established as a higher-order reference method, in which an isotope-labeled fatty acid is used to correct the recovery and detection sensitivity of the target fatty acid during the sample preparation steps and instrumental measurements. The developed ID-GC/MS method affords metrologically traceable measurement results and ensures accurate determination of fatty acid content in foods.

The aim of this study is to develop an accurate analytical method as a higher-order reference method using IDMS for fatty acid analysis in the food. The developed ID-GC/MS method as a primary method provides measurement results with traceability to the International System (SI) of units. Our research laboratory at Korea Research Institute of Standards and Science as the National Metrology Institute of Korea has been developing food-matrix certified reference materials (CRMs) along with a higher-order reference method under a project of nutritional metrology program. (Lee et al., 2017; Lee et al., 2016; Lee et al., 2019b; Shin et al., 2013) The food-matrix CRMs provide certified values for the target nutrients and can be used to verify the nutrient measurements. Under this program, the IDMS methods have been developed as candidate reference methods for the quantitative analysis of individual nutrients in various foods. Recently, accurate amounts of several fatty acids in infant formula were determined using the developed IDMS approach by our research group. (Lee et al., 2019a) There are a few food CRMs to provide certified values for nutrients including fatty acids. (Lee et al., 2019b; Wise & Phillips, 2019; Sharpless et al., 2019) However, to the best of our knowledge, no CRM for the essential fatty acid analysis in cereal or vegetable matrix is currently available. As cereals and vegetables are highly consumed foods in the diet, which are the main source of nutrition including fatty acids, the IDMS method is expanded to

analyze the essential fatty acid content in raw food matrices in the present study. The repeatability and reproducibility of the developed IDMS method for the analysis of essential fatty acids in cereals and green vegetables are evaluated. As a result, the repeatability and reproducibility were within 2% relative standard deviation for all samples. The developed ID-GC/MS method provides higher-order metrological measurement values, which can be a candidate reference method.

Experimental

Materials and chemicals

Linoleic acid and α -linolenic acid were purchased from MilliporeSigma Corporate (St. Louis, MO, USA). These fatty acids were used as primary reference materials for sample analysis. The purity assays of the fatty acids were performed according to the protocols based on a mass-balance approach, maintained in the laboratory at the National Metrology Institute of Korea. (Kim et al., 2013; Lee & Kim, 2014) The purity determination can be applied to the primary reference materials for linking traceability of their certified values of target organic substances in matrix CRMs to the SI units. The purities of linoleic acid and α -linolenic acid were determined to be $96.2 \pm 1.1\%$ and $99.1 \pm 0.2\%$, respectively. $^{13}\text{C}_{18}$ -linoleic acid and $^{13}\text{C}_{18}$ - α -linolenic acid were obtained from Medical Isotopes Inc. (Pelham, NH, USA). Anhydrous sodium sulfate, butylated hydroxytoluene (BHT), hydrochloric acid, sodium chloride, sodium hydroxide, 14% boron trifluoride in methanol solution, heptane, and petroleum ether were obtained from MilliporeSigma Corporate. Chloroform, ethanol, methanol, and *n*-hexane were purchased from Honeywell Burdick & Jackson (Muskegon, MI, USA). Cereal and green vegetable samples were purchased from the local markets.

Standard solutions

Standard solutions and isotope ratio standard solutions were prepared by the procedure maintained in our laboratory as described in previous studies. (Lee et al., 2019a; Kim et al., 2010) Four standard solutions containing approximately 500 mg/kg of the target fatty acid(s) were gravimetrically prepared by dissolving each fatty acid in ethanol including 0.1% BHT as an antioxidant. An isotope-labeled standard solution containing $^{13}\text{C}_{18}$ -linoleic acid (and $^{13}\text{C}_{18}$ - α -linolenic acid in case of vegetable samples) was also prepared in the same manner. For each standard solution, two isotope ratio standard solutions were prepared by gravimetrically mixing it with the isotope-labeled solution (1:1 isotope ratio). A total of eight isotope ratio standard solutions was analyzed by GC/MS to evaluate the repeatability of the preparing standard solutions and isotope ratio standard solutions.

One isotope ratio standard solution was selected as a calibration standard for sample analysis.

Lipid extraction, saponification, and methylation

Approximately 0.3 g of each sample was added into a 50-mL glass tube and spiked with the isotope-labeled standard solution to obtain a 1:1 ratio of the isotope-labeled fatty acid to the target essential fatty acid. To extract the bound lipid from the grain samples, acid hydrolysis using the procedure described by Taylor et al. (Taylor & Nelson, 1920) was performed by adding 5 mL of 1 N HCl solution to the glass tube and heating at 100 °C in a hot water bath for 30 min. After cooling the sample, 3 mL of 3 N NaOH solution was added. Thereafter, 10 mL of chloroform-methanol (2:1, v/v) mixture was added in the case of cereal samples according to the Folch procedure, and the sample tube was heated at 100 °C for 30 min to extract the free lipids. (Folch et al., 1957) For green vegetable samples, 10 mL of *n*-hexane was added to the sample tube and the sample solution was heated at 60 °C for 1 h.

After lipid extraction, saponification and methylation reactions were performed to obtain FAMES for GC/MS analysis. The content in the sample tube was mixed with 5 mL of 0.5 N NaOH in methanol and heated at 100 °C for 10 min to obtain free fatty acids (FFAs). Then, the FFAs were derivatized using 5 mL of 14% boron trifluoride in methanol at 100 °C for 5 min. After adding 10 mL of the saturated sodium chloride solution, 5 mL of heptane and 10 mL of petroleum ether were used to extract the FAMES. The extract (upper organic layer) was transferred into another tube, and residual water was removed by adding sodium sulfate. The final extract was dried at 50 °C under nitrogen gas and diluted with *n*-hexane to obtain a concentration of ppm level suitable for GC/MS analysis.

ID-GC/MS instrument conditions and calculations

A triple quadrupole mass spectrometer with a GC system (TSQ8000 and TRACE1310, Thermo Fisher Scientific, Waltham, MA, USA) was utilized for ID-GC/MS analysis. The chromatographic separation of FAMES was performed using a HP-88 capillary column (length 60 m, ID 0.25 mm, film thickness 0.2 μm, Agilent Technologies, Inc., Santa Clara, CA, USA). Helium at a constant flow rate of 1.0 mL/min was used as the carrier gas. The injector temperature was set to 250 °C and the sample was introduced into the GC in splitless mode with a splitless time of 0.5 min. The oven temperature was programmed from 80 °C to 180 °C at a rate of 40 °C/min and maintained for 14 min. The final oven temperature was increased to 230 °C at a rate of 5 °C/min and held for 5 min. The temperatures of the mass transfer line and ion source were 230 °C and 200 °C, respectively.

FAMES were ionized in the electron impact ionization (EI) mode employing 70 eV of electron energy. The quantitative analysis of FAMES was performed using the selected ion monitoring (SIM) mode. The molecular ions of methylated ester forms of essential fatty acids including methyl linoleate and α-methyl linolenate with their isotopic analogues, ¹³C₁₈-methyl linoleate and ¹³C₁₈-α-methyl linolenate, were detected at *m/z* = 294.2, 292.2, 312.2, and 310.2, respectively.

A detailed description of the calculation and uncertainty evaluation applied for the quantitative analysis of fatty acids through ID-GC/MS measurements has been reported in previous studies. (Kim et al., 2010; Choi et al., 2003) The mass fractions of the individual fatty acids (*C*_{sample}) in cereals and green vegetables (mg/kg) were calculated using the following equation.

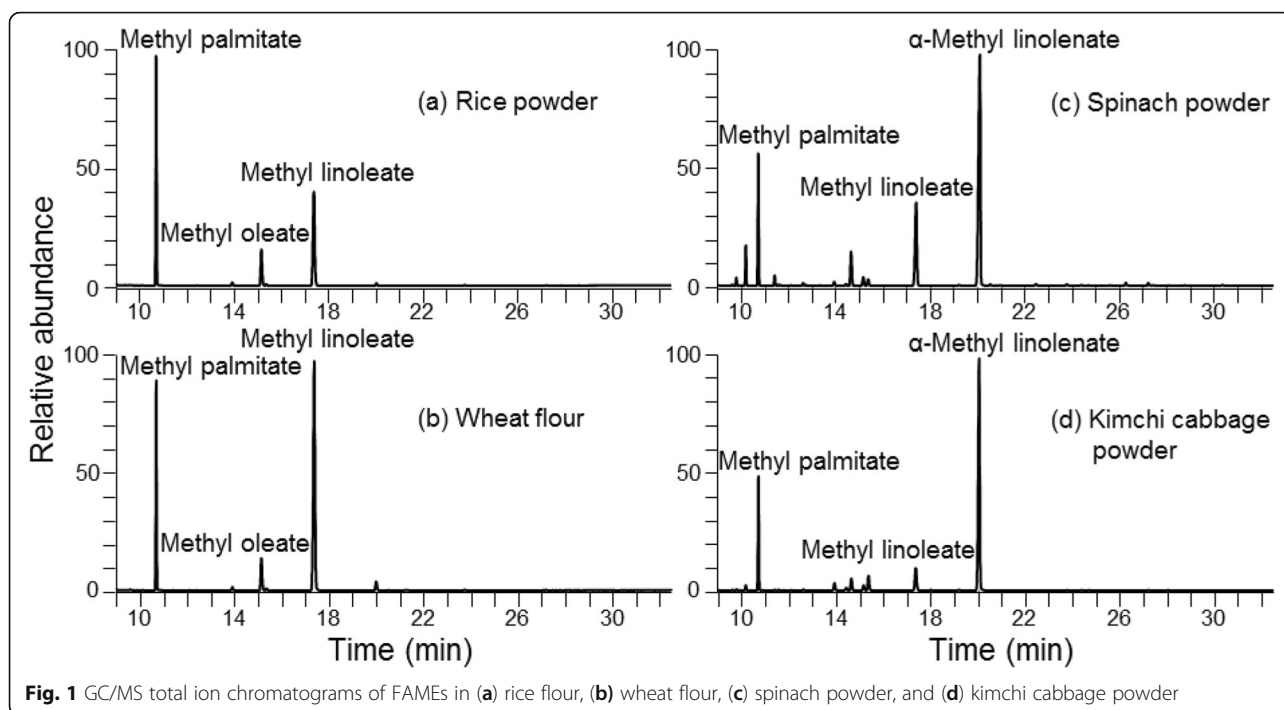
$$C_{\text{sample}} = \frac{M_{\text{is-sol,spiked}} \cdot AR_{\text{sample}} \cdot M_{\text{s-sol,std.mix.}} \cdot C_{\text{s-sol}}}{M_{\text{sample}} \cdot AR_{\text{std.mix.}} \cdot M_{\text{is-sol,std.mix.}}}$$

Here, *M*_{is-sol, spiked} is the mass of the isotope-labeled fatty acid solution added to the sample, *AR*_{sample} indicates the ratio of the FAME and isotope-labeled FAME peak areas in the GC/MS chromatogram of the test sample, *M*_{s-sol,std.mix.} and *M*_{is-sol,std.mix.} are the masses of the fatty acid standard solution and isotope-labeled fatty acid solution, respectively, added to the isotope ratio standard solution, *C*_{s-sol} corresponds to the concentration of the fatty acid in the fatty acid standard solution, *M*_{sample} is the mass of the sample subjected to analysis, *AR*_{std.mix.} corresponds to the ratio of the FAME and isotope-labeled FAME peak areas observed in the GC/MS chromatogram of the isotope ratio standard solution.

Results and discussion

Fatty acid analyses by ID-GC/MS

Homogenized cereal and green vegetable samples, which were developed as candidate CRMs in our laboratory, were tested to determine the overall fatty acid contents in the scan mode of GC/MS. We have used the HP-88 column which shows a good separation for the FAMES in our previous studies. (Lee et al., 2019a; An et al., 2013) The total ion chromatograms (TICs) using the HP-88 column were obtained under optimized extraction and GC conditions described in the experimental section. Figure 1 shows the TICs of the FAMES extracted from rice and wheat flours, as well as spinach and Kimchi cabbage powders. The peaks in the TICs were identified based on the mass spectrum library and GC/MS analysis of the fatty acid standard solutions. As shown in Fig. 1, the cereal samples predominantly contain palmitic acid, which is a saturated fatty acid. Linoleic acid is present as a dominant essential fatty acid component in both cereal



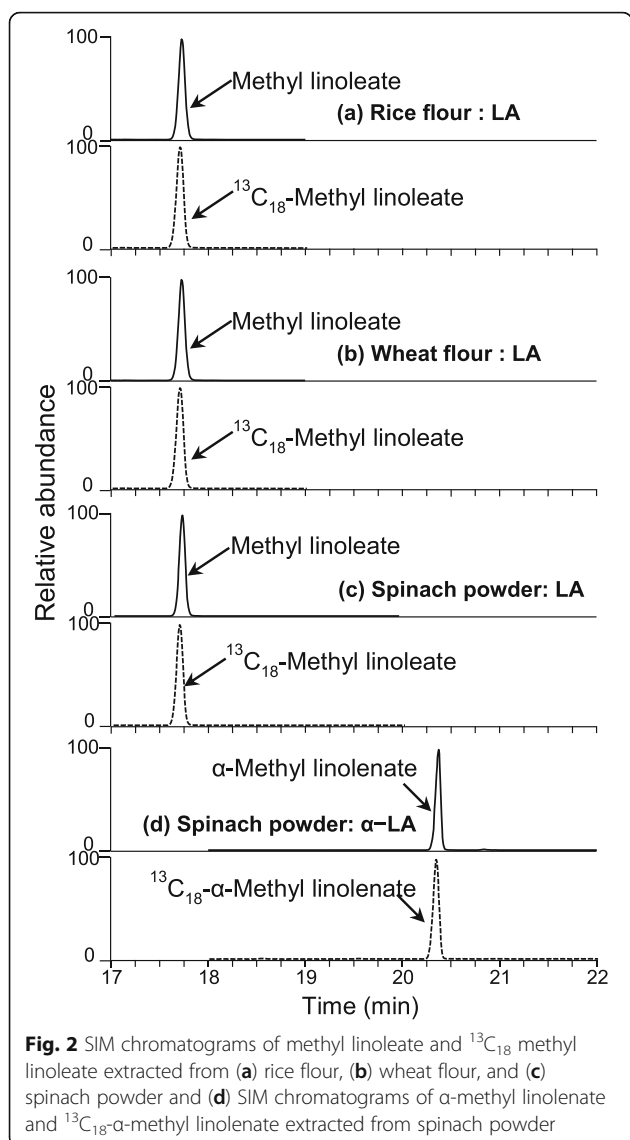
lipids. The results agree with those reported in previous studies. (Yoshida et al., 2011; Yasumatsu & Moritaka, 1964) Wheat flour contains a higher content of linoleic acid than that present in the rice flour. Each cereal sample also contains a low content of α -linolenic acid. For the green vegetable samples, spinach and Kimchi cabbage (which is the main ingredient for Kimchi) were examined. Two essential fatty acids, linoleic acid and α -linolenic acid, are present as major components in the vegetables. Lee et al. also reported that a high content of α -linolenic acid presents in the spinach lipids. (Lee et al., 2000) The vegetable oils have a higher content of unsaturated fatty acids than those contained in the cereal oils. In particular, α -linolenic acid is the most abundant fatty acid in both vegetable samples.

In this study, the essential fatty acids with high contents, i.e., linoleic acid in the cereal samples and linoleic acid and α -linolenic acid in the vegetable samples, were quantitatively analyzed using ID-GC/MS. Figure 2 shows the SIM chromatograms of the target FAMES and their isotope-labeled analogues, which were derivatized from rice flour, wheat flour, and spinach powder extracts. A previous study by our research group demonstrated that molecular ion intensities of the target FAMES with relatively short carbon chains (<C20) and three or less C=C double bonds were sufficient for monitoring in the EI mode. (Lee et al., 2019a) Methyl linoleate and $^{13}\text{C}_{18}$ -methyl linoleate are both observed at 17.7 min, and methyl linolenate and $^{13}\text{C}_{18}$ - α -methyl linolenate are both detected at 20.3 min by monitoring the molecular ions of

FAMES and their isotope-labeled forms. No noticeable interference from the sample matrix is observed. As mentioned above, the fatty acid content was calculated using the ratio of the observed FAME and its isotope-labeled analogue peak areas.

Method validation

As neither well-characterized reference material nor fortified sample for comparison is available, the validation of the developed ID-GC/MS method as a high-order reference method was tested by evaluating the repeatability, reproducibility, and uncertainty of target fatty acid analysis in the homogenized cereal and green vegetable samples. The repeatability of the ID-GC/MS method was assessed by analyzing three subsamples of each candidate reference material within a day. To determine the reproducibility of the developed approach, the same repeatability test for the individual sample was performed on three different days. The measurement results for the target fatty acids in the cereal and green vegetable samples with expanded uncertainties are listed in Tables 1 and 2, respectively. The relative standard deviations (RSD) of the repeatability tests using the ID-GC/MS method are in the 0.23–1.61% range for the cereal samples and 0.39–1.89% range for the vegetable samples. Therefore, the developed method demonstrates good repeatability for fatty acid analysis. The RSDs of the mean values of linoleic acid content obtained at three different time periods are 1.48 and 0.95% for rice and



wheat flours, respectively. The RSDs of the measurement values for spinach and Kimchi cabbage powders show similar results ($\leq 1.6\%$), which indicate good reproducibility of the developed method. The relative expanded uncertainties with a confidence level of 95% for the cereal and vegetable samples are between 1 and 4%, as shown in Tables 1 and 2. The small range of the measurement uncertainties indicates that the developed ID-GC/MS approach can be used as a higher-order reference method for the precise and accurate analysis of the target fatty acids.

As mentioned above, the well-characterized reference material including any fatty acid value with cereal or vegetable matrix is not available for comparison with measured values by the developed method. In this study, an interlaboratory comparison among collaborating laboratories, including Namyang

Table 1 Results of ID-GC/MS measurements for homogenized samples of rice and wheat flours at three different time periods

| Periods | Subsample No. | Results by ID-GC/MS (mg/kg) ^a | |
|-------------------------------|-----------------|------------------------------------------|------------------------------|
| | | Rice flour Linoleic acid | Wheat flour Linoleic acid |
| 1 | 1 | 3599 \pm 64 | 8341 \pm 130 |
| | 2 | 3717 \pm 71 | 8254 \pm 129 |
| | 3 | 3660 \pm 54 | 8335 \pm 112 |
| | Mean | 3659 | 8310 |
| | SD ^b | 59 (1.61%) | 49 (0.58%) |
| 2 | 1 | 3538 \pm 43 | 8400 \pm 88 |
| | 2 | 3554 \pm 59 | 8494 \pm 96 |
| | 3 | 3573 \pm 58 | 8437 \pm 102 |
| | Mean | 3555 | 8437 |
| | SD ^b | 18 (0.49%) | 50 (0.59%) |
| 3 | 1 | 3623 \pm 67 | 8474 \pm 120 |
| | 2 | 3616 \pm 43 | 8461 \pm 170 |
| | 3 | 3650 \pm 61 | 8436 \pm 115 |
| | Mean | 3630 | 8457 |
| | SD ^b | 18 (0.49%) | 19 (0.23%) |
| Overall mean | | 3614 | 8401 |
| SD among periods ^c | | 53 (1.48%) | 80 (0.95%) |

^aThe number following " \pm " is the expanded uncertainty with a 95% confidence level for the measurement value.

^bThe standard deviation (SD) was determined among three values obtained within a day.

^cThe standard deviation (SD) was determined among three mean values obtained at different time points.

Dairy Products Company and Korea Food Research Institute (KFRI), was carried out to evaluate conformity and commutability of the ID-GC/MS method for the analysis of homogenized rice sample. The collaborating laboratories used a gas chromatography-flame ionization detection (GC-FID) approach based on Method 2.1.5.4 in the Korean food code. The GC-FID method used another FAME as an internal standard, which cannot eliminate possible bias resulted from inappropriate recovery correction during sample preparation steps and improper sensitivity calibration during instrumental measurements. The developed ID-GC/MS method as a primary method provides measurement results with traceability to the SI of units, which ensures the recovery correction and instrument sensitivity. The linoleic acid content in multiple subsamples ($n = 10$) of rice using the developed method was 3621 ± 17 mg/kg (RSD = 0.5%). The values obtained from Namyang and KFRI laboratories were 3478 ± 191 mg/kg (RSD = 5.5%) and 3603 ± 64 mg/kg (RSD = 1.8%), respectively. The measurement results by the developed method are comparable to those obtained by the collaborating laboratories. This

Table 2 Results of ID-GC/MS measurements for homogenized samples of spinach and kimchi cabbage powders at three different time periods

| Periods | Subsample No. | Results by ID-GC/MS (mg/kg) ^a | | | |
|-------------------------------|-----------------|------------------------------------------|--------------------------|-----------------------|--------------------------|
| | | Spinach powder | | Kimchi cabbage powder | |
| | | Linoleic acid | α -Linolenic acid | Linoleic acid | α -Linolenic acid |
| 1 | 1 | 6447 \pm 136 | 19947 \pm 820 | 1387 \pm 30 | 9441 \pm 338 |
| | 2 | 6350 \pm 187 | 19694 \pm 793 | 1362 \pm 30 | 9523 \pm 376 |
| | 3 | 6385 \pm 131 | 19882 \pm 753 | 1366 \pm 30 | 9444 \pm 306 |
| | Mean | 6394 | 19841 | 1372 | 9469 |
| | SD ^b | 49 (0.77%) | 131 (1.32%) | 13 (0.98%) | 47 (0.49%) |
| 2 | 1 | 6248 \pm 173 | 19657 \pm 368 | 1373 \pm 32 | 9447 \pm 281 |
| | 2 | 6338 \pm 173 | 19850 \pm 482 | 1365 \pm 29 | 9673 \pm 144 |
| | 3 | 6429 \pm 177 | 19337 \pm 592 | 1337 \pm 33 | 9319 \pm 180 |
| | Mean | 6338 | 19615 | 1358 | 9480 |
| | SD ^b | 91 (1.43%) | 259 (1.32%) | 19 (1.39%) | 179 (1.89%) |
| 3 | 1 | 6301 \pm 246 | 19980 \pm 470 | 1320 \pm 56 | 9645 \pm 224 |
| | 2 | 6333 \pm 244 | 19764 \pm 461 | 1318 \pm 51 | 9609 \pm 227 |
| | 3 | 6350 \pm 252 | 19965 \pm 493 | 1349 \pm 57 | 9698 \pm 226 |
| | Mean | 6328 | 19903 | 1329 | 9651 |
| | SD ^b | 25 (0.39%) | 121 (0.61%) | 17 (1.31%) | 45 (0.46%) |
| Overall mean | | 6353 | 19786 | 1353 | 9533 |
| SD among periods ^c | | 36 (0.56%) | 152 (0.77%) | 22 (1.61%) | 102 (1.07%) |

^aThe number following “ \pm ” is the expanded uncertainty with a 95% confidence level for the measurement value.

^bThe standard deviation (SD) was determined among three values obtained within the period.

^cThe standard deviation (SD) was determined among three mean values obtained at different time points.

implies the ID-GC/MS approach is commutable with the analytical method used by the collaborating laboratories.

The limit of detection (LOD) was evaluated by testing the standard solutions of the target essential fatty acids with signal-to-noise ratio (S/N) of 3. The LODs of linoleic acid and α -linolenic acid using the ID-GC/MS method were 0.15 mg/kg and 0.1 mg/kg, respectively. The limit of quantification (S/N = 10) of linoleic acid and α -linolenic acid were 0.5 mg/kg and 0.3 mg/kg, respectively.

Application of ID-GC/MS method to commercial products

The developed ID-GC/MS method was applied to analyze essential fatty acid contents in commercially available products including cereal and vegetable powders purchased from the local market. Of the various fatty acids, cereal products contained the highest amount of linoleic acid. As shown in Table 3, the commercial rice and wheat flours contain similar amounts of linoleic acid as those observed in the homemade homogenized rice and wheat samples. The rice snacks made with 100% rice show differences in linoleic acid contents depending on the rice varieties. For the vegetable products, linoleic acid and α -

linolenic acid are found as the two major essential fatty acids. Similar to the measurement results for the homogenized vegetables, α -linolenic acid contents are much higher than those of linoleic acid extracted from broccoli and green tea powders (Table 3).

Table 3 Linoleic acid and α -linolenic acid contents of commercial cereal and leaf vegetable products by the developed ID-GC/MS method

| Samples | | Results by ID-GC/MS (mg/kg) ^a | |
|------------------|---|------------------------------------------|--------------------------|
| | | Linoleic acid | α -Linolenic acid |
| Rice flour | A | 3354 \pm 54 | - |
| | B | 3167 \pm 67 | - |
| | C | 3772 \pm 68 | - |
| Wheat flour | A | 6699 \pm 53 | - |
| | B | 6771 \pm 79 | - |
| | C | 7207 \pm 110 | - |
| Rice snack | A | 4196 \pm 36 | - |
| | B | 9827 \pm 55 | - |
| | C | 3544 \pm 30 | - |
| Broccoli powder | | 4967 \pm 385 | 13144 \pm 491 |
| Green tea powder | | 4030 \pm 220 | 9488 \pm 378 |

^aThe number following “ \pm ” is the expanded uncertainty with a 95% confidence level for the measurement value.

Among the vegetable samples, spinach contains the highest contents of both essential fatty acids.

Conclusions

An ID-GC/MS method, which can be used as a reference method for the accurate determination of essential fatty acid contents in cereals and green vegetables is described. Isotope-labeled fatty acids as internal standards were added ahead of the sample preparation steps for the GC analysis, which eliminate possible bias resulting from sample preparation and instrumental measurements. The developed ID-GC/MS method is validated by determining the repeatability, reproducibility, and measurement uncertainty. The SI-traceable measurement results of the essential fatty acids in raw food matrices provides a higher-order metrological quality. A comparison of the target fatty acid analysis results among the collaborating laboratories shows a good agreement within the expanded uncertainties. The developed method can be used to evaluate the reliability of analytical procedures for the quantitative analysis of fatty acids by comparing the measurement results. Additionally, cereal and vegetable CRMs are currently being developed using the ID-GC/MS approach.

Abbreviations

ID-GC/MS: Isotope dilution-gas chromatography/mass spectrometry; GC: Gas chromatography; FAMES: Fatty acid methyl esters; MS: Mass spectrometry; CCQM: Comité Consultatif pour la Quantité de Matière; IDMS: Isotope dilution mass spectrometry; SI: International System; CRMs: Certified Reference Materials; BHT: Butylated hydroxytoluene; FFAs: Free fatty acids; EI: Electron impact ionization; SIM: Selected ion monitoring; TICs: Total ion chromatograms; RSD: Relative standard deviations; GC-FID: Gas chromatography-flame ionization detection; LOD: Limit of detection

Authors' contributions

SL designed and carried out ID-GC/MS analysis and wrote the manuscript. DKL conducted sample preparation. SYB carried out purity analysis. DS, JSP, BMK, and JW carried out the comparative analysis. JL and BK supervised the entire work and edited the manuscript. All authors have read, corrected, and approved the final manuscript.

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Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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