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Monitoring and exposure assessment of ricinine in castor plant-based foods and dietary supplements

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ABSTRACT

Ricinine, a toxic compound found in castor plants (*Ricinus communis)*, causes abdominal pain, nausea, and vomiting. Castor leaves and castor oil-based dietary supplements are commonly consumed, but their ricinine content has not been evaluated for safety. This study aimed to determine the ricinine concentration in these products and to evaluate the ricinine exposure level from their consumption. The developed method combines the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) technique and liquid chromatography–tandem mass spectrometry (LC–MS/MS). A matrix-matched calibration method for castor leaves is then proposed, which demonstrates high linearity over a wide concentration range (50–1000 μg/kg; $r^2 > 0.995$). The intraday and interday accuracies ranged from 93.6% to 113.7%; the precisions were within 10%. The limits of detection and quantitation were ≤ 8.9 μg/kg and ≤ 26.9 μg/kg, respectively, and this validated method was successfully applied to commercial castor plant-based products. Ricinine exposure from the consumption of these foods was estimated to be 0.0001–0.2792 μg/kg body weight/day. This study assesses the safety of castor plant-based food products and could help prevent potential health risks associated with ricinine consumption.

1. Introduction

Throughout their evolution, plants have coped with unfavorable environments by generating a variety of toxic compounds such as alkaloids, glycosides, proteins, terpenes, and steroids (Hussein and El-Anssary, 2019; Matsuura and Fett-Neto, 2015). Ricinine (3-cyano-4-methoxy-N-methyl-2-pyridone) is an alkaloid phytotoxin found in castor plants (*Ricinus communis*) (Musshoff and Madea, 2009; Waller et al., 1965). The leaves of the castor plant contain varying concentrations of ricinine, ranging from 2.3 to 32.9 g/kg. The amount of ricinine in the leaves can vary depending on several factors such as the seasonal stage of the plant and environmental conditions. Ricinine is mainly present in young leaves, and its concentration decreases as the leaves mature. Additionally, the ricinine contents in the flowers, stem, shoots, roots, and seeds of the castor plant are approximately 10.7, 2.4, 0.16, 3, and 0.43–7.0 g/kg, respectively (Waller et al., 1965; Severino et al., 2012). In other words, the ricinine content of the castor plant has different distribution patterns in different parts, with the leaves and flowers containing particularly high levels of ricinine. In an experimental mouse model, ricinine causes abdominal pain, nausea, vomiting, and death, and the lethal dose at which 50% of the mouse population dies is 340 mg/kg for intraperitoneal administration (Ferraz et al., 1999) and 3 g/kg for oral administration (Worbs et al., 2011). In addition, ricinine administered at 20 mg/kg causes seizure-like central nervous system effects in rats (Ferraz et al., 2000; 2002). Laboratory studies on animals have been conducted to investigate the toxic effects of ricinine. In contrast, human studies of ricinine are limited, and to date, the toxicological effects of ricinine in humans have not been specifically examined in any study. Literature includes only case and accident studies of people who were poisoned or died because of ricin after

Abbreviations: d-SPE, dispersive solid phase extraction; LOD, limit of detection; LOQ, limit of quantitation; QuEChERS, quick easy cheap effective rugged, and safe.

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accidental ingestion or exposure to castor beans or their derivatives (Audi et al., 2005).

Although toxic, ricinine has important biological activities, including insecticidal, analgesic, and hepatoprotective effects. (Rana et al., 2012). Castor leaves also serve many medicinal purposes (Scarpa and Guerci, 1982), including relief from itching, swelling, and wounds (Akbar, 2020). In India, castor leaves are considered to alleviate a wide range of disorders, such as viral illnesses bile, burns, glans headaches, malaria, and night blindness (Nemudzivhadi and Masoko, 2014; Patel and Patel, 2016; Poddar et al., 2020). In South Korea, the custom of blanching and drying soft castor leaves and eating them as vegetables has developed uniquely. Although various commercial food products based on castor leaves and castor oil/leaf-based dietary supplements are available on the market, none of these products provide information on the safe limits of ricinine contents. The appropriate doses and reference levels of plant toxins are less well established than those of other toxic substances such as pesticides, veterinary drugs, and environmental pollutants (Dolan et al., 2010). These plant toxins must be evaluated through quantitative analytical methods in various complex matrices for risk assessment, quality, and safety management.

In a previous study, a qualitative liquid chromatography (LC)–based method was developed that simultaneously detects multiple plant toxins (including ricinine) in various foods and feed matrices using orbitrap mass spectrometry (MS) (Mol et al., 2011). Castor plants also contain ricin, a highly toxic protein that can potentially be used in bioterrorism. Exogenous ricinine (a biomarker of ricin) in blood, urine, and plasma has been detected using quantitative LC–MS/MS methods (Johnson et al., 2005; Hamelin et al., 2012; Mouser et al., 2007; Pittman et al., 2013; Verougstraete et al., 2019). In another study, the LC–MS/MS method was developed for the quantification of ricinine in ricinine-spiked animal feed, biopesticides, beverages, cooking oils, and four food matrices (ground beef, chicken breast, hot dogs, and beaten egg) (Black et al., 2015; Cai et al., 2014; Choi et al., 2016; Oishi et al., 2019; Wang et al., 2009). However, castor leaves are a matrix that has not been studied to date for the purpose of method validation. Previous studies have mostly focused on the development of analytical methods for determining artificially added ricinine in biological or food matrices. Notably, no studies have monitored ricinine levels in commercially available food products and assessed dietary exposure; therefore, little is known about the potential risks of consuming foods that may contain ricinine. In this study, we developed a quantitative analysis method using matrix-matched calibration with castor leaves that can more rapidly and accurately detect ricinine and measure its concentration in various commercially available castor leaf products and castor oil/leaf-based dietary supplements to assess ricinine exposure from consumption of these foods.

2. Materials and methods

2.1. Reagents and materials

Ricinine (purity > 95%, CAS No. 524–40–3) was purchased from Sigma Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (CAS No. 67–68–5), which was used to prepare the standard stock solution with ricinine was purchased from Sigma Aldrich. The acetonitrile (CAS No. 75–05–8) used as the extraction solvent was purchased from J.T. Baker (Phillipsburg, NJ, USA). Water with 0.1% formic acid (v/v) and acetonitrile with 0.1% formic acid (v/v) used as mobile phase solvents were purchased from Fisher Scientific (Pittsburgh, PA, USA). 0.1% formic acid in water, which served as the solvent for dilution of the samples, was purchased from Thermo Fisher Scientific (Waltham, MA, USA). HPLC or analytical grade solvents and reagents were used in all experiments. The deionized water (18.2 MΩ•cm) was supplied by a Milli Q pure water system (MilliQ EQ 7000, Merck Millipore, USA). A Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) EN extraction kit Cat #5982–6650 (4 g magnesium sulfate ($MgSO_4$), 1 g sodium chloride (NaCl), 1 g sodium citrate ($Na_3C_6H_5O_7$), 0.5 g sodium hydrogen citrate sesquihydrate ($C_6H_6Na_2O_7{\cdot}1.5\ H_2O$)) and two dispersive solid phase extraction (D-SPE) kits: (i) Cat No. 5982–5221 (2 mL; 25 mg primary secondary amine (PSA), 2.5 mg graphitized carbon black (GCB), 150 mg $MgSO_4$), and (ii) Cat No. 5982–5121 (2 mL; 25 mg PSA, 25 mg octadecylsilane, end-capped (C18EC), 150 mg $MgSO_4$) were obtained from Agilent Technologies (Santa Clara, CA, USA).

2.2. Standard solution

To prepare the standard stock solution at 1 mg/mL, the ricinine standards were precisely weighed on an analytical balance (XSR205DU; Mettler Toledo, Columbus, OH, USA) and dissolved in dimethyl sulfoxide (DMSO), and stored at − 20 °C before use. Standard stock solutions were diluted to 0.1, 1, 10, and 100 μg/mL in acetonitrile to prepare standard working solutions. The solutions prepared in this way were stored at − 20 °C.

2.3. Preparation of blank matrix

The matrix-matched calibration method is a useful tool for the accurate and precise quantification of analytes in complex food matrices. To remove ricinine from castor leaves for the matrix-matched calibration method, dried castor leaves were soaked in water overnight, rinsed three times with fresh water, and boiled for one hour. The rinsing and boiling processes were triplicated. The castor leaves were then squeezed and homogenized in a blender (Aio vacuum blender UB-1000, 1000 Watt, Gyeonggi-do, Korea) for 10 min and aliquoted into a 50 mL polypropylene tube (conical tube, SPL Life Sciences Co., Gyeonggi-do, Korea) for 10 g and stored at − 20 °C.

2.4. Sample collection

Natural and processed products of castor leaf were sourced from Korea and China and purchased through major producers or online stores. Dietary supplements of castor leaf or oil were purchased directly from online markets (Table 2). We have tried to collect as many castor plant-based foods as possible that are available in South Korea.

2.5. Modular extraction procedure

This technique involved extraction with acetonitrile, followed by a salting out step. The extracted sample was purified by D-SPE using sorbents based on the presence of pigment (Module 1) or fat (Module 2) in the sample (Fig. 1).

2.5.1. QuEChERS extraction stage (Step 1)

For extraction of the samples, 10 mL acetonitrile was added into a

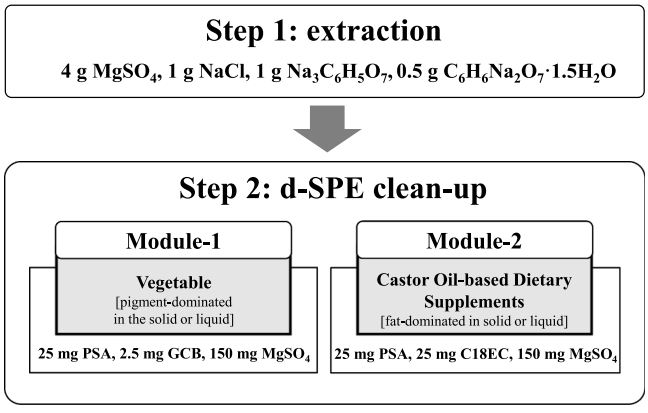

Fig. 1. Modular extraction process for various sample characteristics.

50 mL tube containing a 10 g homogenized sample and mixed for 30 s using a Vortex-Genie 2 T (Scientific Industries, Bohemia, NY, USA). Powders containing 4 g magnesium sulfate ($MgSO_4$), 1 g sodium chloride (NaCl), 1 g sodium citrate ($Na_3C_6H_5O_7$), and 0.5 g sodium hydrogen citrate sesquihydrate ($C_6H_6Na_2O_7{\cdot}1.5\ H_2O$) were then added, the tube was vortexed for 30 s, agitated for 10 min, and centrifuged at 3220 g at 4 °C for 10 min in a cooled high-speed centrifuge (5810 R; Eppendorf, Hamburg, Germany). The 1 mL supernatant, in the acetonitrile phase, was transferred to 2 mL polypropylene tubes packed with various dispersive-SPE sorbents.

2.5.2. Sample clean-up by dispersive-SPE (D-SPE) stage (Step 2)

The extracts were cleaned using the dispersive-SPE purification technique with different sorbents (PSA, C18EC, and GCB) and combinations of these sorbents (Table 1). PSA removes polar pigments, various polar organic acids, and some sugars and fatty acids, GCB removes sterols and pigments such as chlorophyll, and end-capped octadecylsilane (C18EC) removes nonpolar interferents such as lipids. Simultaneously, $MgSO_4$ was added to eliminate excess water and improve analytical splitting. The filling material for each sample was selected according to the sample characteristics (pigment-dominated or fat-dominated). The sorbent compositions were as follows: 25 mg PSA, 2.5 mg GCB, and 150 mg $MgSO_4$ (module-1) or 25 mg PSA, 25 mg C18EC, and 150 mg $MgSO_4$ (module-2). The 1 mL QuEChERS extract, in the acetonitrile phase, was transferred to 2 mL polypropylene tubes containing the selected D-SPE sorbent and vortexed for 30 s. The tubes were centrifuged at 18,213 g for 10 min at 4 °C in a centrifuge (5427 R; Eppendorf, Hamburg, Germany). The 20 μL supernatant was transferred to a clean tube and diluted with 180 μL water with 0.1% formic acid. The final solution was ready for LC–MS/MS analysis.

2.6. LC–MS/MS analysis

LC–MS/MS analyses were performed in ultra-high-performance liquid chromatography (UHPLC) (Vanquish, Thermo Fisher Scientific, Waltham, MA, USA)–triple stage quadrupole (TSQ) mass spectrometry (Altis, Thermo Fisher Scientific, Waltham, MA, USA) system. Chromatographic separation was carried out using a Kinetex 2.6 μm C18 column (100 mm × 2.1 mm) and mobile phases A (water with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid). The chromatographic total run time was 5 min, with the gradient program consisting of different compositions of mobile phases A and B at specific time intervals. A gradient program is 0–0.5 min with 5% B, 0.5–3 min with 5–95% B, 3–4 min with 95% B, 4–4.1 min with 95–5% B, and 4.1–5 min with 5% B. The column temperature was maintained at 40 °C and the final solution injected was 10 μL. The mass spectrometer was operated in the positive mode of electrospray ionization (ESI) and the quantification was performed in the multiple reaction monitoring (MRM) mode. Three transition reactions of ricinine were monitored with a target scan time of 48 ms (Supplementary Table 1). The first transition typically represents the most abundant or intense precursor/product ion pair, which is used as the quantitative ion for the analyte (*m/z* 165→138). The second and third transitions are typically less abundant or intense than the first one and are used as qualitative ions to identify the analyte and to help reduce false positives or negatives (*m/z* 165→118 and *m/z* 165→82). The sheath gas = 50 Arb (arbitrary units), auxiliary gas = 10 Arb, sweep gas = 1 Arb, ion spray voltage = 3500 V, ion transfer tube temperature = 325 °C, evaporation temperature = 350 °C were used as source parameters for the mass spectrometric analysis. MS parameters are listed in detail in Supplementary Table 2.

2.7. Method validation

2.7.1. Calibration curve, limit of detection, and limit of quantitation

The matrix-matched calibration curve was constructed by plotting the peak area (*y*-axis) under the quantitative ion (*m/z* 165→138) against the standard concentration spiked into the matrix (x-axis). For validation purposes, matrix-matched calibration curves were constructed using spiked blank sample extracts at five concentrations. (50, 100, 200, 500, and 1000 μg/kg). The linearity was tested by computing the coefficient of determination (r^2) of the calibration curve using the least squares regression approach. Excellent linearity is affirmed if r^2 is 0.99 or above. Weighting is usually applied to reduce the effect of large variations in data and to improve the accuracy of the calibration curve. Herein, a weighting of 1/x was applied to the calibration curve, assigning more weight to lower concentrations and reducing the effect of large variations in the data. This approach, particularly for low concentrations, yields more accurate, reliable calibration curves than those obtained without weighting. The limit of detection (LOD) and limit of quantitation (LOQ) were obtained using the standard deviation σ of the response and slope S of the calibration curve. σ represents instrument noise or variability, whereas S reflects instrument sensitivity or response to the analyte. σ is determined from seven repeated analyses of the lowest concentration.

The LOD and LOQ were respectively calculated as follows:

$$\mathrm{LOD} = 3{\cdot}3 \times \sigma/S \quad (1)$$

$$\mathrm{LOQ} = 10 \times \sigma/S \quad (2)$$

The above formulas were suggested by the International Council on Harmonization (Borman and Elder, 2017).

2.7.2. Accuracy and precision

The validation factors were evaluated based on performance standards suitable for quantitative analyses, which are presented in the CODEX standard method performance requirements. The accuracy and precision were evaluated as elements of intraday and interday validation. Accuracy refers to the degree to which a measured value approaches the actual value and is calculated as (*the mean of the measured concentration*)/(*the mean of the target concentration*) × 100. Precision refers to the closeness (variance) between the results of each repeated experiment under specified conditions and is calculated as (*the relative standard deviation*)/ (*the mean of the measured concentration*) × 100 (see Table 1).

Table 1
Validation results for the accuracy and precision of quality control (QC) samples ($n = 3$) at three selected levels (50, 500, and 1000 μg/kg).

Concentration Added (μg/kg)		Intraday ($n = 3$)[a]					Interday ($n = 3$)[b]				
		Concentration Found (μg/kg)			Accuracy (%)[c]	CV (%)[d]	Concentration Found (μg/kg)			Accuracy (%)[c]	CV (%)[d]
50	(low)	52.8	±	0.6	105.6	0.9	52.9	±	2.3	105.8	3.0
500	(medium)	568.0	±	5.4	113.7	0.9	568.0	±	48.9	107.0	8.7
1000	(high)	997.6	±	11.7	99.7	1.1	936.0	±	82.7	93.6	8.6

[a] Average of multiple measurements taken on the same day.
[b] Average of measurements each day for three days within a week.
[c] Accuracy (%) = mean of the measured concentration / mean of the target concentration × 100.
[d] CV (coefficient of variation, %) = relative standard deviation / mean of the measured concentration × 100.

2.7.3. Dilution factor

The samples were diluted to adjust the concentration of ricinine if it exceeded the upper limit of the calibration range. For this purpose, a known volume of the sample extract with an appropriate volume of diluent (water with 0.1% formic acid) was mixed with the samples. The diluted sample was then analyzed using LC–MS/MS by employing the same analytical method as for the original sample. Finally, the calculated concentrations of the analytes were corrected for the dilution factor.

2.8. Statistical analysis

The targeted screening quantitative data were processed using TraceFinder software (ver.4.1, Thermo Fisher Scientific, Waltham, MA, USA) and statistical work was performed on a Microsoft Office Excel 2016 (Microsoft Corporation, Seattle, WA, USA). Each experimental result was reported as the mean ± standard deviation of triplicate values (Table 1).

2.9. Assessment of ricinine exposure

The assessment of ricinine exposure was based on the monitoring data for ricinine content in food, daily consumption, and the average body weight of the Korean population (57.6 kg) was derived using the National Health and Nutrition Survey in Korea (Korea Disease Control and Prevention Agency, 2020). The estimated daily intake (EDI) of ricinine was determined as follows:

EDI (μg/kg bw/day) = *daily consumption of a food product* (g/day) × *ricinine concentration in the food product* (μg/kg) / *average body weight of the population* (kg). (3).

3. Results

3.1. Method development

3.1.1. Mass spectrometry

In the positive ionization mode, the MS spectrum of ricinine showed a dominant peak at *m/z* 165, which can be attributed to the protonated molecule of ricinine. The *m/z* 165 ion was then selected as the precursor ion for further fragmentation (Fig. 2 A). Fragmentation of *m/z* 165 resulted in the generation of three product ions with the following relatively high intensities: *m/z* 138, *m/z* 108, and *m/z* 82. Among these product ions, the *m/z* 165→138 ion exhibited the highest intensity and was, therefore, selected as the quantitative ion (Fig. 2B). The *m/z* 138 ion was generated by the elimination of hydrogen cyanide (HCN) molecule, while the *m/z* 108 ion was generated by the elimination of HCN and CHO. Finally, the *m/z* 82 ion was formed by the sequential elimination of HCN, CO, and CHO free radicals from the precursor ion

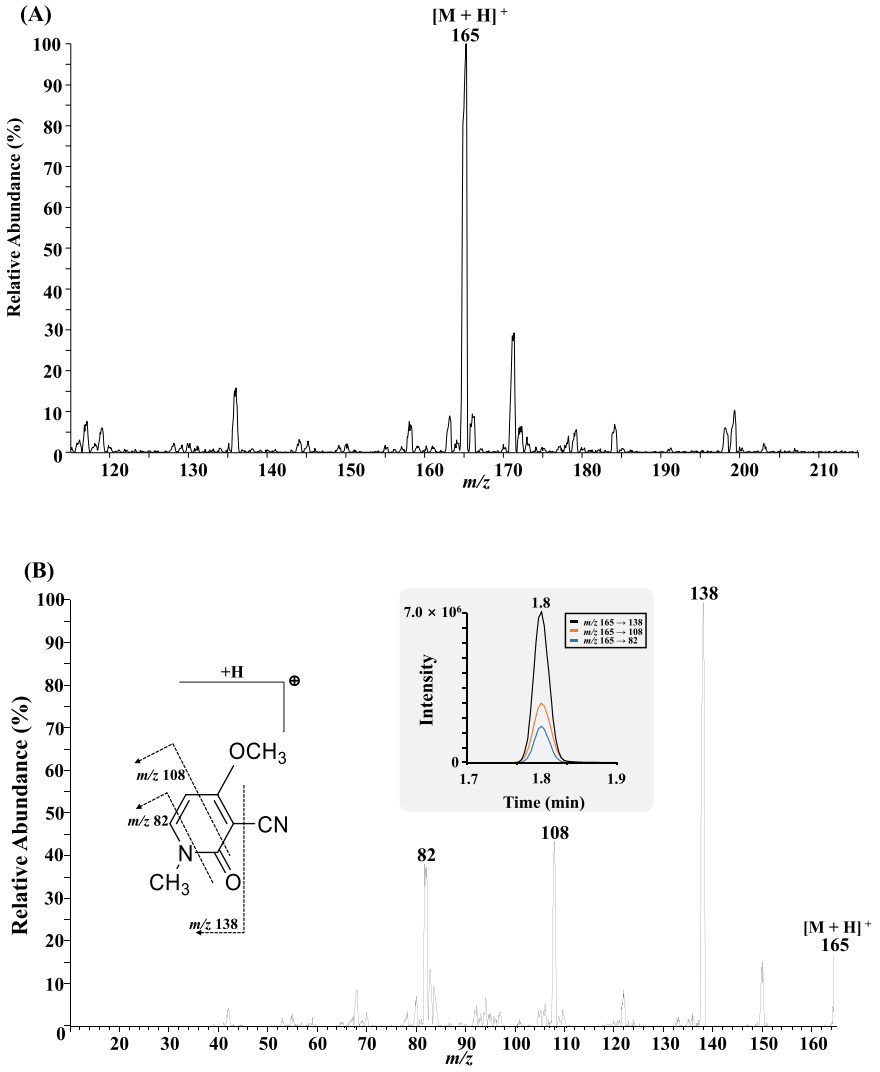

Fig. 2. (A) Full scan of the ricinine standard working solution. (B) The product ion mass spectrum of the ricinine (*m/z* 165) ion. Three major product ions at *m/z* 82, *m/z* 108, and *m/z* 138 were observed in the LC–MS/MS spectrum.

(Cai et al., 2014).

3.1.2. Optimization of LC–MS/MS analysis conditions

It is necessary to optimize the chromatography conditions to achieve separation and specificity of ricinine by LC–MS/MS. This study used different parameters, including the column temperature, flow rate, and mobile phase composition, to determine the optimal conditions for the separation and detection of ricinine. Increasing the column temperature to 50 °C decreased the retention time but led to a poor peak shape. Decreasing the column temperature to 35 °C increased the retention time; therefore, a column temperature of 40 °C was selected. A flow rate of 0.5 mL/min was chosen because a flow rate higher than 0.6 mL/min reduced the total analysis time to 3 min but increased the backpressure and stress on the column and instrument. In contrast, reducing the flow rate to 0.3 mL/min increased the analysis time, which is unfavorable for rapid analysis. Additionally, ricinine is polar due to the presence of polar functional groups, such as amine and carbonyl groups, which have hydrogen bonds and dipole–dipole interactions with polar solvents. A higher proportion of mobile phase A (water with 0.1% formic acid) indicates better retention of polar analytes and can result in better ionization efficiency. To achieve this, we tested different linear slopes of mobile phase A (water with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid). Following initial optimization of the MS detection parameters and chromatographic conditions, the analyte was isolated within the shortest possible time (Supplementary Table 2).

3.2. Method validation

3.2.1. Selectivity

The endogenous analyte was removed from castor leaves in a representative matrix (see subsection 2.3). This matrix, from which more than 99.9% of the ricinine was removed, was used as the blank matrix. After adding a standard solution, the analyte was clearly identified without interference by the matrix components. Fig. 3 shows the MRM chromatograms of the blank matrix (Fig. 3A), standard working solution (0.1 μg/mL, Fig. 3B), the spiked sample in the blank matrix (100 μg/kg, Fig. 3C), and the selected castor plant-based natural product (sample ID A; see Table 2 and Fig. 3D). The blank matrix showed a minor peak at retention time (RT) 1.8 min, but this was at a trace level well below the LOQ and, therefore, met the acceptance criteria of the bioanalytical guidelines. The chromatogram of the standard working solution of ricinine (Fig. 3B) showed sharp and symmetrical peaks, indicating successful chromatographic separation at the 1,8 min RT. Castor leaf-based samples were diluted 10,000-fold to account for high concentrations (Fig. 3D). Despite the dilution, a distinct peak corresponding to ricinine was still clearly observed in the chromatogram, indicating successful detection. The target analyte (i.e., ricinine) was well separated from all other samples and detected under the optimized LC–MS/MS conditions (Fig. 3).

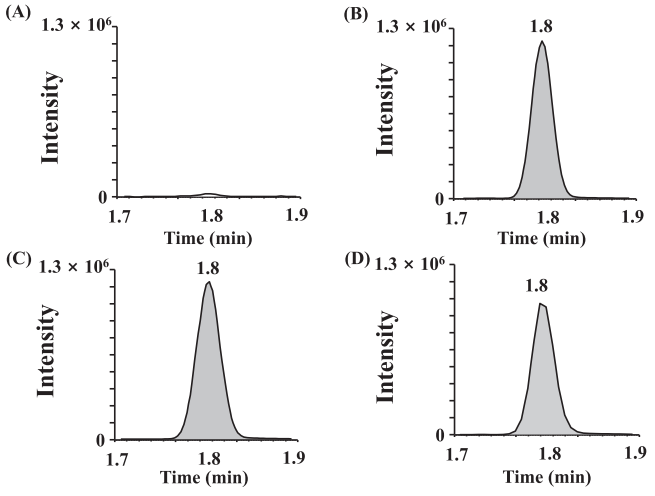

Fig. 3. Representative chromatograms of ricinine under the multiple reaction monitoring mode for the (A) blank matrix, (B) standard working solution (0.1 μg/mL), (C) spiked sample in the blank matrix (100 μg/kg), and (D) castor plant-based natural product (sample ID: A (see the Table 2), diluted additionally 10,000 times).

3.2.2. Linearity, limit of detection, and limit of quantitation

The linearity of the method was evaluated using the matrix-matched calibration curve with castor leaves. As calibrators, the standard samples at different concentrations (50, 100, 200, 500, and 1000 μg/kg) were spiked with the ricinine standard working solution (1, 10, and 100 μg/mL) in the blank matrix. The ricinine concentration was strongly linearly expressed as y (concentration) = 14,850 x + 387,000 (correlation coefficient $r^2 = 0.9969$, using a weighing factor of 1/x). The LOD and LOQ were determined as 8.9 and 26.9 μg/kg, respectively (Supplementary Table 3).

3.2.3. Accuracy and precision of the method

The accuracy of the method was evaluated by averaging the quality control (QC) measures at three selected concentrations (low, medium, and high); meanwhile, the precision was evaluated from the repeatability (intraday) and reproducibility (interday over three consecutive days) measures. In this evaluation, ricinine standard working solutions at different concentrations were added to the blank matrix to obtain final QC concentrations of 50, 500, and 1000 μg/kg (Table 1). The intraday and interday accuracies of the QCs at the three levels ranged from 99.7% to 113.7% and from 93.6% to 107.0%, respectively. The intraday and interday precisions were 0.9–1.1% and 3.0–8.7%, respectively.

3.2.4. Cross-validation

Cross-validation was performed by two external organizations. (referred to as Organizations A and B). In both cross-validations, the same concentrations of QCs under the same analytical conditions as those for the method validation were evaluated on a Vanquish UHPLC–Altis TSQ system. The accuracies and precisions were calculated as 85.5–105.1% and 0.3–0.7%, respectively, by Organization A and as 89.9–104.3% and 0.3–1.1%, respectively, by Organization B.

3.3. Ricinine contents in various food products

3.3.1. Ricinine concentration in natural products

Natural products refer to products that are dried or boiled without further processing or additives. The ricinine contents were monitored in 16 commercial (11 dried and 5 boiled) castor plant-based natural products purchased from domestic online markets in South Korea. The ricinine concentrations in the dried and boiled castor leaves ranged from 0.0107 to 2.3456 g/kg and from 0.0012 to 0.0495 g/kg, respectively (Table 2). The 11 kinds of dried castor leaves were labeled ID A–K and the five kinds of boiled castor leaves were labeled ID L–P. In general, the ricinine concentration was higher in the dried castor leaves than in the boiled leaves.

3.3.2. Ricinine concentration in processed foods

Only two processed castor plant-based foods (both from Korea) were found during our search for commercial castor plant-based products. Sample ID Q (Table 2) is sold frozen and can be heated in a microwave prior to consumption. The ricinine concentration of ID Q was relatively high (1.7047 g/kg) but will likely be reduced by cooking. The other product (sample ID R; Table 2) is vinegar made from castor leaf extract, with a ricinine concentration of 0.0555 g/kg.

3.3.3. Ricinine concentration in dietary supplements

The six commercially available dietary supplements analyzed in this

Table 2
Results of the monitoring of ricinine content in foods derived from castor plants, including natural products, processed products, and dietary supplements, and the assessment of ricinine exposure.

Category[a]	ID[b]	Raw materials	Modular extraction	Area[c]	Ricinine (g/kg)	Exposure estimation (μg/kg bw/day)
Natural products	**A**[d]	castor leaves	Module-1	Sancheong-gun, Korea	2.3456	0.2792
	B[d]	castor leaves	Module-1	Domestic (not specified)	0.5880	0.0182
	C[d]	castor leaves	Module-1	Domestic (not specified)	0.6161	0.0733
	D[d]	castor leaves	Module-1	Domestic (not specified)	0.5957	0.0709
	E[d]	castor leaves	Module-1	China	0.5877	0.0700
	F[d]	castor leaves	Module-1	Yanggu-gun, Korea	0.5743	0.0684
	G[d]	castor leaves	Module-1	Domestic (not specified)	0.8714	0.1037
	H[d]	castor leaves	Module-1	Domestic (not specified)	0.3277	0.0390
	I[d]	castor leaves	Module-1	Domestic (not specified)	0.6731	0.0801
	J[d]	castor leaves	Module-1	Hapcheon-gun, Korea	1.0715	0.1275
	K[d]	castor leaves	Module-1	Domestic (not specified)	0.0107	0.0021
	L[e]	castor leaves	Module-1	Domestic (not specified)	0.0427	0.0054
	M[e]	castor leaves	Module-1	China	0.0099	0.0012
	N[e]	castor leaves	Module-1	Domestic (not specified)	0.0105	0.0013
	O[e]	castor leaves	Module-1	Domestic (not specified)	0.0012	0.0001
	P[e]	castor leaves	Module-1	Domestic (not specified)	0.0495	0.0059
Processed products	**Q**[f]	castor leaves	Module-1	Namyang-ju, Korea	1.7047	0.0528
	R[g]	castor leaves	Module-1	Domestic (not specified)	0.0555	0.0007
Dietary supplements	**S**[h]	castor leaves (30%)	Module-1	Domestic (not specified)	0.3978	0.0049
	T[i]	castor oil	Module-2	USA	0.0157	0.0019
	U[i]	castor oil	Module-2	Canada	0.0022	0.0003
	V[i]	castor oil	Module-2	USA	<LOQ	0.0000
	W[i]	castor oil	Module-2	Germany	0.0082	0.0010
	X[i]	castor oil	Module-2	Germany	0.0043	0.0005

[a] Different types of products
[b] Sample ID
[c] Producing district
[d] Dried castor leaves
[e] Boiled castor leaves
[f] Frozen food
[g] Vinegar made from castor leaves
[h] Dietary supplements made from castor leaves
[i] Dietary supplements made from castor oil

study were all available on domestic online markets; one is domestically manufactured (containing 30% castor leaves) and five are manufactured overseas (castor oil-based products). The ricinine concentrations detected in the six dietary supplements exceeded the LOQ and the highest detected ricinine concentration was 0.3978 g/kg (Sample ID S; Table 2), similar to that of dried castor leaves. As dietary supplements are generally chosen without consulting a professional such as a pharmacist or doctor, they may pose a potential risk to the human body. Therefore, guidelines for the appropriate use of dietary supplements containing ricinine should be established.

3.4. Assessment of dietary exposure to ricinine

Food intake and the average body weight were calculated using the data from the Korea National Health and Nutrition Examination Survey to assess dietary exposure to ricinine. Table 2 shows the estimated exposure values based on the ricinine content in food. The dietary exposure to ricinine was estimated to be 0.0001–0.2792 μg/kg bw/day from natural products, 0.0007–0.0528 μg/kg bw/day from processed products, and 0.0000–0.0049 μg/kg bw/day from dietary supplements. Supplementary Table 4 lists the exposure to ricinine in diet, considering gender and age. The data also show age-related differences in the exposure of people to ricinine. In particular, dietary exposure to ricinine was only observed in individuals aged 65 years and older, suggesting that older adults may be more likely to be exposed to ricinine than other age groups through their diet. Alternatively, there was no remarkable difference between exposure of men and women to ricinine, suggesting that gender may not be an important determinant of dietary exposure to ricinine.

3.5. Reduction of ricinine

Ricinine was found in most commercial castor leaf-based foods that we tested. To reduce the levels of ricinine in existing products and develop safe ways to consume them, we conducted a ricinine reduction study. The dried castor leaf samples, which yielded the highest detected ricinine concentrations, were processed and analyzed through five steps. In Step 1, the ricinine concentration under the initial sample conditions of the dried castor leaves (Sample ID A) was expressed as 100%. In Step 2, the dried castor leaves (Step 1) were soaked in lukewarm water for 16 h. The ricinine concentration after soaking was unchanged from that of Step 1. In Step 3, the castor leaves were rinsed three times in cold water and their ricinine concentration dropped significantly. In Step 4, the leaves were boiled for 10 min in water, and in Step 5, they were rinsed in cold water and fried for three minutes. After Step 5, the ricinine concentration was 89% lower than in the initial sample. Apparently, the toxin concentration was found to be substantially reduced by the rinsing process (Step 3) after full immersion in water in Step 2. The relative concentrations of ricinine for different steps are compared in Fig. 4.

4. Discussion

The analytical method developed in this study enables the fast and accurate quantitation of endogenous ricinine in commercially available castor plant-based foods. Although the LC–MS/MS method is widely used for its high sensitivity and selectivity, its accuracy, precision, and sensitivity can be degraded by matrix effects. As we focused on food sample analyses, we expected potential matrix effects because of the complex composition of the food matrix. Although we did not perform any concentration-specific validation of the matrix effect, we found a weak matrix effect of about 80% in our high concentration-based assays

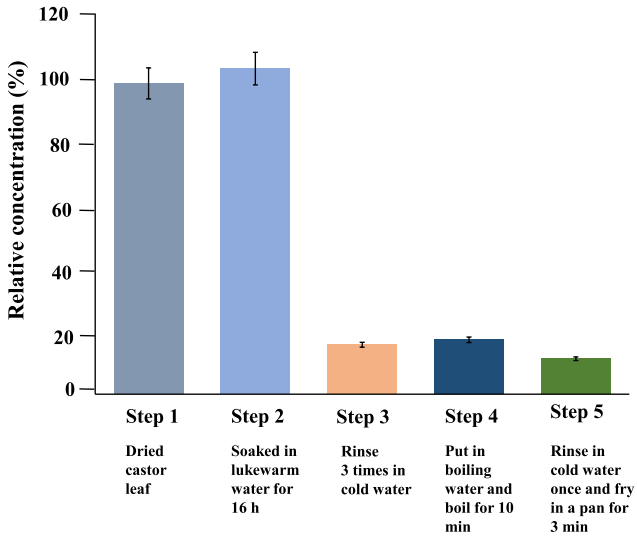

Fig. 4. Process of reducing the plant toxin, ricinine. The ricinine content was analyzed after each step, and the concentrations of ricinine were compared to that of the first step (i.e., relative ricinine concentrations). After the last step, the ricinine content in the leaves decreased by 89% compared to that in the first step. The values of the relative concentration are the average of three replicates, and the error bars represent standard deviations ($n = 3$).

(Matrix effect (%) = (The peak area of the analyte in the sample /the peak area of the analyte in the standard working solution) × 100). To compensate for potential matrix effects, a widely used method in food analysis is a matrix-matched calibration based on the same matrix as the sample, but for castor leaves, matrices free of the target endogenous analytes are very difficult to obtain. a variety of proven methods were considered to compensate for the lack of blank matrices in the quantitative analysis of endogenous compounds: background subtraction, surrogate matrix approaches, and isotopically labeled internal standards (Li and Cohen, 2003; Thakare et al., 2016). Quantification by background subtraction (Gachet et al., 2015) is difficult because the endogenous levels of ricinine vary among the samples. Deuterium-substituted ricinine (d_3-ricinine) was not adopted as an isotope-labeled internal standard because large amounts of d_3-ricinine were detected in real samples. In addition, the concentration of ricinine in castor leaves was very high, exceeding the upper LOD of the device, so none of the above-mentioned approaches could solve the problem. Therefore, for the removal of the endogenous analyte ricinine, we applied a simple immersion, soak, blanch, and rinse procedure, which removed 99.9% of the ricinine from the matrix. In fact, the samples from which we quantified ricinine are not raw castor leaves but commercial food products that have already undergone a pretreatment (e.g. drying or blanching in water), depending on the manufacturer or retailer. Therefore, the steps that we took to prepare a blank sample using castor leaves are reasonable. The FDA guidelines state that "the biological matrix used to prepare the calibration standard must be identical to the sample used in the study and must be free of endogenous analytes" but do not specify how to obtain an endogenous analyte-free biological matrix (Thakare et al., 2016). As described above, our quantification strategy adopts a matrix-matched calibration approach using the same matrix as the real sample after removing the endogenous analytes. Good accuracy and precision were maintained at low, medium, and high concentrations, demonstrating that the endogenous compounds were quantified without compromising the validation criteria. All validation parameters of the method satisfied the CODEX criteria and were applied to the determination of endogenous ricinine in several castor plant-based samples.

The main objective of this study was to evaluate the safety of commercially available castor plant-based foods, with a focus on their classification as food under domestic food regulations. Castor oil is derived from the castor bean and may contain high levels of ricinine, but it is not primarily consumed as a food product in South Korea; instead, it is widely used in cosmetics and industrial applications. Therefore, as the study focused on castor plant-based foods, we carefully decided to exclude castor oil from the scope of the study, though castor oil-based dietary supplements were considered as food products and included in the scope of the study. Unusually, castor leaves are consumed as a side dish in South Korea, and many castor leaf-based food products are available in the market. The majority of our survey sample comprised castor leaf-based foods. The leaves of the castor plant contain varying concentrations of ricinine ranging from 2.3 to 32.9 g/kg, which are higher levels of ricinine compared to that present in the other parts of the plant. The highest ricinine concentration in the castor plant-based products we examined (2.3456 g/kg) was similar to the concentration in raw castor leaves reported in previous studies (1.5–2.3 mg/g) (de Melo Cazal et al., 2009; Nebo et al., 2019). The similarity of the ricinine concentrations of raw castor leaves and products, despite the products being subjected to pretreatment such as washing and boiling, suggests that the ricinine in castor based foods and supplements may pose a health risk if consumed in excessive amounts. Therefore, it is important to develop appropriate processing and QC measures to ensure the safety of these products and to suggest safe consumption methods. Experiments on ricinine reduction showed that soaking dried castor leaves in lukewarm water for 16 h did not change the ricinine concentration compared to the initial sample. This observation suggests that ricinine may have limited solubility in water. In contrast, subsequent washes remarkably reduced the ricinine concentration, suggesting that ricinine may be removed by physical washing with water or dilution effects caused by water rather than by complete dissolution. Although the specific aqueous solubility of ricinine is uncertain, our results indicate that aqueous treatments are effective in reducing ricinine concentrations, and the reduction in ricinine observed after soaking and rinsing suggests that aqueous treatments can partially remove ricinine from castor plant leaves, thereby reducing the toxin concentrations. Our findings may provide insight into safer ways to consume castor plant-based products.

Data from the dietary exposure assessment of ricinine-containing foods show an interesting pattern of individual exposure to ricinine. Dietary exposure was observed only in individuals aged 65 years and older, whereas no dietary exposure was observed in younger-aged groups. These results may be attributed to factors related to dietary habits and consumption patterns in different age groups. It is necessary to target specific age groups or populations and implement appropriate measures to minimize the potential health risks associated with ricinine consumption.

Five castor oil-based samples were included among the samples examined. These five samples comprised castor oil-based dietary supplements in the capsule form. We acknowledge that we were unable to use blank matrices for the five castor oil-based supplements because it is difficult to remove the complex compounds found in some supplements and sophisticated pretreatment is required for this removal. Given these constraints and limitations, we focused primarily on castor plant leaves, which comprised the majority of the samples studied and provided a convenient and accessible source of ricinine for our analyses. We conducted recovery studies on edible oils other than castor oil and found recoveries in the range of 80–120%. Conducting a recovery study on castor oil itself would have provided more direct insight into the quantification of ricinine in castor oil-based products. We recognize this limitation and aim to conduct further research and validation in this area in the future.

In this study, we did not directly evaluate whether the estimated intake of foods containing ricinine corresponded to urinary or serum levels associated with adverse effects in humans or animals. Evaluation of urinary or serum levels associated with the estimated intake typically involves conducting specific toxicological studies in which ricinine

intake levels are correlated with compound levels in biological fluids (i.e., urine or serum). A limitation of conducting controlled experiments in human subjects to determine the toxic effects of ricinine is that they are difficult to perform in human populations, as exposure to potentially harmful compounds can pose serious health risks. However, toxicological data from existing animal studies, as well as poisoning incidents and case studies in humans, are helpful in understanding the potential toxic effects of ricinine in humans. As mentioned in the introduction, in animal studies, the lethal dose in rats is 3 g/kg (body weight), and the dose that causes seizures in rats is 20 mg/kg (body weight). Assuming a weight of approximately 60 kg for humans, the lethal dose for ricinine is 180 g, and the dose that causes seizures is 1.2 g. The highest concentration of ricinine in the castor plant-based foods we examined was 2.3 g/kg. That is, our calculations show that a person weighing 60 kg can be killed by ingesting about 78.3 kg of castor plant-based foods, and 0.5 kg of this food can cause seizures.

While previous studies have focused on developing analytical methods to measure ricinine intentionally added to biological or food matrices, this study is the first to attempt and succeed in a matrix-matched calibration method using castor leaves to determine ricinine levels in a variety of commercially available castor plant-based foods and assess dietary exposure.

The importance of our study is its contribution to the understanding of ricinine levels in commercially available castor plant-based foods and dietary supplements. It provides valuable information for risk assessment and consumer safety by quantifying ricinine content and assessing potential exposure levels. This research can help to inform regulatory authorities, food manufacturers, and consumers about the potential risks associated with exposure to ricinine. Furthermore, this research can support the development of appropriate processing and QC measures and guidelines for the safe consumption of castor plant-based products.

5. Conclusions

We present a novel approach using QuEChERS and LC–MS/MS with matrix-matched calibration for rapid and reliable quantification of ricinine in commercial castor plant-based foods. This optimized method is fully validated and shows excellent linearity, accuracy, and precision. This study further provides important information on ricinine concentrations and dietary exposure levels in commercially available castor plant-based foods; the findings suggest that further attention and monitoring are necessary to ensure the safety of consumers who may be exposed to ricinine through food consumption. The pretreatment of soaking castor leaves in water and rinsing them three times was found to effectively remove approximately 80% of the endogenous ricinine based on the highest detected sample, suggesting that there is a need to provide consumers with a safe way to consume castor plant-based foods. The developed analytical method and the data that we obtained from it may serve as a useful tool for future safety management of phytotoxins in foods.

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CRediT authorship contribution statement

Hana Park: Conceptualization, Resources, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Junghyun Son:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing. **Yoeseph Cho:** Investigation. Changmin Sung: Resources. Resources. **Hophil Min:** Resources. **Ki Hun Kim:MinSun Jung:** Exposure assessment. **Shinai Choi:** Exposure assessment. **Yong-Sun Bahn:** Supervision. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data that has been used is confidential.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105484.

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